PROTEIN SCIENCE

37th Annual Symposium of The Protein Society

SPECIAL ISSUE

PS37

July 13 - 16, 2023
Boston, MA

PROTEIN SOCIETY

Mission

The Protein Society is a not-for-profit scholarly society with a mission to advance state-of-the-art science through international forums that promote communication, cooperation, and collaboration among scientists involved in the study of proteins.

For 37 years, The Protein Society has served as the intellectual home of investigators across all disciplines - and from around the world - involved in the study of protein structure, function, and design. The Society provides forums for scientific collaboration and communication and supports professional growth of young investigators through workshops, networking opportunities, and by encouraging junior researchers to participate fully in the Annual Symposium. In addition to our Symposium, the Society's prestigious journal, *Protein Science*, serves as an ideal platform to further the science of proteins in the broadest sense possible.

#PS37

1986 - 2023

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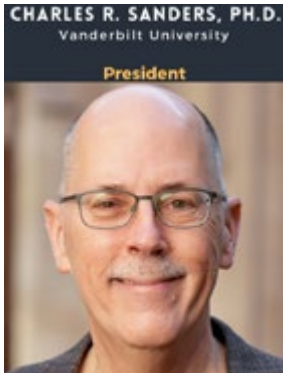
Welcome



Welcome to Boston and the 37nd Annual Symposium of the Protein Society! We are excited to bring you this year's Symposium comprising 12 exceptional scientific sessions covering a wide range of scientific achievement in the field of protein science. Our program committee, chaired by Mary Munson, Ph.D and co-chaired by Ruben Gonzalez, has convened a host of stimulating speakers and topical areas of current research. This year's Symposium continues our commitment to open participation, with a number of Symposium talks coming from contributed sessions and speakers across a broad range of topics. We are proud of the amazing line-up of poster presentations, and our ability to recognize outstanding young scientists through specially-designated sessions and awards. Protein Society Award-winners will also present their work throughout the Symposium, including the inaugural edition of our Marie Maynard Daly Award lecture! Moreover, if you cannot make it to all talks, you can read about their work in a future special issue of *Protein Science*, the Society journal. A special treat in this year's edition of the Annual Symposium will be a Plenary Lecture by Susan Marqusee.

While we celebrate more than 3 decades of impact in the field of protein science, future challenges drive us to advocate for the importance of scientific research throughout the world and to continue to strive for diversity, equity and inclusivity in all of our endeavors. I urge you to engage in important dialogues within our community and, of growing importance, with the public, on the critical need for scientific research.

Thank you for joining us at our 2023 Symposium in Boston. We hope you will take advantage of everything our event has to offer. Finally, we would appreciate it greatly if you will take a few moments to give us your feedback and suggestions for improvement in the survey you'll receive at the end of the conference. We are committed to strengthening our events to meet the needs of members and constituents, and your honest feedback will directly shape future events.

Wishing you all a fruitful and engaging Symposium. Please take a moment to introduce yourselves to me during the meeting; I would love to meet you.

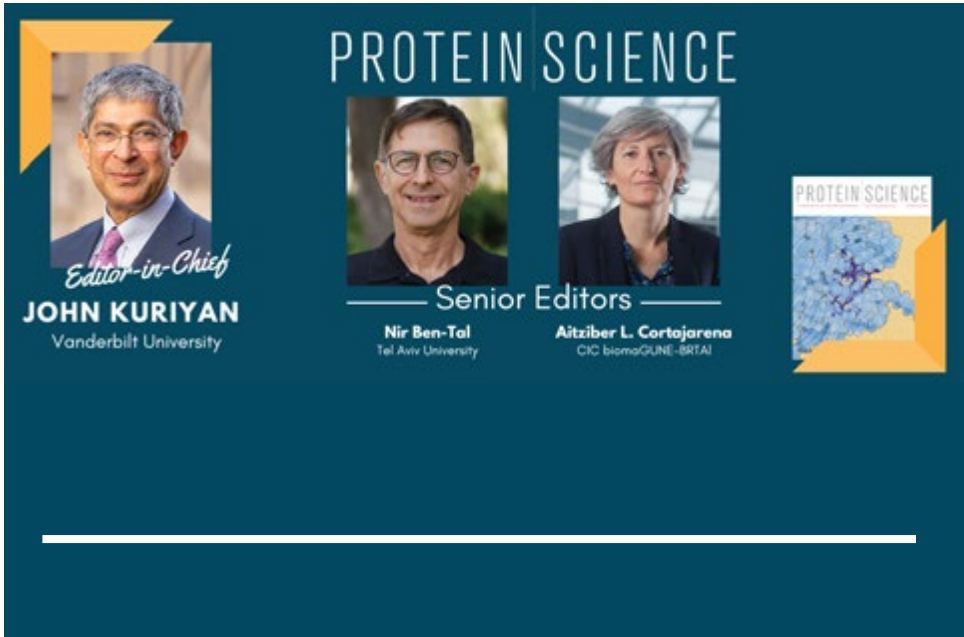
Sincerely,

Charles R. Sanders,
President of The Protein Society

Program Planning Committee

Boston | July 13 - 16, 2023

A special thanks to our Program Planning Committee, chaired by Mary Munson, UMass Chan Medical School, and co-chaired by Ruben Gonzalez, Columbia University. This year's PPC has assembled a program that spans the broad range of protein science, combining critical underpinnings of the field, the latest developments and a vision into the future with career development and technical workshops as well as ample networking opportunities.



At A Glance

37th Annual Symposium

July 13 - 16, 2023

	July 12	July 13		July 14		July 15		July 16	
7 a.m.		**Registration** (7 a.m. - 3:30 p.m.)		**Registration** (8 a.m. - 3 p.m.)		**Registration** (8 a.m. - 3 p.m.)		**Registration** (8 - 11:30 a.m.)	
7:30 a.m.									
						TPS New Member Business Meeting (8:15 - 9 a.m.)			
8:30 a.m.		**2023 Opening Plenary** Susan Marqusee, UC Berkeley		**2023 Marie Maynard Daly:** Renã Robinson, Vanderbilt U				**Session 1:** Engineering Protein Fate & Function	**Session 2:** Aggregates, Amyloids or Condensates?
9:10 a.m.		**Session 1:** Modern Anti-Viral Strategies	**Session 2:** Protein Folding & Function in Context	**Session 1:** RNA-Protein Machines: Ancient Synergies	**Session 2:** Peptide Modalities: Size Doesn't Matter	**Session 1:** Structure Prediction & Design	**Session 2:** Proteins in Motion		
11:20 a.m.								**2023 Stein & Moore:** Kevin Gardner, CUNY Advanced Structural Research Center	
Noon		**Networking Tables Event w/DEI, Careers & More** (RSVP Required)		**Protein Science Workshop/ Educator's Panel**		**Undergrad Research Session/**		**2023 Hans Neurath:** Elena Conti, Max Planck Institute	
1:45 p.m.		**Session 1:** Structures of Mega-Complexes	**Session 2:** Protein Evolution: Lessons from the Past	**Session 1:** Capturing Protein Interactions	**Session 2:** Membrane Proteins: From Natural to De-signed	**2021 Stein & Moore:** David Agard, UCSF			
3 p.m.						**2023 Carl Branden:** Arthur Lesk, Penn State U			
4 p.m.	**Registration** (4 - 7:30 p.m.)					**2023 Christian B. Anfinsen:** Mei Hong, MIT			
						2023 Dorothy Crowfoot Hodgkin: Patricia Clark, U of Notre Dame			
5 p.m.		**Poster Presentations & Exhibits Networking Reception** (5 - 7 p.m.)				**2023 Emil Thomas Kaiser:** Jason Gestwicki, UCSF			
7 p.m.	**Student Welcome Orientation (RSVP Only)**								
8 p.m.						**Night At The Museum (RSVP Only)** (8:30 - 11:30 p.m.)			

Industry Visits will take place July 12, 13, and 14

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Industry Visits

Corporate Support

The Protein Society is extremely grateful to the following sponsors for their generosity and continued support.

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WEIGHING MOLECULES WITH LIGHT

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See change

WILEY

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Thank you for helping us celebrate 37 years of impact.

Registration

Pick Up Your Badge

Though the registration desk will be open throughout the Symposium, we encourage you to pick up your badge on Wednesday, July 12, from 4 - 7:30 p.m (and be entered to win one of three $100 gift cards). This allows you plenty of time to get a seat for the Opening Plenary.

Attendees are required to wear their badge at all times. In addition to being a means of identification, your badge is required for admission to all scientific sessions, networking/social events, and exhibits.

Registration Hours

Wednesday, July 12: 4 - 7:30 p.m.
Thursday, July 13: 7 a.m. - 3:30 p.m.
Friday, July 14: 8 a.m. - 3 p.m.
Saturday, July 15: 8 a.m. - 3 p.m.
Sunday, July 16: 8 - 11:30 a.m.

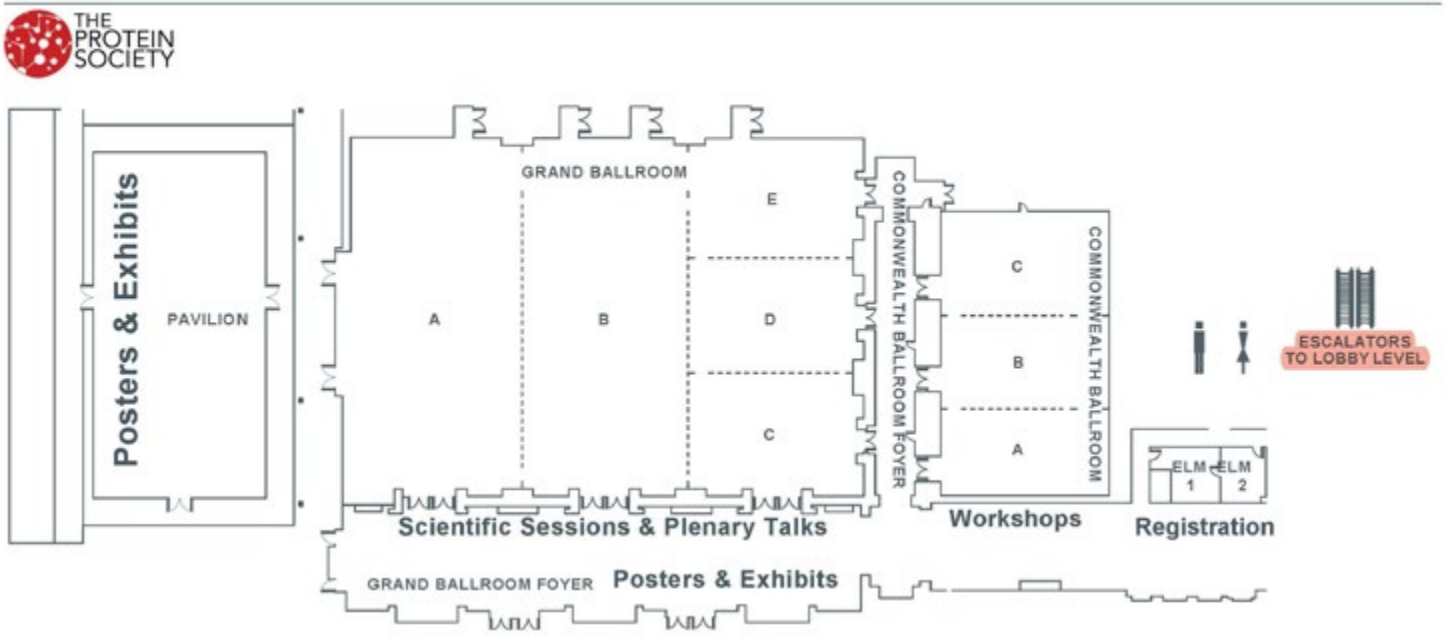
Wifi

Custom Network: Westin_Conference
Password: Vancouver24

Get Your Swag Here!

Be sure to stop by our exhibit booth next to registration, chat with TPS staff, and pick up some cool swag!

Hotel Floor Plan



Diversity

HER

Protein Society DEI Statement:

his

Protein science is an integrative and inclusive endeavor that utilizes concepts and methods from a diverse array of disciplines to strive for a more complete understanding of the role that proteins play in biological structure and function across many levels. As a membership-based society and leader in its field, The Protein Society values and is committed to diversity, equity, and inclusion in all aspects of its societal endeavors. We, therefore, strive to provide a safe and supportive environment for all of our constituents, where everyone is treated with respect and is encouraged to contribute their unique strengths and abilities to our shared mission. We are committed to acting on these principles for the betterment of the field of protein science and all activities with which The Protein Society is engaged.

them

The Protein Society Diversity Committee
Chair

Bil Clemons
California Institute of Technology

Charles L. Brooks III
University of Michigan

THEY

Jeanne Hardy
University of Massachusetts Amherst

She

Sheila Jaswal
Amherst College

Heather Pinkett
Northwestern University

We Care About Your Pronouns!

Stop by The Protein Society's booth outside of the Exhibit Hall and grab a pronoun pin for your name badge!

These let us all know how to address one another, and help everyone feel more comfortable.

When you meet someone, look for their pronoun pin!

He

Posters

Poster Set Up & Removal

All posters will be displayed in the Pavilion of the Westin Boston Seaport, and will be available for viewing during lunch hours and for presentations on the following days:

Thursday, July 13:	**5 - 7 p.m**.
Friday, July 14:	**5 - 7 p.m.**
Saturday, July 15:	**5 - 7 p.m.**

Instructions for Preparing Posters

Posters are displayed on a standard poster board with the dimensions of 90 inches (2.3 meters) wide by 42 inches (1.1 meters) high of usable space.

This year, due to the number of posters received, each poster will be up for 1 day only. It is important that you install it the morning of your presentation and remove it after the end of the entire session. If it is not removed by the end of the day, we will discard in order to install other posters.

Exhibitor Hours

July 13:
Coffee Breaks and Exhibits: 10:35 - 11:05 a.m.
Lunch: 12:15 - 1:45 p.m.
Coffee Breaks and Exhibits: 3 - 3:30 p.m.
Mix & Mingle Poster Reception:* 5 - 7 p.m.

July 14:
Coffee Breaks: 10:25 - 10:55 a.m.
Lunch: 12:05 - 1:45 p.m.
Coffee Breaks and Exhibits: 3 - 3:30 p.m.
Mix & Mingle Poster Reception:* 4:45 - 6:45 p.m.

July 15:
Coffee Breaks 10:20 - 10:50 a.m.
Lunch: 12 - 1:45 p.m.
Coffee Breaks and Exhibits: 3:15 - 3:40 p.m.
Mix & Mingle Poster Reception:* 5 - 7 p.m.

Mix & Mingle Poster Reception:* 5 - 7 p.m.

*Reception will feature snacks/bar

General Info

Public Transportation

Take The "T"

Boston's public transportation system is operated by the Massachusetts Bay Transportation Authority (MBTA), but locals know it simply as the "T." It offers subway, bus, trolley car and boat service to just about everywhere in the Greater Boston area and beyond. Subway stops are color coded - Red Line, Green Line, Blue Line, Orange Line or Silver Line.

SERVICE UPDATES - SPRING 2023

Several updates for all transportation options and stations can be located on the MBTA's service alert page.

Take the T from the Airport - Boston Logan Airport Wayfinder

Boston Wayfinder
To ride the T, you'll need a CharlieCard or CharlieTicket, which can be purchased at most subway station vending machines, and at select convenience stores. The basic fare for a one-way ride is $2.40 and kids 11 years and younger ride free. Plus, your Charlie Card will provide you with more than 75 discounts on attractions during your visit!

The "T's" LinkPass gives visitors unlimited travel on Subway, Local Bus, Inner Harbor Ferry and Commuter Rail Zone 1A for 1 day ($11.00) or 7 days ($22.50). More than 500 fare-vending machines are located throughout all subway stations.

Local buses feature expanded late-night service; fares are $1.70.

Taxi Information
Taxi service is available throughout the city. From Logan International Airport to most hotels in Boston and Cambridge, current fares are approximately $25-$35.00, one way. You may also book online or call 617-410-7500.

Bus
Service is widely available throughout Massachusetts. All intercity/interstate buses depart from South Station. Ticket counters are located on the third level of the Transportation Center. For information, call the South Station Bus Terminal at 617-737-8040.

SUMNER TUNNEL CONSTRUCTION - SPRING 2023
The Sumner Tunnel will close every weekend, from 11 P.M. on Friday to 5 A.M. on Monday, excluding holidays. More information.

General Info

Safety

As with any large city, there can be problems with petty theft on the public transit systems. Crowded buses, or mass transit stops, make for the perfect environment for those stealing purses, bags or wallets. You will doubtless notice a number of people experiencing homelessness in areas around the hotel. We recommend you exercise the same common-sense precautions as you would visiting any unfamiliar city. Keep the following common sense tips in mind when moving around the city:

- Keep an eye on your belongings when out and about.
- Carry wallets in your front pockets.
- Be award of your surroundings.
- Don't carry expensive or unnecessary items with you.
- Park in an attended garage.

Social Media

The Society staff will be updating its Facebook page, Instagram, and Twitter feed with Annual Meeting information throughout the meeting.

Follow @ProteinSociety on Twitter, Facebook, and Instagram, and @the-protein-society on LinkedIn for Symposium updates and coverage during the event. You can contribute to the conversation by using the hashtag #ps37.

General Info

Live Mobile App

The NEW PS37 Mobile App (search Protein Society Symposium) provides on-the-go Symposium information including a program planner, poster presentations info, exhibitor list, social media updates, #PS37 alerts, and maps. The Protein Society's "PS37" mobile application is available for download in the Apple App Store and Google Play. You can view/create schedules; view abstracts, and interact virtually with speakers using the app. Use the QR code at right to dowload, for both iPhone and Android.

Cameras/Video Recording

The unauthorized use of cameras/video recording inside session rooms or among the posters is prohibited.

Mobile Devices

As a courtesy to your fellow attendees, please silence all cell phones prior to entering a session room.

Certificates of Attendance

All attendees will receive a certificate of attendance via email in PDF format after the Symposium.

Internet Access

There is complimentary wi-fi internet access for the Symposium in the meeting space. **Please use the following information to gain access:**
Network Name: Westin_Conference Password: Vancouver24

Photography

Registration for the meeting implies consent to having photographs taken and to their use by officials of The Protein Society, or their representatives, for editorial and promotional purposes, on the Society website, social media outlets, and publications. Recordings of any kind (audio taping, videotaping, camera, tablets, or cell phones) in the session rooms, Exhibit Hall, and poster areas are strictly prohibited, unless accompanied by a member of the Society staff. Any individual seen taking photographs of any session or presentation will be escorted out by security.

General Info

COVID-19 Precautions

The Protein Society and PS37 Symposium adhere strictly to the most recent CDC guidelines regarding prevention of transmission of SARS-CoV-2. Should a change in the risk level increase, we will adhere strictly to health directives from local, state, and national authorities.

- Masks: We strongly encourage PS37 attendees to wear a mask, regardless of vaccination status, except when eating, drinking, or presenting. **Need a Mask?** TPS will provide complimentary 3M Aura N95 masks at registration.

- Handouts: In order to minimize touchpoints, materials will be available mostly electronically. Attendees should use the Mobile App to access program information.

- Food & Beverage: Where possible, we will favor food served pre-packaged, in individual portions, or served by hotel staff. We will also ensure to place several identical food distribution points so as to minimize proximity.

- Cleaning & Hygiene: High-touch areas will be cleaned and sanitized regularly. Hand sanitizer stations will be positioned at key locations throughout the event. Microphones will be sanitized after each use.

- Distancing: Our meeting rooms are designed for social distancing. We ask that you always:

- Leave an empty chair on either side of you.

- Leave an adequate distance when you are lining up, such as registration, food counters, etc.

TPS Membership

Member Rates

TPS Membership (Jan 1 - Dec 31)					Meeting Reg (Before May 30)		Meeting Reg (After May 30)	
Category		1-Year	2-Year	5-Year	Member	Non-Member	Member	Non-Member
Undergraduate	For undergraduate students pursuing a course of study in protein science.	$30	$50		FREE	$50	FREE	$60
Graduate	For graduate students pursuing a course of study in protein science.	$30	$50		$100	$200	$120	$240
Early Career	For those who have received their first professional degree in the last 6 years.	$75	$130	$300	$250	$400	$300	$480
Lab Staff	For academic lab staff in the field of protein science without a graduate degree.	$50	$85	$200	$100	$250	$120	$300
Emeritus	For those who are at least 62 years of age and are retired in the field of protein science	$30	$50	$120	$100	$200	$120	$240
Full	For those who have held a professional degree for 7 or more years and are active in their respective fields.	$150	$255	$600	$350	$600	$420	$720
Corporate	For anyone who works in industry associated with the field of protein science.	$150	$255	$600	$600	$850	$700	$1,000

Individual Memberships

TPS members represent an international community of all those who share an interest in the structure, function, design, synthesis, and utilization of proteins. In fact, it is this diversity of disciplines and perspectives represented by TPS members that is the group's defining characteristic.

Members include chemists, biologists, physicists, and mathematicians - researchers of all stripes, whose collaboration and communication comprise the Society's core mission. They represent academia, industry, government, non-profits, and leading institutions in more than 50 nations.

Benefits Include:

Annual Symposium and Awards

- Members save as much as 50% for the Annual Symposium.
- Get funding for your local protein-centered mini-symposium, workshop, or other event with a Member Mini-Grant.
- Connect with TPS leaders and have a say in the direction of your Society.
- Nominate your colleagues for one of eight prestigious TPS awards.
- Eligibility to submit a contributed talk or be considered for a Young Investigator Talk.
- Design your own session at an upcoming Symposium.

Protein Science Benefits

- Complimentary online access to the premier Journal focused on all aspects of protein science.
- $250 discount on publication fees.
- Pain-Free Publishing: Fast turnaround under the guidance of Editor-in-Chief John Kuriyan and Senior Editors Aitziber Lopez Cortajerena and Nir Ben-Tal.
- Reduced open-access fees from publisher Wiley Blackwell.

Networking and Leadership

- Connect and collaborate privately with other members through the Member Directory or the members-only LinkedIn group
- Be eligible to vote – or stand yourself – for TPS Executive Council, Nominating Committee, and other leadership roles
- Stay informed with the monthly member e-news

2023 Award Winners

Renã Robinson, Ph.D., Vanderbilt University
2023 Marie Maynard Daly Award Winner

Plenary: July 14, 8:30 a.m.

The Protein Society announced its newest annual award in 2023: The Marie Maynard Daly Award. This award recognizes Dr. Daly, who conducted pioneering studies of protein synthesis, histone biochemistry, and the relationships between hypertension, high cholesterol, and atherosclerosis. Dr. Daly was a trailblazer as the first black woman in the United States to receive a Ph.D. in chemistry (Columbia University); she also actively promoted diversification in STEM. The Marie Maynard Daly Award will recognize groundbreaking research at the interface between protein science and human health.

The 2023 inaugural awardee, Renã Robinson, Vanderbilt University, has pioneered the application and development of mass spectrometry-based proteomics to study aging and neurodegeneration, with a particular focus on health disparities in minority populations.

Specific Requirement: Groundbreaking research at the interface between protein science and human health

2024 Awards

TPS awards recognize excellence across diverse disciplines that collectively advance our understanding of proteins; their structure, function, design, and application. They honor researchers who have distinguished themselves with significant achievements in protein research and those who have made outstanding contributions in leadership, teaching, and service. TPS members AND non-members may submit nominations, which are awarded by Executive Council, and recipients will be honored at next year's Annual Symposium (**July 23 - 26, in Vancouver, Canada**).

Membership is not required to **submit a nomination**, and nominees do not have to be a member of the Society. Members can also submit more than one nomination. Deadline for nominations is **October 15**.

Arthur Lesk, Ph.D., Penn State University
2023 Carl Brändén Award Winner - Sponsored by Rigaku Corp.

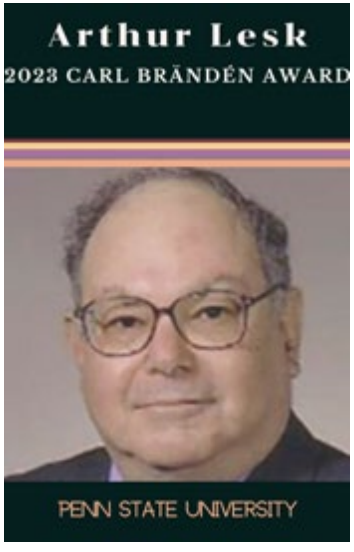
Plenary: July 15, 2:15 p.m.

In the tradition of Carl Brändén, pioneer in structural biology, co-author of the seminal text Introduction to Protein Structure, and leader of the world-class synchrotron facility at Grenoble, the Carl Brändén Award, sponsored by Rigaku Corporation, honors an outstanding protein scientist who has also made exceptional contributions in the areas of education and/or service.

The 2023 awardee, computational biologist Arthur Lesk of Penn State University, influenced generations of scientists with his analyses of protein structure and evolution, and his textbooks on protein science, genomics, and bioinformatics.

from qualitative and descriptive to quantitative and predictive. Through his desire to answer deep fundamental questions, Dr. Herschlag has been on the frontier of developing and applying cutting edge techniques that illuminate new aspects of protein behavior and their biological consequences. His dedication as mentor is apparent his trainees' success, his accomplishments as Dean of graduate students, and his support of young and diverse scientists.

Specific Requirement: Sustained, high-impact research contributions to the field and additional contributions to education/service



Previous Carl Brändén Award Winners

2022 - David Goodsell
2021 - Sheila Jaswal
2020 - Karen Fleming
2019 - Minoru Kanehisa
2018 - Jane & Dave Richardson
2017 - Billy Hudson
2016 - Gary Pielak
2015 - C. Robert Matthews
2014 - Stephen White
2013 - Sheena Radford
2012 - Helen Berman
2011 - Michael Summers
2010 - Nobuhiro Go
2009 - Bruce Alberts
2008 - Howard Schachman
2007 - Lubert Stryer

2023 Award Winners

Mei Hong, Ph.D.; Massachusetts Institute of Technology
2023 Christian B. Anfinsen Award Winner

Plenary: July 15, 2:45 p.m.

Established in 1996 and named for Nobel laureate Christian Boehmer Anfinsen, whose research on the structure and function of enzyme proteins contributed to the general acceptance of the "thermodynamic hypothesis," The Christian B. Anfinsen Award recognizes significant technological achievements and/or methodological advances in protein research.

2023 awardee Mei Hong of MIT created innovative tools to interrogate protein structure and dynamics using solid-state NMR spectroscopy.

Specific Requirement: Technological achievement or significant methodological advances

Previous Christian B. Anfinsen Award Winners

2022 - Jin Zhang
2021 - Petra Fromme
2020 - Stephen Sligar
2019 - Anthony Kossiakoff
2018 - Yifan Cheng
2017 - Lewis Kay
2016 - Andreas Pluckthun
2015 - Sachdev Sidhu
2014 - Robert Tycko
2013 - Tom Alber
2012 - Barry Honig
2011 - Wayne Bolen
2010 - Yoshinori Fujiyoshi
2009 - Wayne Hubbell
2008 - Carol Robinson
2007 - Carl Frieden
2006 - John R. Yates, III
2005 - Matthias Mann
2004 - Meir Wilchek
2003 - Ada Yonath
2002 - Roger Tsien
2001 - Martin Karplus
2000 - Stephen Benkovic
1999 - Alan Fersht
1998 - James Wells
1997 - Wayne Hendrickson
1996 - Donald Hunt

Patricia Clark, Ph. D., University of Notre Dame
2023 Dorothy Crowfoot Hodgkin Award Winner

Plenary: July 15, 3:40 p.m.

Dorothy Crowfoot Hodgkin was a founder of protein crystallography as well as a Nobel laureate. The Dorothy Crowfoot Hodgkin Award, supported by a grant from Genentech, a member of the Roche Group, is granted in recognition of exceptional contributions in protein science which profoundly influence our understanding of biology.

2023 awardee Patricia Clark of the University of Notre Dame has shed light on mechanisms of co-translational folding, codon usage, and other factors that influence protein structure in vivo.

Specific Requirement: Profound influence on our understanding of biology

Previous Dorothy Crowfoot Hodgkin Award Winners

2022 - Sun Hur
2021 - Janet Smith
2020 - Catherine Drennan
2019 - Hao Wu
2018 - Susan Marqusee
2017 - Juli Feigon & Manajit Hayer-Hartl
2016 - Rachel Klevit
2015 - Eva Nogales;
2014 - Judith Frydman
2013 - Christopher Hill & Cynthia Wolberger
2012 - Mark Lemmon
2011 - Brenda Schulman & Wei Yang
2010 - Lila Gierasch
2009 - Janet Thornton
2008 - Douglas Rees
2007 - Leemor Joshua-Tor

2023 Award Winners

Jason Gestwicki, Ph.D., University of California San Francisco
2023 Emil Thomas Kaiser Award Winner:

Plenary: July 15, 4:10 p.m.

In 2002, The Protein Society established The Emil Thomas Kaiser Award. Dr. Kaiser's highly original research, including the profoundly significant discovery of the necessity amphiphilic helices to biological life, can be said to have introduced a new field of chemistry. In this tradition, The Emil Thomas Kaiser Award recognizes a recent, highly significant contribution in applying chemistry to the study of proteins.

The Emil Thomas Kaiser Award, sponsored by generous individual contributions, recognizes a recent and highly-significant application of chemistry to the study of proteins. The 2023 recipient is Professor Jason Gestwicki (University of California San Francisco). Dr. Gestwicki has developed innovative tools and approaches to target diseases of protein misfolding.

Specific Requirement: Application of chemistry to the study of proteins

Previous Emil Thomas Kaiser Award Winners

2022 - Philipp Kukura
2021 - Lei Wang
2020 - Shuguang Zhang
2019 - Shahriar Mobashery
2018 - Michael Rosen
2017- Thomas Muir
2016 - Charles Craik
2015 - Anna Mapp
2014 - Carol Fierke
2013 - Wilfred van der Donk
2012 - No Award Given This Year
2011 - Jeffery Kelly
2010 - Suzanne Walker
2009 - Donald Hilvert
2008 - JoAnne Stubbe
2007 - Michael Marletta;
2006 - Barbara Imperiali
Previous recipients, sponsored by SynPep Corporation, include:

2005 - Ronald Raines
2004 - Homme Hellinga
2003 - Michael Hecht
2002 - Steve Kent

Kevin Gardner, Ph. D., CUNY Advanced Science Research Center
2023 Stein & Moore Award Winner

Plenary: July 16, 11:20 a.m.

The Stein and Moore Award, named for Nobel laureates Dr. William Stein and Dr. Stanford Moore, venerates their contribution to understanding the connection between chemical structure and catalytic activity of the active center of the ribonuclease molecule. Established in 1986, sponsored by The Protein Society with support from Wiley, the Stein and Moore Award is given to recognize eminent leaders in protein science who have made sustained high impact research contributions to the field.

The 2023 recipient is Professor Kevin Gardner, CUNY Advanced Science Research Center. Dr. Gardner has studied the structure, dynamics, and function of ligand-regulated protein/protein interaction domains from bacteria, plants, and humans to develop innovative optogenetic tools and cancer therapeutics.

Specific Requirement: Sustained, high-impact research contributions to the field

Previous Stein & Moore Award Winners

2022 - Daniel Herschlag
2021 - David Agard
2020 - Jim Bowie
2019 - Dame Carol Robinson
2018 - Raymond Stevens
2017 - John Kuriyan
2016 - Jane Clarke
2015 - William DeGrado
2014 - Nikolaus Pfanner
2013 - Robert T. Sauer
2012 - No Award Given This Year
2011 - Gerhard Wagner
2010 - Peter Wright
2009 - Peter Walter
2008 - Susan Lindquist
2007 - Paul Schimmel
2006 - Arthur Horwich & F. Ulrich Hartl
2005 - Avram Hershko & Alexander Varshavsky
2004 - Wolfgang Baumeister
2003 - Chris Dobson
2002 - Paul Sigler
2001 - Alan Fersht
2000 - Brian Matthews
1999 - Mo Cleland
1998 - David Davies
1997 - Mildred Cohn
1996 - David Eisenberg
1995 - Harold Scheraga
1994 - Michael Rossman
1993 - Walter Kauzmann
1992 - Robert Baldwin
1991 - Russell Doolittle

2023 Award Winners

Elena Conti, Ph.D., Max Planck Institute
2023 Hans Neurath Award Winner - Sponsored by Wiley

Plenary: July 16, 11:55 a.m.

Hans Neurath played an integral role in the early life of the Society, as a founding member and later -at age 81- as founding editor of Protein Science. His contributions to the early success of the Society were surpassed only by his larger contributions to the field of biochemistry and our early understanding of proteins.

Reflective of his prolific contributions to the understanding of the physical chemistry of proteins, The Hans Neurath Award, sponsored by the Hans Neurath Foundation, seeks to honor individuals who have made a recent contribution of exceptional merit to basic protein research.

2023 awardee Elena Conti from the Max Planck Institute for Biochemistry is recognized for her discovery of mechanisms of RNA export, processing, and quality control in the cell.

Specific Requirement: A recent contribution of unusual merit to basic protein science

Previous Hans Neurath Award Winners

2022 - Squire Booker
2021 - Toshiya Endo & Amy Rosenzweig
2020 - Martin Gruebele
2019 - Dave Thirumalai
2018 - David Baker
2017 - Kazuhiro Nagata
2016 - H. Eric Xu
2015 - Marina Rodnina
2014 - James Hurley
2013 - Jennifer Doudna & Chuck Sanders
2012 - Charles Brooks
2011 - Johannes Buchner
2010 - Wendell Lim
2009 - William Eaton
2008 - Robert Stroud
2007 - Robert Sauer
2006 - Christopher Dobson
2005 - Roderick MacKinnon
2004 - Carlos Bustamante
2003 - James Wells
2002 - Ad Bax
2001 - Arthur Horwich
2000 - Janet Thornton
1999 - Peter Kim
1998 - Ken Dill

Polly Fordyce, Ph.D., Stanford University
2023 Protein Science Young Investigator Award Winner - Sponsored by Wiley

Will Speak at PS38 in Vancouver, 2024

The Protein Science Young Investigator Award, sponsored by Wiley, formerly known as The Irving Sigal Young Investigator Award, recognizes a scientist in the first 8 years of an independent career who has made an important contribution to the study of proteins.*
*With allowances for familial leave or other exigent circumstance.

The 2023 recipient is Professor Polly Fordyce, Stanford University. Dr. Fordyce has developed innovative microfluidic systems to study how protein sequence encodes function at a large scale.

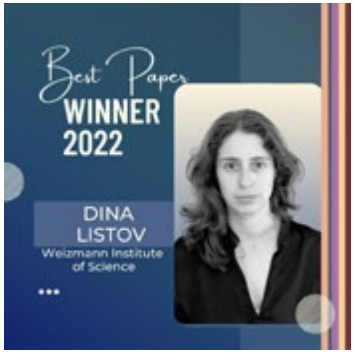
Specific Requirement: Within 8 years of starting an independent career

Previous Protein Science Young Investigator Award Winners

2021 - Bruno Correia
2020 - Mohammad Seyedsayamdost
2019 - Gabriel Lander
2018 - Brandon Ruotolo
2017 - David Pagliarini
2016 - Benjamin Garcia
2015 - Nieng Yan
2014 - M. Madan Babu
2012 - Mei Hong & Tarun M. Kapoor;
2011 - Shu-ou Shan
2013 - Feng Shao
2010 - Charalampos Kalodimos
2009 - Virginia Cornish
2008 - Jamie H. Doudna Cate
2007 - Benjamin Cravatt, III
2006 - Vijay Pande
2005 - Thomas Muir
2004 - Erin O'Shea & Jonathan Weissman
2003 - Yigong Shi
2002 - Carolyn Bertozzi
2001 - Kevan Shokat
2000 - David Baker
1999 - Jeffery Kelly
1998 - Nikola Pavletich
1997 - John Kuriyan
1996 - Michael Summers
1995 - Stuart Schreiber
1994 - Peter Kim
1993 - Ad Bax & Marius Clore
1992 - Peter Schultz
1991 - Carl Pabo
1990 - Rachel Klevit
1989 - William DeGrado

Best Paper Award Winners

Dina Listov, Ph.D., Weizmann Institute of Science
2022 Best Paper Award Winner - Sponsored by Wiley



Dina Listov is a graduate student in the Fleishman Lab in the Department of Biomolecular Sciences at Weizmann Institute of Science.

Her paper in *Protein Science* concerns the design of artificial sequences of amino acids that produce novel proteins. The paper describes research advances that facilitate the evaluation of whether or not a novel protein sequence will fold into a stable three-dimensional structure.

Her mentor, Sarel Fleishman, has this to say about Dina Listov: "I'm delighted that Dina is being awarded the Protein Society's Best Paper Prize for 2022. Her award-winning paper is the result of her unwavering dedication to designing new enzymatic activities within natural protein scaffolds. Dina's work demonstrates beautifully that a profound understanding of biophysical principles can effectively overcome significant obstacles in protein design and engineering."

Gwendell Thomas, Ph.D., Georgia Institute of Technology
2022 Best Paper Award Winner - Sponsored by Wiley

Gwendell Thomas is a graduate student and Graduate Research Assistant in the Lieberman Lab in the School of Chemistry & Biochemistry at Georgia Institute of Technology.

Her paper in *Protein Science* concerns the development of plastics that can be degraded by enzymes, an attribute that will help ameliorate the environmental impact of plastics in trash. The paper presents a biochemical and structural analysis of a novel enzyme with promising properties regarding the degradation of a biodegradable plastic substitute.

Her mentor, Raquel Lieberman, has this to say: "We are so grateful to TPS for recognizing our collaborative study with Kimberly Clark Corporation on enzymatic plastic degradation. Hopefully we will see a transformation in our everyday plastic use in our lifetime."

Travel Awards

Congratulations to the following outstanding students and early-career investigators for receiving travel assistance to attend The 37th Annual Symposium of The Protein Society.

Under the strong belief that our Symposia presents an invaluable opportunity for future protein scientists, The Protein Society is committed to making it possible for young scientists to participate and benefit from our Annual Meeting by awarding the DEI, Finn Wold Travel Awards and *Protein Science* Young Investigator Awards. The leadership and Executive Council of The Protein Society also thank the recent donors to the Finn Wold Travel Awards Fund.

2023 DEI Awards

Mia Louis Abramsson, Karolinska Institute

Roksana Azad, CUNY Advanced Science Research Center

Arumay Biswas, Penn State University

Amir Bitran, University of California Berkeley

Cassandra Chartier, Columbia University

Lennie Ka Yan Cheung, The University of British Columbia

Jamaine Davis, Meharry Medical College

Shankar Devkota, Monash University Clayton Campus

Nidhi Dhama, South Asian University

Riley Eisert-Sasse, Pennsylvania State University

Jonathan Giudice, University of California, Berkeley

Madiha Habib, The Chinese University of Hong Kong

Timothy Hasse, Wayne State University

Sparsh Makhaik, University of Massachusetts Amherst

Angel Vazquez Maldonado, University of California, Berkeley

Jose Martinez, The University of Texas at Austin

Prithviraj Nandigrami, Albert Einstein College of Medicine

Anika O'Brian, Colorado State University

Sargent Rachel, Wichita State University

Mohammad Imtiazur Rahman, Arizona State University

Carmen Giovana Granados Ramírez, Universidad Distrital Francisco José de Caldas

Karen Ramirez, University of Georgia

2023 DEI Awards (cont.)

Ignacio Retamal, Pontificia Universidad Catolica de Chile

Kelly Risch, Texas A&M University

Kristen Rivera, Northwestern University

Leah Spangler, Virginia Commonwealth University

Dominique Stephens, Fisk and Vanderbilt University

Nishanti Sudhakar, Pennsylvania State University

Xiaojing Sui, Northwestern University

Uthayasuriya Sundaramoorthy, The Australian National University

Saacnicteh Toledo, Okinawa Institute of Science & Technology Graduate University

Phoebe Tou, Bio21 Molecular Science & Biotechnology Institute

Luis Pablo Velazquez, Universidad de Sonora

Priscilla Villalona, Pennsylvania State University

Anne van Vlimmeren, Columbia University

Jimin Yoon, Massachusetts Institute of Technology

Kenneth Young II, Virginia Tech

Travel Awards

2023 Finn Wold & Protein Science Young Investigator Travel Award Recipients

Jessica Allen, University of Massachusetts Amherst
Dimitra Apostolidou, Duke University
Joshuah Arellano, Chapman University
Matt Begley, University of North Carolina at Chapel Hill
Soumendu Boral, Indian Institute of Technology Kharagpur
Amanda Brown, Clark University
Stephen Buckley, Swiss Federal Institute of Technology Lausanne
Evan Buechel, Northwestern University
Cady Burnside, University of Delaware
Nathaniel Carl, Chapman University
Abdul Castillo, Virginia Commonwealth University
Lauren Chisholm, King's College London
Leang-Chung Chris Choh, Purdue University
Andra-Elena Coșoreanu, Institute of Biochemistry of the Romanian Academy
Kristalle Cruz, University of Massachusetts Amherst
Elizabeth D'Lauro, Drexel University
Jacob DeRoo, Colorado State University
Angela Develin, Virginia Commonwealth University
Farid Ghelichkhani, University of Delaware
Alex Grigas, Yale University
Aransa Griñen, Pontificia Universidad Católica de Chile,
Aakash Gupta, University of Massachusetts Dartmouth
Megan Hill, Clark University
Ziyuan Jiang, Columbia University
Andrew Johns, Columbia University
Alec Jones, Colorado State University
Megan Juba, The University of Alabama
Kanchana Karunarathne, University of South Florida

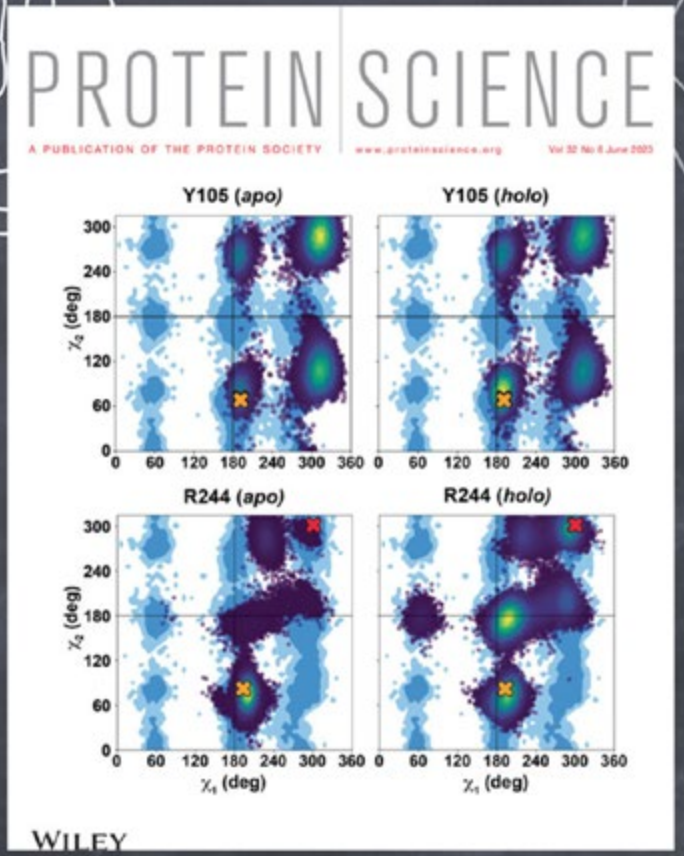
2023 Finn Wold & Protein Science Young Investigator Travel Award Recipients

Keisuke Kasahara, The University of Tokyo
Courtney Labrecque, Virginia Commonwealth University
Taylor Laflamme, Clark University
Ryan Lawrence, King's College London
Yuxuan LI, The Chinese University of Hong Kong
Elizabeth H. Liakos, Clark University
Ao Liu, Dartmouth College
Taylor Lundgren, University of Notre Dame
Anthony Marchand, Swiss Federal Institute of Technology Lausanne
Jane McCallum, Furman University
Duc Minh Nguyen, University of Toronto
Trilok Neupane, Dalhousie University
Yoshiki Ochiai, Okinawa Institute of Science and Technology Graduate University
Iman Omar, University College London
Anthony Rish, The Ohio State University
Ioannis Riziotis, European Bioinformatics Institute
Vida Robertson, Fisk University
Zhe Sang, Icahn School of Medicine at Mount Sinai
Roopashi Saxena, Purdue University
Arne Schneuing, Swiss Federal Institute of Technology Lausanne
Harsimranjit Sekhon, SUNY Upstate Medical University
Ankita Shrivastava, South Asian University
Andrew Shultz, University of Massachusetts Amherst
Adam Simard, Dartmouth College
Hannah Skaggs, University of Louisville
Abby Stapleton, Furman University
Grace Sturrock, Duke University
Taichi Sumikawa, The University of Tokyo

Travel Awards (cont.)

2023 Finn Wold & Protein Science Young Investigator Travel Award Recipients

Danielle Swingle, CUNY Advanced Science Research Center
Weimin Tan, Texas A&M University
Roxana Tarabuta, University of Montreal
Jenny Tran, Vanderbilt University
Cat Vesely, Oregon State University
AJ Vincelli, University of Massachusetts Dartmouth
Elena Voisin, Loyola University
Sara Walters, Virginia Commonwealth University
Virgil Woods, CUNY Advanced Science Research Center
Tianyi Yang, Clark University
Owen Young, University of North Carolina at Chapel Hill
Takanori Yokoo, The University of Tokyo
Jeffrey Youn, University of Toronto Mississauga
Jie Yu, Pennsylvania State University



Protein Science Workshop

JULY 14, 2023 | 12:15 - 1:30 P.M.
COMMONWEALTH BALLROOM A - B

PROTEIN SOCIETY

NIGHT AT THE MUSEUM

RSVP Only

July 15
8:30 - 11:30 p.m.

Boston Museum of Science
Museum Of Science Driveway
Boston, MA 02114

PS37

Included: 1 Drink Ticket, hors d'oeuvres, Tour, Free Parking, DJ

Program

July 12, 2023

4:30 - 7:30 p.m. | Registration
Grand Ballroom Foyer | Concourse Level

Pick up your badge on July 12 and be entered to win a gift basket or $100 Amazon gift card.

9 a.m. - 5 p.m. Industry Visits (RSVP only) (Transportation Not Provided)

C4 Therapeutics - July 12, 9 -11 a.m.
490 Arsenal Way, Suite 120 Watertown, MA 02472 (30 min. by car / 60 min. by local transit from Westin)

Scholar Rock - July 12, 12:30 - 2:30 p.m.
301 Binney Street, 3rd Floor, Cambridge, MA 02142 (30 min. car/ local transit from Westin)

Novartis - July 12, 2:45 - 5 p.m.
250 Massachusetts Ave, Cambridge, MA 02139 (30 min. by car/local transit from Westin)

7:30 - 8:15 p.m. | Welcome/Orientation For Students (RSVP only)

Commonwealth Ballroom A-B-C

Hosted by Dr. Bil Clemons (Caltech) and Dr. Margaret Stratton (UMass Amherst)

Description: Take advantage of the PS37 orientation, where the hosts will share insider information related to the program & opportunities offered at the meeting. Get ready to network and mingle with other attendees, including speakers and meeting organizers. Bring your appetite and questions! Recommended for undergraduate, graduate students and postdocs.

July 13, 2023

7:30 a.m. - 5 p.m. | Registration
Grand Ballroom Foyer | Concourse Level

8:30 - 8:35 a.m. | Opening Remarks:
Grand Ballroom A - B

Charles (Chuck) R. Sanders, Vanderbilt University | President, The Protein Society

8:35 - 9:05 a.m. | Introduction, Opening Plenary | Dr. John Kuriyan,
Vanderbilt University; Editor-in-Chief, *Protein Science*

Opening Plenary:
Dr. Susan Marqusee, MD, Ph.D., | University of California, Berkeley
Interrogating a Protein's Energy Landscape: From Protein Folding to Coronavirus

9:20 a.m. - 12:15 p.m.
Parallel Session 1: Modern Anti-viral Strategies
Grand Ballroom A - B

Session Chair: **Jeanne Hardy**; University of Massachusetts, Amherst

9:20 - 9:50 a.m.
Peijun Zhang, Diamond Light Source |
CryoEM Investigation of Virus Infection and Vaccines in Cells

9:50 - 10:20 a.m.
Yufei Xiang, Icahn School of Medicine at Mount Sinai |
Nanobody Technologies Against the Evolving COVID-19 Pandemic

10:20 - 10:35 a.m.
Alex Guseman, University of Pittsburgh |
Targeting Spike Glycans to Inhibit Viral Entry

10:35 - 11:05 a.m. Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

11:05 - 11:35 a.m.
Christopher Barnes, Stanford University |
Structural Insights Into the Development of Pan-Coronavirus Immunotherapies

11:35 a.m. - 12:05 p.m.
Celia Schiffer, University of Massachusetts Chan Medical School |
Pre-Emptively Avoiding Drug Resistance: Lessons From Viral Proteases

12:05 - 12:15 p.m.
Lorenzo Casalino, University of California San Diego |
Whole Virion Simulations Illuminate the Vulnerabilities of Influenza Glycoproteins

Program

Vincent Azzolino, University of Massachusetts Chan Medical School |
Experimental and Computational Characterization of Enterovirus 68 3C Protease for the Development of Robust, Direct-Acting Antiviral Inhibitors

Alec Jones, Colorado State University |
Wastewater Viral Surveillance Using Modified Porous Protein Microcrystals

9:20 a.m. - 12:15 p.m.
Parallel Session 2: Protein Folding and Function in Context
Grand Ballroom C - D - E

Session Chairs: **Mary Munson**, University of Massachusetts Medical School; and **Breann Brown**, Vanderbilt University

9:20 - 9:50 a.m. | Caitlin Davis, Yale University |
Structure and Activity of Metabolic Proteins in Living Cells

9:50 - 10:20 a.m. | Marcelo Sousa, University of Colorado Boulder |
Protein Mechanical Stability as a Determinant for Type Three Secretion

10:20 - 10:35 a.m. | Jonathan Giudice, University of California, Berkeley |
Understanding Efficient Endosomal Escape: A Structural and Biochemical Approach

10:35 - 11:05 a.m. Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

11:05 - 11:35 a.m. | Bil Clemons, California Institute of Technology |
A Universal Mechanism for the Catalytic Cycle of the Tail-anchor Chaperone Get3

11:35 a.m. - 12:05 p.m. | Christian Kaiser, Johns Hopkins University |
Co-Translational Stabilization Drives Folding Of A Kinetically Stable Protein

12:05 - 12:15 p.m.| Zachary Wood, University of Georgia |
Using Hydrodynamics, Crowders and Kosmotropes to Turn a kcan't Into a kcat

Jorge González-Higueras, Pontificia Universidad Católica de Chile |
Elucidating the all-α to all-β Refolding of RfaH Bound to Transcription Elongation Complexes Using Dual-basin Structure-Based Models

Ume Tahir, Yale University |
FRET as a High-Throughput Reporter for Fold Switching in the NusG Family

12:15 - 1:45 p.m. | Lunch

12:15 - 1:30 p.m.
NETWORKING TABLES with DEI TOPICS (RSVP Required)
Commonwealth Ballroom A - B - C

Chair: **Dr. Margaret Stratton** (UMass Amherst)
The networking event is a unique experience that brings student and early career attendees up close and personal with protein science veterans and peers to discuss various career and diversity, equity and inclusion topics.

12:45 - 1:30 p.m. | INDUSTRY VISIT: PSIVANT (RSVP ONLY)
Address: 451 D Street, Boston MA (5 min. walk from Westin)

1:45 - 4:40 p.m.
Parallel Session 3: Structures of Mega-Complexes
Grand Ballroom A - B

Session Chair: **Margaret Cheung**, Pacific Northwest National Laboratory

1:45 - 2:15 p.m.
Alan Brown, Harvard University |
Axonemal Structures Reveal Mechanoregulatory and Disease Mechanisms

2:15 - 2:45 p.m.
Joey Davis, MIT |
Visualizing Massive Macromolecular Complexes in Motion with Cryo-EM, Cryo-ET, and Deep Learning

2:45 - 3 p.m.
Friederike Maria Carola Benning, Massachusetts General Hospital |
Helical Reconstruction of VP39 Reveals Principles for Baculovirus Nucleocapsid Assembly

3 - 3:30 p.m. Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

3:30 - 4 p.m.
André Hoelz, Caltech |
Structure And Function Of The Nuclear Pore Complex

4 - 4:30 p.m.
Eva Nogales, UC Berkeley |
Structural Insights into the Regulation of the Gene Silencer PRC2

Program

4:30 - 4:40 p.m.
Alireza Ghanbarpour, MIT |
Visualizing the AAA+ protease ClpXP With a Fully Engaged Substrate

Kripa Nand, Rensselaer Polytechnic Institute |
Bacterial Production of Recombinant Contraceptive Vaccine Antigen from CatSper Displayed on a Human Papilloma Virus-like Particle

Matt Begley, University of North Carolina at Chapel Hill |
Molecular Determinants of AP-3 Membrane Engagement and Cargo Selection

1:45 - 4:40 p.m.
Parallel Session 4: Protein Evolution: Lessons from the Past
Grand Ballroom C - D - E

Session Chair: **Denise Okafor**, Penn State University; and
Connie Jeffery, University of Illinois at Chicago

1:45 - 2:15 p.m.
Betul Kacar, University of Wisconsin-Madison |
Enigmatic Evolution of Nitrogenases: Insights From Earth's Past

2:15 - 2:45 p.m.
Margaret Glasner, Texas A&M University |
Leveraging Catalytically Promiscuous Enzymes to Understand the Basis for Enzyme Specificity and Epistasis Among Homologs

2:45 - 3 p.m.
Sabab Khan, Pennsylvania State University |
Ligand-induced Shifts in Conformational Ensembles that Describe Transcriptional Activation

3 - 3:30 p.m. Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

3:30 - 4 p.m.
Shelley Copley, University of Colorado Boulder |
Evolution of Novel Metabolic Pathways: Insights from Laboratory Evolution

4 - 4:30 p.m.
José Manuel Sánchez Ruiz, Universidad de Granada |
Ancestral Proteins as Scaffolds for Enzyme Engineering and Evolution

4:30 - 4:40 p.m.
Dia Ghose, MIT |
Marginal Specificity in Protein Interactions Constrains Evolution

Kendra Marcus, Vanderbilt University |
Rescuing from the DEAD: Understanding Substrate-Mediated ATP Hydrolysis through Deep Mutagenesis of a DNA Polymerase Clamp Loader

Jimin Yoon, MIT |
Pro283 Substitution on Influenza Nucleoprotein Allows Immune Escape but Also Introduces Folding Defects

5 - 7 p.m. | Posters & Exhibits Networking
Grand Ballroom Foyer & Pavilion

July 14, 2023

8 - 4 p.m. | Registration
Grand Ballroom Foyer | Concourse Level

8 -10 a.m. | Industry Visit: Ginkgo (RSVP ONLY)
Address: 27 Drydock Ave 8th Floor, Boston, MA (15 min. walk from Westin)

8:30 - 9 a.m.
Introduction, Marie Maynard Daly Award |
Steven Damo, Fisk University
Grand Ballroom A - B

2023 Marie Maynard Daly Award Plenary:
Dr. Renã Robinson, Vanderbilt University |
Large-Scale Analysis Of Proteins To Solve Equity Issues In Alzheimer's Disease Research

9:10 a.m. - 12:05 p.m.
Parallel Session 1: RNA-Protein Machines: Ancient Synergies
Grand Ballroom A - B

Session Chair: **Ruben Gonzalez,** Columbia University

9:10 - 9:40 a.m.
Jingyi Fei, University of Chicago |
Context-dependent RNA Localization and Organization in Nuclear Speckles

Program

9:40 - 10:10 a.m.
Ganesh S. Anand, Pennsylvania State University |
Asymmetric Genomic RNA Egress and Virus Disassembly Driven by Capsid Genome Rearrangements by HDXMS and cryo-EM

10:10 - 10:25 a.m.
Sandra Byju, Northeastern University |
Dependence of P/E tRNA Hybrid Formation on Subunit Rotation in the Ribosome

10:25 - 10:55 a.m. | Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

10:55 - 11:25 a.m.
Aaron Hoskins, UW-Madison |
How snRNAs and Proteins Collaborate to Recognize Introns during pre-mRNA Splicing

11:25 - 11:55 a.m.
Rachel Green, Johns Hopkins University |
Colliding Ribosomes Function as a Signaling Hub to Dictate Cell Fate Decisions

11:55 a.m. - 12:05 p.m.
Yoshiki Ochiai, Okinawa Institute of Science and Technology |
SUPREM: Super RNA EcoGII Methyltransferase Engineered by Ancestral Sequence Reconstruction

Arjun Kanjarpane, University of Maryland |
Insights into HIV-1 Gag-RRE Interactions: Exploring Rev and Gag Binding & Competition on RRE Stem 1

Roopashi Saxena, Purdue University |
Elucidating the Mechanism of Ebolavirus Matrix Protein Dimer Stability and Oligomerization using Peptidomimetics

9:10 a.m. - 12:05 p.m.
Parallel Session 2: Peptide Modalities: Size Doesn't Matter
Grand Ballroom C - D - E

Session Chair: **Jeanine Amacher**, Western Washington University

9:10 - 9:40 a.m.
Christina I. Schroeder, Genentech |
Recifin A, a Novel and Selective Allosteric Inhibitor of Tyrosyl-DNA phosphodiesterase 1 (TDP1)

9:40 - 10:10 a.m.
Peter T. Beernink, University of California, San Francisco |
Gonococcal Peptide Mimetic Vaccine Antigen Forms a Beta-hairpin Structure and Binds to a Chimeric Antibody Primarily through Hydrophobic Interactions

10:20 - 10:25 a.m.
Conan Wang, University of Queensland |
More Contacts, Copies, and Stability - Optimizing Efficacy of a PCSK9 Peptide Inhibitor

10:25 - 10:55 a.m. | Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

10:55 - 11:25 a.m.
Joshua Schwochert, Unnatural Products |
Tales of Macrocycle Medchem: Balancing Cell Permeability and Target Binding

11:25 - 11:55 a.m.
Lauren Monovich, Novartis |

11:55 a.m. - 12:05 p.m.
Maria-Augustina Rossi, UMass Amherst |
Mapping the Conformational Change that Accompanies Functional Interaction of the E. coli Hsp70, DnaK, with its Nucleotide Exchange Factor, GrpE

Damla Surmeli, Tufts University |
Tuning GLP-1 and GIP "Dual Agonist" Activation Using N-Terminal Modifications

Jen Kosmatka, MIT |
Implementing a Novel Proteome-Wide Biochemical Screen to Define Sequence Motifs That Support Binding to the Core Autophagy Protein LC3B

12:05 - 1:45 p.m. | Lunch

Program

12:15 - 1:30 p.m.
Protein Science Workshop: Demystifying Peer Review with Protein Science
Commonwealth Ballroom A - B

Chairs: **Melissa Asaro** (Wiley) and **April Rodd** (Wiley)
Speakers: **John Kuriyan**, Vanderbilt University | **Nir Ben-Tal**, Tel Aviv University | **Aitziber L. Cortajarena**, CIC biomaGUNE

Description: Join the editors, reviewers, and authors of the Protein Society's journal, *Protein Science*, as we talk about peer review and how it has evolved to tackle modern challenges. In this workshop, we'll cover topics ranging from how to write an effective review to grappling with the threat of paper mills.

12:15 - 1:30 p.m.
Educators Panel
Commonwealth Ballroom C

Chair: **Dr. Donald Spratt**, Clark University

Professional Development for Protein Science Students – Discussions on the Importance of Soft Skills, Networking, and Resume Building.

12:30 - 1:30 p.m. | Industry Visit: Vertex, (RSVP ONLY)
316 - 318 Northern Ave. Boston, MA (12 min. walk from Westin)

1:45 - 4:40 p.m.
Parallel Session 3: Capturing Protein Interactions
Grand Ballroom A - B

Session Chair: **Raquel Lieberman**, Georgia Institute of Technology; and **Matthew Gage**, U Mass Lowell

1:45 - 2:15 p.m.
James Munro, UMass Chan Medical School |
Single-molecule Imaging of Viral Glycoprotein Conformational Dynamics and Membrane Interactions

2:15 - 2:45 p.m.
James Murphy, The Walter and Eliza Hall Institute of Medical Research (LORNE) |
Tales From The Crypt: How RIPK3 KINASE Unleashes the Zombie Protein, MLKL, to Kill Cells by Necroptosis

2:45 - 3 p.m.
Lauren Jackson, Vanderbilt University |
Tepsin Binds LC3B To Promote ATG9A Export And Delivery At The Cell Periphery

3 - 3:30 p.m. | Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

3:30 - 4 p.m.
Giulia Palermo, University of California Riverside |
Dynamics and Mechanisms of CRISPR-Cas9 Through the Lens of Computational Methods

4 - 4:30 p.m.
Neel Shah, Columbia University |
Rewired Interaction Specificity Biases Ligand-dependent Activation in a Mutant Tyrosine Phosphatase

4:30 - 4:40 p.m.
Dimitra Apostolidou, Duke University |
Thermal Stability of Monomeric, Dyad and Triad NanoLuc Constructs and Their Assisted Refolding Via Addition of E. coli Chaperones (DnaK, DnaJ, GrpE)

Jie Yu, Pennsylvania State University |
Investigation of the Molecular Determinants of Picornavirus 3C Protease and 3D RNA Polymerase Interactions with Phosphoinositide-enriched Replication Membranes

Roksana Azad, CUNY Advanced Science Research Center |
Structural Basis of Signal Transduction Within Environmental Sensing PAS Regulated Ser/Thr Kinases

1:45 - 4:40 p.m.
Parallel Session 4: Membrane Proteins: From Natural to Designed
Grand Ballroom C - D - E
Session Chair: **Shuguang Zhang**, MIT

1:45 - 2:15 p.m.
Tae-Young Yoon, Seoul National University |
Watching Single Helical Membrane Proteins Fold With Magnetic Tweezers

Program

2:15 - 2:45 p.m.
Rachelle Gaudet, Harvard University |
Visualizing the Gymnastics of a Transition Metal Importer and Other LeuT-fold Transporters

2:45 - 3 p.m.
Chrystal Starbird, UNC Chapel Hill |
Insights into Intercellular Bridging via TAM Receptor Structures

3 - 3:30 p.m. | Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

3:30 - 4 p.m.
Rui Qing, Shanghai Jiao Tong University |
Solubility-Based Protein Design for Biomedical Applications

4 - 4:30 p.m.
Shuguang Zhang, MIT|
The Simple QTY Code for Protein Design

4:30 - 4:40 p.m.
Mia Louis Abramsson, Karolinska Institute |
Exploration of Lipid-Membrane Protein Interactions a Study of an Evolutionary Deprived in Silico Designed Protein

Niek Dekker, AstraZeneca |
Protein Engineering of Extracellular Vesicle for Delivery of Complex Drugs

Ryan Lawrence, King's College London |
Cry(o)ing over Multidrug Resistance: Structural Analysis of the Anaerobic Efflux Pump MdtF

4:45 - 6:45 p.m. | Posters & Exhibits Networking Reception
Grand Ballroom Foyer & Pavilion

July 15, 2023

8 a.m. - 4 p.m. Registration
Grand Ballroom Foyer | Concourse Level

8:15 - 9 a.m. | TPS Members Meeting
Commonwealth Ballroom A - B - C

9:05 a.m. - 12 p.m.
Parallel Session 1: Structure Prediction and Design
Grand Ballroom A - B

Session Chair: **Cesar Ramirez Sarmiento**, Pontificia Universidad Catolica de Chile

9:05 - 9:35 a.m.
Eva-Maria Strauch, University of Georgia |
How Phase Separation is Encoded in Intrinsically Disordered Low-Complexity Domains

9:35 - 10:05 a.m.
Lauren Porter, National Institutes of Health |
Predictions of Protein Fold Switching From Sequence

10:05 - 10:20 a.m.
Dina Listov, Weizmann Institute of Science (Protein Science Best Paper) |
Assessing and Enhancing Foldability in Designed Proteins

10:20 - 10:50 a.m. | Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

10:50 - 11:20 a.m.
Kresten Lindorff-Larsen, University of Copenhagen |
Towards Structure Prediction and Design of Disordered Proteins

11:20 - 11:50 a.m.
Sergey Ovchinnikov, Harvard University |
Recent Advances in Protein Structure Prediction and Design

11:50 a.m. - 12 p.m.
Arne Schneuing, Swiss Federal Institute of Technology Lausanne |
Flexible Structure-based Design of Small Molecules with Equivariant Diffusion Models

Program

Amelia McCue, University of North Carolina at Chapel Hill |
Masking T-Cell Engaging Bispecific Antibodies for Safer Cancer Immunotherapy

Amir Khan, Trinity College Dublin |
Structure of a Beta-lactamase Variant From an Evolution-informed Design Strategy

9:05 a.m. - 12 p.m.
Parallel Session 2: Proteins in Motion
Grand Ballroom C - D - E

Session Chair: **Lynn Kamerlin**, Uppsala University

9:05 - 9:35 a.m.
Jane Dyson, Scripps Research Institute |
Role of Disorder and Dynamics in Protein Function

9:35 - 10:05 a.m.
Daniel Keedy, CUNY Advanced Science Research Center |
Perturbing Protein and Ligand Conformational Landscapes to Link Dynamics and Function in Protein Tyrosine Phosphatases

10:05 - 10:20 a.m.
Colin Kinz-Thompson, Rutgers University |
Elevator Mechanism Dynamics in a Sodium-coupled Dicarboxylate Transporter

10:20 - 10:30 a.m. | Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

10:50 - 11:20 a.m.
Rodrigo Maillard, Georgetown University |
Conformational Dynamics Play a Catalytic and an Allosteric Role During the Activation Cycle of Protein kinase A

11:20 - 11:50 a.m.
David Rueda, Imperial College London |
Searching and Finding Targets on Genomes

11:50 a.m. - 12 p.m.
Stephanie Wankowicz, University of California San Francisco |
Assessing How Side Chain Conformational Heterogeneity Changes Upon Ligand Binding

Jamie Towle-Weicksel, Rhode Island College |
Research at a PUI: Elucidating the Mechanism of DNA Pol Theta in Cancer

Eunjeong Lee, University of Colorado |
Modulating Enzyme Function via Dynamic Allostery and Structure Changes Within Biliverdin Reductase B Family

12 - 1:45 p.m. | Lunch

12:05 - 1:35 p.m.
Undergraduate Research Session
Commonwealth Ballroom A - B

Chair: **Dr. Matthew Gage**, U Mass Lowell

The undergraduate research session showcases outstanding scientific research by undergraduate students.

Speakers:

Megan Juba, The University of Alabama | **Colin Burdette**, Furman University | **Sydney Green**, Rhode Island College | **Isabella Valentino**, Villanova University |**David Liu**, Wichita State University |**Taylor Laflamme**, Clark University

1:45 - 5 p.m. | Plenary Award Talks
Grand Ballroom A - B

1:45 - 2:15 p.m.
Introduction, 2021 Stein & Moore Award |
Dr. Elizabeth Meiering, University of Waterloo
President-Elect, The Protein Society

2021 Stein & Moore Award Plenary:
Dr. David Agard, University of California San Francisco |
To Fold Or Not To Fold: Chaperone-Mediated Proteostasis Revealed In Atomic Detail

2:15 - 2:45 p.m.
Introduction, 2023 Carl Branden Award |
Dr. Raquel Lieberman, Georgia Tech

2023 Carl Branden Award Plenary:
Dr. Arthur Lesk, Penn State University |
Some People and Molecules I Have Known

Program

2:45 - 3:15 p.m.
Introduction, 2023 Christian B. Anfinsen Award:
Dr. Jeanne Hardy, UMass Amherst

2023 Christian B. Anfinsen Award Plenary:
Dr. Mei Hong, MIT |
Structure and Dynamics of Channels and Transporters in Infectious Diseases from Solid-State NMR Spectroscopy

3:15 - 3:40 p.m. | Coffee Break & Exhibits
Grand Ballroom Foyer & Pavilion

3:40 - 4:10 p.m.
Introduction, 2023 Dorothy Crowfoot Hodgkin Award |
Dr. Tobin Sosnick, The University of Chicago

2023 Dorothy Crowfoot Hodgkin Award Plenary:
Dr. Patricia Clark, University of Notre Dame |
Co-Translational Folding Suppresses Protein Aggregation In The Crowded Cellular Milieu

4:10 - 4:40 p.m.
Introduction, 2023 Emil Thomas Kaiser Award |
Dr. Cesar Ramirez Sarmiento, Pontificia Universidad Católica de Chile

2023 Emil Thomas Kaiser Award Plenary:
Dr. Jason Gestwicki, University of California San Francisco |
High Throughput Methods for Studying Protein Stability

4:40 - 5 p.m. | Poster Competition Winners |
Dr. Christopher Snow, Colorado State University

5 - 7 p.m. | Posters & Exhibits Networking Reception
Grand Ballroom Foyer & Pavilion

8:30 - 11:30 p.m. | PS37 Night At the Museum (RSVP ONLY)
Boston Museum of Science

July 16, 2023

8 - 10 a.m. | Registration
Grand Ballroom Foyer | Concourse Level

8:30 - 11:15 a.m.
Parallel Session 1: Engineering Protein Fate and Function
Grand Ballroom A - B

Session Chair: **Erin Dueber**, Genentech

8:30 - 9 a.m.
Ray Deshaies, Amgen | *Multispecificity – The Future of Molecular Medicines*

9 - 9:30 a.m.
Liskin Swint-Kruse, The University Of Kansas Medical Center |
Rheostats, Toggles, and Neutrals, Oh My!

9:30 - 9:45 a.m.
Gwendell Thomas, Georgia Tech, Protein Science Best Paper |
Biochemical Characterization of New Archaeal Intramembrane Aspartyl Proteases (IAPs)

9:45 - 10:05 a.m. | Coffee Break
Grand Ballroom Foyer

10:05 - 10:35 a.m.
Dan Nomura, University of California, Berkeley |
Reimagining Druggability Using Chemoproteomic Platforms

11:05 - 11:15 a.m.
Mark van Zee, University of California, Los Angeles |
PicoShells: A Tool for Screening Millions of Protein Variants in Application-Relevant Environments

Thomas Groseclose, Los Alamos National Laboratory |
Engineering of a Highly Efficient Polyethylene Terephthalate Hydrolase via High-throughput Enzyme Evolution and Screening

Harsimranjit Sekhon, SUNY Upstate Medical University |
A DNA-Triggered Protein Biosensor for Point-of-care Diagnostics

Program

8:30 - 11:15 a.m.
Parallel Session 2: Aggregates, Amyloids, or Condensates?
Grand Ballroom C - D - E

Session Chair: **Tobin Sosnick,** University of Chicago

8:30 - 9 a.m.
Ibrahim Cisse, MIT |
Super-resolution Imaging of Transcription in Living Cells

9 - 9:30 a.m.
Kendra Frederick, UT Southwestern Medical Center |
Structural Determination of Neurodegenerative Disease-associated Proteins Inside Cells

9:30 - 9:45 a.m.
Marisa Barilla, Yale University |
Protein Stability, Folding, and Confinement in CAHS Hydrogels

9:45 - 10:05 a.m. | Coffee Break
Grand Ballroom Foyer

10:05 - 10:35 a.m.
Rohit Pappu, Washington University in St. Louis |
Molecular Grammars that Contribute to Organizing Nucleolar Sub-phases

10:35 - 11:05 a.m.
Amy Gladfelter, University of North Carolina at Chapel Hill |
RNA Control of Biomolecular Condensates

11:05 - 11:15 a.m.
Sabareesan Ambadi Thody, University of Texas Southwestern Medical Center |
Small Molecule Properties Define Partitioning into Biomolecular Condensates

Jianzheng Wu, Stowers Institute for Medical Research |
Cis-Gatekeeper: Isomerization of A Single Proline Governs TDP-43 Aggregation

Nadia El Mammeri, MIT |
Understanding Tau's Interactome: Microtubules, Lipid Membranes, and Anionic Co-factors

11:20 - 11:25 a.m.
TPS Service Awards | Dr. Charles (Chuck) R. Sanders, Vanderbilt University
President, The Protein Society

11:25 - 11:55 a.m.
Introduction, 2023 Stein & Moore Award |
Dr. Charles (Chuck) R. Sanders, Vanderbilt University
President, The Protein Society

2023 Stein & Moore Award Plenary:
Kevin Gardner, CUNY Advanced Structural Research Center |
Inspiration From Nature's Switches to Develop Biotech Tools and Therapeutics

11:55 a.m. - 12:30 p.m
Introduction, Hans Neurath Award | George Steers, Hans Neurath Foundation

Introduction, 2023 Hans Neurath Award | Dr. Lynn Kamerlin, Uppsala University

2023 Hans Neurath Award Plenary:
Dr. Elena Conti, Max Planck Institute |
To Degrade Or Not To Degrade: Molecular Mechanisms Of Rna Homeostasis

12:30 p.m. | Closing - Dr. Charles (Chuck) R. Sanders, Vanderbilt University
President, The Protein Society

Exhibitor List

Agilent	**Booth 6**
ASAP Bio	**Booth 4**
Dynamic Biosensors	**Booth 8**
Gator Bio	**Booth 3**
RedShift Bio	**Booth 2**
Refeyn	**Booth 5**
St. Jude Children's Research Hospital	**Booth 7**
The Protein Society/Wiley	**Booth 1**

Exhibitor Hours

July 13:
Coffee Break: 10:35 - 11:05 a.m.
Lunch: 12:15 - 1:45 p.m.
Coffee Break: 3 - 3:30 p.m.
Mix & Mingle: 5 - 7 p.m.

July 14:
Coffee Break: 10:25 - 10:55 a.m.
Lunch: 12:05 - 1:45 p.m.
Coffee Break: 3 - 3:30 p.m.
Mix & Mingle: 4:45 - 6:45 p.m.

July 15:
Coffee Breaks 10:20 - 10:50 a.m.
Lunch: 12 - 1:45 p.m.
Coffee Break: 3:15 - 3:40 p.m.
Mix & Mingle: 5 - 7 p.m.

Exhibitor Diretory

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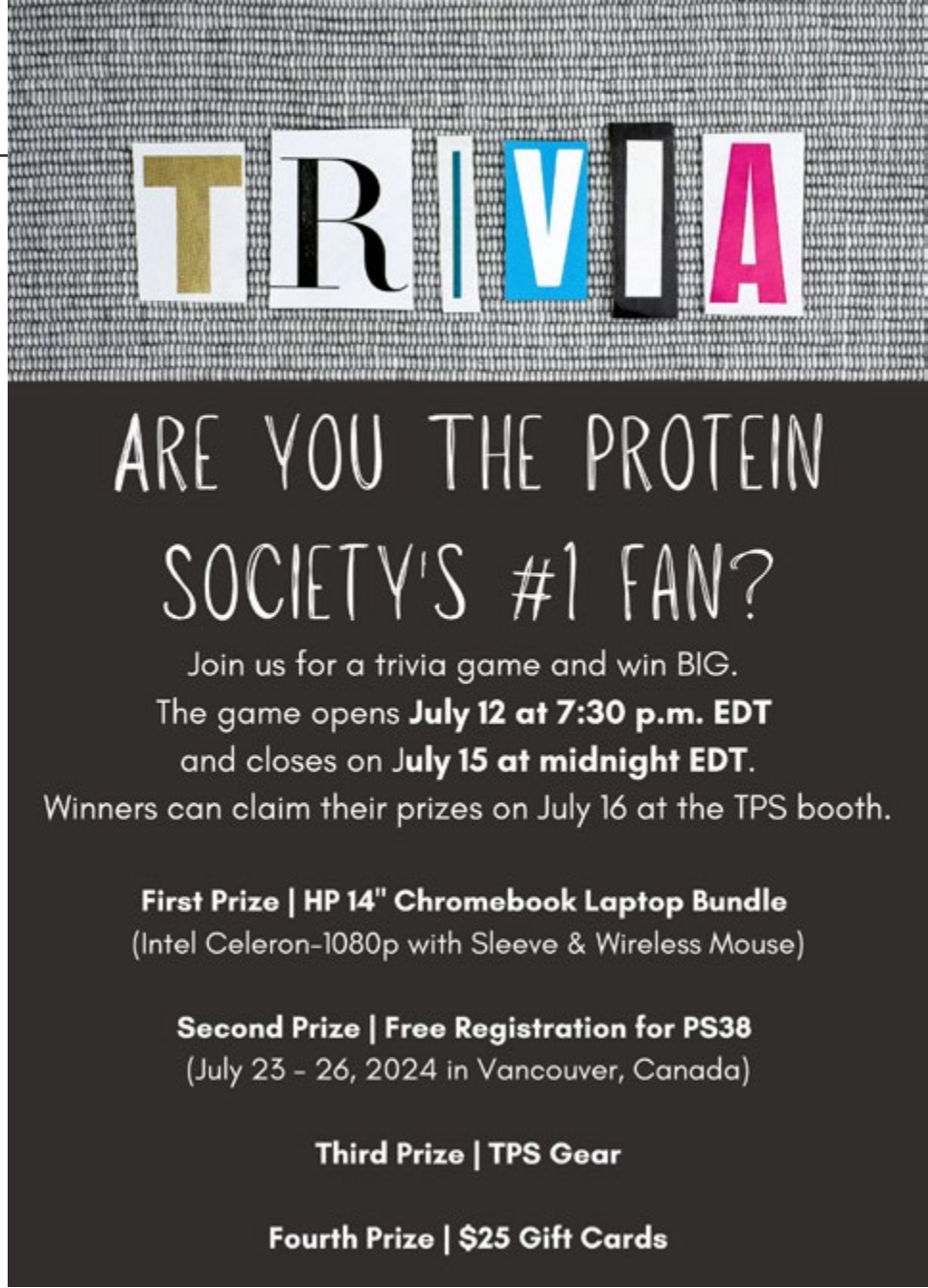

UNDERGRAD RESEARCH SESSION

Chaired by Matthew Gage,
U Mass Lowell

Groundbreaking Research with Undergrad Scholars

July 15, 12:05 - 1:35 p.m.
Commonwealth Ballroom A - B - C

PS38 ANNUAL SYMPOSIUM

Vancouver, Canada | July 23 - 26, 2024

THE PROTEIN SOCIETY

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ABSTRACT

Abstracts presented at the 37th Annual Meeting of The Protein Society

ABS#7

Poster session, July 15

Using Hydrogen-Deuterium Exchange (HDX) Mass Spectrometry (MS) to Investigate Cotranslational Folding

Thomas Wales (1); Aleksandra Pajak (2); Alzbeta Roeselova (2); Santosh Shiwakumaraswamy (2); Steven Howell (3); F. Ulrich Hartl (4); David Balchin (2); John Engen (1)
(1) Chemistry & Chemical Biology, Northeastern University, Boston, United States of America; (2) Protein Biogenesis Laboratory, Francis Crick Institute, London, United Kingdom; (3) Proteomics Science Technology Platform, Francis Crick Institute, London, United Kingdom; (4) , Max Planck Institute of Biochemistry, Martinsried, Germany

Cotranslational folding and chaperone activity are critical for efficient protein biogenesis, but how they direct the maturation pathway of nascent proteins remains poorly understood. Here we have used hydrogen-deuterium exchange (HDX) mass spectrometry (MS) to investigate, at peptide resolution, the cotranslational chaperone-assisted folding pathway of Escherichia coli dihydrofolate reductase.

On the ribosome, the nascent polypeptide folds along an unanticipated pathway, via structured intermediates not populated during refolding from denaturant. Association with the ribosome allows these intermediates to form, as otherwise destabilizing C-terminal sequences remain confined in the ribosome exit tunnel, suggesting a general role for the tunnel in facilitating folding.

We find that partially-folded nascent chains recruit the endogenous chaperone Trigger factor, which uses a large composite hydrophobic/hydrophilic interface to engage folding intermediates without disrupting their structure. As a result, the nascent chain bound by Trigger factor is poised to complete folding immediately upon emergence of the C-terminus from the exit tunnel.

We further mined our HDX MS data to study ribosomal protein dynamics at the peptide level. We comprehensively map interactions between the nascent chain and several ribosomal proteins, tracing the path of the emerging polypeptide during synthesis. Our work provides a peptide-level description of de novo protein folding dynamics, thereby revealing new mechanisms by which cellular factors shape the conformational search for the native state.

ABS#8

Poster session, July 14

The 3D modules of catalysis: Identifying recurring functional entities in enzyme active sites

Ioannis Riziotis (1)
(1) EMBL's European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom

Enzyme catalysis is governed by a limited toolkit of residues with specific physical-chemical properties. It is expected that recurring residue arrangements will be found across the enzyme space, which are structurally similar, perform a defined function and occur in unrelated enzymes. Leveraging curated information from the Mechanism and Catalytic Site Atlas (M-CSA) (enzyme structure, sequence, catalytic residue annotations, catalysed reaction, detailed mechanism description), 3D templates were generated to represent compact groups of catalytic residues. A fuzzy template-template search allowed us to identify conserved and convergent motifs which we define as the "modules of enzyme catalysis". We show that a large fraction of modules facilitates binding of metal ions, co-factors and substrates, and have emerged through convergent evolution. A smaller number of convergent modules perform a well-defined catalytic role, such as the variants of the catalytic triad (i.e. Ser-His-Asp/Cys-His-Asp) and the saccharide-cleaving Asp/Glu triad. We also observe that enzymes of

divergent function retain regions of their active site unaltered during evolution, as shown by modules performing similar or identical steps of the catalytic mechanism. We have compiled a comprehensive library of catalytic modules that characterise a broad spectrum of enzymes. These modules can be used as templates in enzyme design and for better understanding of catalysis in 3D.

ABS#9

Poster session, July 13

Disruption of a highly conserved hydrogen-bond network within human aquaporin-1 causes instability and misfolding

Philip Drewniak (1); Xiao Peng (1); Vladimir Ladizhansky (1); Leonid Brown (1)
(1) Department of Physics, University of Guelph, Guelph, Canada

Aquaporins (AQPs) are a class of water-transporting integral membrane proteins, which are crucial for many cellular processes and involved in several diseases including many types of cancers. Aquaporin-1 (AQP1) contains two structural features within its pore that allow for such efficient water transport – two Asn-Pro-Ala (NPA) motifs on either side of the channel, and an aromatic/Arginine selectivity filter. In addition to these, AQP1 contains a uniquely rigid loop C region that seems to make essential contacts with several residues from surrounding helices and loops adjacent to both the second NPA motif and the selectivity filter. Prior experimental evidence and structural conservation analysis have revealed a highly conserved hydrogen-bond network that may not only anchor the loop C region, but directly stabilize the helical bundle within the membrane. We have set out to explore this network, using mutagenesis to replace conserved hydrogen-bonded residues, and then probing the stability of these novel mutants with attenuated total reflectance (ATR)-FTIR spectroscopy. Secondary structure changes caused by these mutations were observed, and the overall stability was assayed using hydrogen/deuterium exchange upon increasing temperature. By monitoring the amide II/II' bands, the degree of solvent accessibility of backbone amides could be directly measured, and thus the degree of misfolding for each mutant. The results indicate that disruption of any of the tested residues leads to either partial instability or outright misfolding. As well, considerable secondary structure deviations from the mainly helical WT are observed among these mutants, with some even exhibiting new partial β-sheet structures. These results provide novel and valuable insight into the structural stability of aquaporins, as these networks are conserved among a variety of human AQPs and even across non-mammalian homologs.

ABS#16

Protein Evolution: Lessons from the Past (July 13, PM)

Marginal specificity in protein interactions constrains evolution

Dia Ghose (1); Kaitlyn Przydzial (1); Emily Mahoney (1); Amy Keating (1); Michael Laub (1)
(1) Massachusetts Institute of Technology, Cambridge, United States of America

The evolution of novel functions in biology relies heavily on gene duplication and divergence, creating large paralogous protein families. Selective pressure to avoid detrimental cross-talk often results in paralogs that exhibit exquisite specificity for their interaction partners. But how robust or sensitive is this specificity to mutation? Here, using deep mutational scanning, we demonstrate that a paralogous family of bacterial signaling proteins exhibits marginal specificity, such that many individual substitutions give rise to substantial cross-talk between normally insulated pathways. Our results indicate that sequence space is locally crowded despite overall sparseness, and we provide evidence that this crowding has constrained the evolution of bacterial signaling proteins. These findings underscore how evolution selects for 'good enough' rather than optimized phenotypes, leading to restrictions on the subsequent evolution of paralogs.

ABS#18

Structure Prediction and Design (July 15, AM)

Predictions of protein fold switching from sequence

Lauren Porter (1); Joseph Schafer (1); Devlina Chakravarty (1)
(1) National Library of Medicine, National Institutes of Health, Bethesda, United States of America

Although most globular proteins fold into a single stable structure, some switch folds by remodeling their

secondary and tertiary structures in response to cellular stimuli. State-of-the-art algorithms, such as AlphaFold2, predict that these fold-switching proteins assume only one stable structure, missing their functionally critical alternative folds. Nevertheless, previous work has demonstrated that fold switching is predictable. Leveraging this work, we show that structural features unique to both conformations of fold-switching proteins have been preserved by natural selection. To do this, we developed an approach that successfully revealed coevolution of amino acid pairs uniquely corresponding to both conformations of 56/58 fold-switching proteins from distinct families. Then, using a set of coevolved amino acid pairs predicted by our approach, we successfully biased AlphaFold2 to predict two experimentally consistent conformations of a candidate protein with unsolved structure. A related approach allowed us to elucidate a covert mutational pathway interconnecting proteins with distinct folds. Our work both paves the way for predictions of diverse protein structures from single sequences and implies that the functionalities of fold-switching proteins provide evolutionary advantage.

ABS#19

Poster session, July 15

The mitochondrial ClpP protease: from basic principles to drug discovery

Walid Houry (1)
(1) Biochemistry, University of Toronto, Toronto, Canada

Mitochondrial matrix ClpP is a serine protease that forms a tetradecameric cylindrical structure. ClpP acts as a complex with the hexameric ATPase ClpX to form the ClpXP ATP-dependent protease. ClpXP is responsible for mitochondrial protein quality control through specific degradation of proteins involved in several metabolic processes. Furthermore, ClpP overexpression is required in many cancer cells to eliminate ROS-damaged proteins and to sustain oncogenesis. Targeting ClpP to dysregulate its function using small molecule agonists is a recent strategy in cancer therapy. Here, we describe the mechanism of function of ClpXP and the development of novel compounds that dysregulate the activity of the protease. Using X-ray crystallography, we found that these compounds bind at surface hydrophobic pockets of ClpP preventing ClpX interaction. N-terminome profiling of cancer cells upon treatment with one of these compounds revealed the global proteomic changes that arise and identified the structural motifs preferred for protein cleavage by compound-activated ClpP. Together, our studies provide the structural and molecular bases by which dysregulated ClpP affects cancer cell viability and proliferation.

ABS#21

Poster session, July 15

Engineering A Photoactivatable O-GlcNac Transferase Library to Explore Adaptor Protein Interactions

Cassandra Joiner (1)
(1) Chemistry, Saint Olaf College, Northfield, United States of America

O-GlcNAcylation, or the transfer of O-linked beta-N-acetylglucosamine (O-GlcNAc) to serine and threonine residues of nuclear and cytoplasmic proteins, is an essential, metazoan post-translational modification. O-GlcNAc transferase (OGT) is the sole enzyme responsible for this modification and targets over one thousand different substrates involved in almost every cellular process. Misregulation of O-GlcNAc levels and O-GlcNAcylation patterns has been implicated in metabolic diseases, cancers, and neurodegenerative diseases. This broad disease activity makes OGT an attractive therapeutic target, however the substrate diversity makes pan inhibition as a therapeutic strategy unfeasible. Rather, a substrate-specific approach to targeting is more advantageous, but how OGT chooses its substates is poorly understood. Recent studies have shown that substrate specificity is affected by interactions between OGT's noncatalytic tetratricopeptide repeat (TPR) domain and adaptor proteins that are proposed to target protein substrates to OGT and alter its glycosyltransferase activity. However, the identity of adaptor proteins and their binding sites along the TPR domain remain poorly characterized, making it challenging to selectively target these interactors and interrogate the mechanism of adaptor-mediated substrate selection. Here, we made a library of recombinant OGT constructs containing site-specifically incorporated photoactivatable unnatural amino acids (UAAs) along the surface of the TPR domain to covalently capture known adaptors and map their interactions along OGT. With this library, we have identified the binding sites of known adaptor proteins along OGT's TPR domain and captured novel

protein interactions along the TPR domain from cellular extracts in hopes to identify new adaptor proteins.

ABS#22

Poster session, July 13

Truncated SUMO1 protein inhibits Aβ aggregation potentially through SUMO-SIM interaction

Yuxuan LI (1)
(1) School of Life Science, The Chinese University of Hong Kong (CUHK), Hong Kong, Hong Kong

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is pathologically characterized by accumulation of aggregates of amyloid beta (Aβ) peptide, a 38-43 amino acid peptide generated by enzymatic cleavage of amyloid precursor protein (APP) by β and γ secretase. Intermolecular interaction between partially folded Aβ initiates the aggregation, resulting in oligomer and fibril formation. According to the amyloid cascade hypothesis, Aβ aggregation plays a primary role in AD pathogenesis. Notably, Aβ oligomers are proven to be highly neurotoxic, indicating the importance of stabilizing Aβ in its monomeric form and preventing Aβ oligomerization at early stage.

In this study, we predicted and confirmed the direct interaction between Aβ with a N terminus and C terminus truncated small ubiquitin like modifier 1 (SUMO1) protein, SUMO1(15-92), by computation prediction and by binding affinity determination. We expected that removing the floppy N-terminal and C-terminal region on SUMO1 could facilitates the binding of SUMO1 (15-92) to Aβ. Microscale Thermophoresis analysis showed that SUMO1(15-92) has high affinity to both Aβ40 and Aβ42. The highly hydrophobic C-terminus of Aβ, which contains two SUMO interaction motifs (SIMs), was identified as the critical binding region on Aβ for its interaction with SUMO1(15-92). Thioflavin T assays were applied to demonstrate the ability of SUMO1(15-92) protein in suppressing Aβ aggregation in vitro. Circular Dichroism Spectrum of Aβ with or without SUMO1 (15-92) was recorded and SUMO1(15-92) was found to inhibit the structural transition of Aβ. The efficacy of SUMO1 in ameliorating toxicity induced by Aβ aggregation was evaluated in transgenic Caenorhabditis elegans (C. elegans) model. Taken together, our findings shows that SUMO1(15-92) could inhibit Aβ aggregation potentially through SUMO-SIM interaction and SUMO1(15-92) could protect transgenic C. elegans against Aβ-induced toxicity.

ABS#23

Structures of Mega-Complexes (July 13, PM)

Helical reconstruction of VP39 reveals principles for baculovirus nucleocapsid assembly

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Baculoviruses are important insect-infecting pathogens with wide applications as biological pesticides, in vitro protein production vehicles and gene therapy tools. Its nucleocapsid encapsulates and protects the circular double-stranded viral DNA encoding proteins for viral replication and entry. The highly conserved major capsid protein VP39 forms a cylindrical nucleocapsid protein assembly. The mechanism for VP39 assembly remains unknown. We determined a 2.6 Å cryo-electron microscopy helical reconstruction of an infectious nucleocapsid of Autographa californica multiple nucleopolyhedrovirus, revealing that the VP39 monomer comprises a novel fold, which includes a putative inward-facing Zinc finger domain. Dimers of VP39 assemble into helical tubes of ˜50 nm diameter and several hundred mm in length. VP39 dimers are stabilized by wrapping an extended loop region around a protruding beta sheet of an adjacent monomer. We discuss how flexible subunit contacts enable the formation of cylindrical VP39 assemblies varying in diameter. This VP39 helical reconstruction reveals potential interaction sites for the viral genome, and general principles for baculoviral nucleocapsid assembly.

ABS#24

Poster session, July 13

Elucidation and control of physicochemical properties of antibodies by supercharging of variable regions

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Mutation with charged amino acids onto the surface of proteins (Supercharge) [1] is expected to improve the properties and functions of antibodies, but there are still few examples of studies, and the details of the molecular mechanisms that modify and control these properties are not known [2-4].
In this study, we aimed to elucidate the mechanism of modification of the properties and functions of Fab antibody by Supercharge. Amino acid sequences of mutants with net charges of +10 and -10 (pos10, neg10) were obtained from a model Fab antibody (WT) with a net charge of -4, by introducing charged amino acid mutations into the variable region using the computational design software Rosetta. The mutants were expressed, purified, and analyzed for thermal stability by DSC (Differential scanning calorimetry), colloidal stability by DLS/SLS (Dynamic/static light scattering) and zeta potential measurements, and the interaction with the antigen by ELISA, ITC (Isothermal titration calorimetry) and SPR (Surface plasmon resonance).
It was suggested that pos10 acquired pH-independent thermal stability by covering the positively charged surface of the antibody with phosphate ions in buffers [5]. The surface charge of pos10 in phosphate buffer was neutralized by the solvation effect of the anions, resulting in reduced colloidal stability, whereas in acetate buffer it was strongly positively charged and colloidal stability was improved. The binding affinity of pos10 for positively charged antigens increased under sufficiently saline conditions but decreased at low salt concentrations. On the other hand, neg10 showed reduced thermal stability, indicating that the colloidal stability and binding affinity were comparable to those of WT. Based on these results, the possibility of controlling the stability and binding affinity of the antibodies by introducing charged amino acids and selecting the solvent environment is discussed.

ABS#26

Poster session, July 14

Surface Fingerprinting for the Design of Novel Protein-Protein Interactions

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Proteins play a fundamental role in nearly all biological processes, from regular cell homeostasis to pathogenicity. Therefore, engineering novel protein-protein interactions (PPI) represent a potential mechanism for biomedical research to develop cell-based therapies, protein-based therapeutics, synthetic biology tools or biosensors. However, most protein therapeutics, and especially antibodies, were developed using experimental procedures and uncertainties remain regarding the way they engage their respective binding sites. Despite recent advances in the field[1], the prediction of a specific amino acid sequence forming novel protein interactions with a specific target site remains a significant challenge. With these considerations in mind, we previously developed MaSIF[2], a deep learning framework using vectorized geometric and chemical features of the protein surface, also referred to as fingerprints. MaSIF demonstrated great performances in predicting PPI interfaces and matching PPI pairs with orders of magnitude faster than other state-of-the-art algorithms. Here, we enhanced the potential of MaSIF[3] for designing de novo PPIs by using a database of small binding motifs, referred to as seeds. Seeds were grafted on different protein scaffolds and refined using the Rosetta modelling suite. Selected designs were then screened using high-throughput approaches, improved by experimental maturation techniques if necessary and characterized with various biophysical methods for folding, stability and binding affinities. With this approach, we computationally designed and validated several protein binders against four protein targets of therapeutic interest: SARS-CoV-2 spike, PD-1, PD-L1, and CTLA-4. While some protein binders reached nanomolar affinities after in vitro maturation, others demonstrated native-like affinities by pure in silico generation. Moreover, all candidates proved highly accurate predictions with structural and mutational characterization. Altogether, our surface-centric approach contributes to a better understanding of PPI design and opens the possibility of developing innovative biologics orother biotechnologies.

ABS#30

Poster session, July 13

Dynamics Dictating Function of Bacterial Enzymes

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As antimicrobial resistance grows more rampant, the need for new therapeutic targets intensifies. Proteins are a prominent target as they tend to be highly conserved if essential for bacterial survival (Luo, Gao et al. 2015). Furthermore, rational drug design benefits from the identification of protein sites which affect activity. We investigate bacterial protein regions of interest by examining how the active site is globally coupled to the surrounding protein structure and movements. Our focus is on a variety of virulent proteins including Staphylococcus aureus exfoliative toxin A (a skin cleaving protease) (Nishifuji, Sugai et al. 2008), Streptococcus pneumoniae sortase A (transpeptidase which attaches proteins required for adhesion and immuno-evasion to the bacterial cell wall) (Bender and Weiser 2006), and Streptococcus pneumoniae high temperature requirement A (associated with host inflammation) (Ibrahim, Kerr et al. 2004). Here, we show that by studying multiple timescales of movement and the effect of multiple mutations we can identify the intrinsic conformational changes of bacterial proteins and how they influence activity. Specifically, we utilize nuclear magnetic resonance, analytical sizing, and catalytic assays to guide our research. These studies highlight how structure and dynamics should be considered in tandem as the shift between multiple conformations may explain how proteins interact with their environment and perform their function. Additionally, it directly addresses the difficulty in therapeutically targeting bacterial proteins with active sites closely related to that of human proteins by allowing us to allosterically target these bacterial proteins instead.

ABS#35

2023 Christian B. Anfinsen

Structure and Dynamics of Channels and Transporters in Infectious Diseases from Solid-State NMR Spectroscopy

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Enveloped viruses and bacteria encode membrane-bound ion channels and transporters that are important for the survival and infectivity of these pathogens. Elucidating the structure and dynamics of these membrane proteins is important for advancing fundamental knowledge about infectious diseases and for developing antiviral and antibiotic drugs. Solid-state NMR spectroscopy is well suited to the study of these membrane proteins, but has been limited by low sensitivity and the difficulty of measuring inter-atomic distances to the nanometer range that is important for defining the oligomeric structure and ligand-binding site of these membrane proteins. We have invented high-sensitivity and nanometer-distance 19F and 1H solid-state NMR techniques (1, 2) to accelerate the structure determination of these viral and bacterial channels and transporters. I will describe these methods and present their application to the influenza B M2 proton channel (3), the SARS-CoV-2 envelope protein cation channel (4, 5), and the bacterial transporter EmrE (6, 7). For each protein, we solved two structures, corresponding to the closed and open states of the ion channels or the putative outward- and inward-facing states of the transporter. We also developed solid-state NMR experiments to detect channel water dynamics and the dynamics of the protein and ligand. These structural and dynamical information gives rich insights into the channel activation mechanism of influenza BM2 and SARS-CoV-2 E and the alternating access motion by EmrE to export substrates.

ABS#37

Poster session, July 13

Cry3Aa-Antimicrobial peptide (AMP) Fusion Crystal Protein as an Improved Anti-leishmanial Agent

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Intracellular infections are caused by pathogens that evade the immune system, persist, and multiply in the host cells. The obligatory parasite Leishmania, is one such intracellular pathogen and causes Leishmaniasis. There is no vaccine available to control leishmaniasis although, existing drugs for leishmaniasis have severe drawbacks in terms of cost, toxicity, resistance, and safety. At present, antimicrobial peptides (AMPs) have attracted the attention due to its advantageous over standard antibiotics. Most of them are cationically charged, and act by penetrating the pathogen's cell membrane. AMPs also have certain drawbacks they can be unstable

and have cytotoxicity. To overcome the limitations of AMPs, our research group previously reported the use of submicron sized crystals of the protein Cry3Aa to deliver the antimicrobial peptide, dermaseptin S1 to macrophages, where parasite resides. In this study, we have explored the targeted delivery of prodrug of antileishmanial peptides (melittin) with Cry3Aa crystal proteins, aids in the entrapment of peptide and its delivery to the lysosome of macrophages. We have developed a melittin prodrug derivative with LPETG linker, that can be cleaved by pepsin enzyme, abundant in the microenvironment of macrophages and effectively kills the leishmania parasites. In order to accomplish these objectives, we have carried out in vitro tests to demonstrate the cytotoxicity of our drug, such as the MTT assay with IC50 > 2uM, red blood cell (RBC) hemolysis assay with 2-3% hemolysis at 0.5mg/ml concentration, in vitro antiamastigotes assay for the Leishmania amazonensis and Leishmania donovani strains, and further performed the animal studies for the cutaneous and visceral leishmaniasis mouse model. These experiments led to the conclusion that Cry3A-mPMLT with an LPETG linker site was successful in reducing the parasite population. These studies provide new insights into how to enhance the therapeutic effectiveness of AMPs.

ABS#40

Poster session, July 15

Targeting a NADPH Oxidase 2 Membrane Anchor by Fragment-Based Drug Design for Selective Inhibition

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Peripheral membrane proteins (PMPs) act as critical mediators of various signaling pathways through their interactions with membranes. Despite this, they remain underutilized as therapeutic targets due to their complex nature of interactions, technical challenges, and general lack of deep ligand binding pockets. The PMP p47phox contains a PX domain which preferentially binds to phosphoinositols located within a membrane. This recognition event induces the translocation of a cytosolic activating complex of the phagocytic transmembrane protein, NADPH Oxidase 2 (NOX2). Overactivation of NOX produces a cytotoxic oxidative burst implicated in cardiovascular diseases, cancers, and neurodegenerative disorders. Current attempts at inhibiting NOXs have been hindered by non-selective compounds across the seven NOX isoforms – all of which contain a conserved catalytic domain. This study aims to overcome this issue by targeting the pre-activation event for NOX2 induced by p47phox-PX translocation. A library containing 2,000 rule-of-three compliant fragments was screened against the PX domain using Nuclear Magnetic Resonance Spectroscopy to discover initial hits. Five fragments fell within our binding parameter limitations of having a binding affinity lower than 5 mM and a ligand efficiency greater than 0.2. Docking simulations of the top two fragments displayed overlapping binding poses in the PX domain, which guided further fragment elaborations. Characterization of initial elaborated fragments has displayed binding affinities in the low micromolar range – an approximate 50-fold enhancement in affinity to the PX domain as compared to the original fragments. This study utilizes a compelling strategy that leverages fragment-based inhibitor design to block membrane anchoring events for therapeutic advantage where previous methods have failed.

ABS#41

Engineering Protein Fate and Function (July 16 AM)

PicoShells: A Tool for Screening Millions of Protein Variants in Application-Relevant Environments

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Current protein screening technologies represent a major bottleneck in our ability to evolve and engineer new proteins because of their low throughput and/or inability to place proteins into their native structure or solutions relevant for their end application. To overcome these limitations, we have developed PicoShell technology, a protein screening tool that enables scientists to screen millions of variants, swap out the soluble environment the proteins are exposed to, and place proteins in solutions that are

useful for their end application (e.g. serum, buffer). PicoShells are spherical microparticles with a hollow center and an outer porous shell made of polyethylene glycol that can be fabricated at a rate of 2000 particles per second. Cells are placed into the hollow center where they can either secrete or be lysed to release the desired proteins. The Picoshells pore size can be engineered to be anywhere between 15 and 250 kDa such that proteins and plasmids are retained within the hollow center. PicoShells can also be engineered to contain capture motifs that can target released proteins. Due to the free diffusion of small molecules across the shell, the interior solution where the proteins are located can be changed by centrifuging down the PicoShells and exchanging the solution the particles are suspended in. This feature enables assays to select proteins that respond to changes in analyte concentrations from other proteins that are less- or non-responsive and place proteins in any desired solution (Figure shows screening of a calcium biosensor). PicoShells can be coupled with fluorescence-activated cell sorting (FACS), which enables us to screen millions of protein variants with throughputs of >50 variants per second. We expect that the PicoShell technology will accelerate the process of protein engineering and increase the range of selection criteria that can be used to guide protein directed evolution efforts.

ABS#42

Poster session, July 15

Exploring the Consequences of Making a Mistake - How Does the Antiviral Galidesivir Derail Flavivirus Replication?

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We are entering a new era of infectious diseases due to the increased size and mobility of human populations(1), drug/vaccine resistance(2) and anthropogenic climate change(3). There is consequently an urgent need to develop effective antivirals(4). An important class of antivirals are nucleoside analogs, which are structural mimics of their endogenous counterparts. Following incorporation into the nascent viral genome by viral polymerases, nucleoside analogs block viral replication by prematurely terminating chain extension or facilitating mutation(5,6). An interesting subset of nucleoside analogs are 'non-obligate' chain terminators, which possess the chemistry required for chain elongation yet are able to terminate viral genome synthesis(7,8). One such antiviral is the adenosine analog, Galidesivir. In cell-based and animal models Galidesivir is active against numerous RNA viruses, including the flaviviruses Dengue and Zika(9-12).

Our objective was to investigate the inhibition of the flavivirus polymerase by Galidesivir. Using an in vitro polymerase assay, we showed that Galidesivir exhibits modest potency against Dengue-2 and Zika virus polymerases with IC50 values in the 40-50 μM range. Next, using a primer extension assay, we showed that the inhibitory effects of Galidesivir depend on the template RNA sequence. The polymerase tends to stall while incorporating a single Galidesivir, leading to the build-up of truncated products. However full length RNA transcripts are still produced in reduced amount. Intriguingly, there is a strong barrier to incorporating Galidesivir nucleotides at two consecutive sites, which results in the near complete abolition of chain extension. This suggests that Galidesivir-induced localized distortion in the dsRNA impedes the incorporation of consecutive Galidesivir nucleotides. The results provide insights into the mechanism of inhibition of Galidesivir and highlight the importance of considering sequence-specific effects when analysing the mechanism of a nucleoside analog.

ABS#43

Membrane Proteins: From Natural to Designed (July 14, PM)

Protein Engineering of Extracellular Vesicle for Delivery of Complex Drugs

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Extracellular vesicles (EVs) have shown promise as biological delivery vehicles, but therapeutic applications require efficient cargo loading and targeted delivery to diseased tissue. We have developed novel methods for loading of CRISPR/Cas9 into EVs. The plant proteins CIBN and CRY2 reversibly heterodimerize as triggered by light. Constructs encoding fusion proteins CRY2-Cas9 and CIBN with an exo-sorting MysPalm motive were

transiently transfected into Expi293 cells, and as a result Cas9 protein was successfully loaded into the lumen of released EVs under light-on conditions at approximately 25 Cas9 molecules per particle. Cas9-loaded EVs displayed CRISPR editing in vitro of an Cre reporter cassette in HEK293. Mechanistical analysis using a Galectin-9 imaging reporter assay revealed that endosomal escape was a limiting factor for the functional delivery of EV cargo. Addition of Chloroquine resulted in increased levels of cargo delivery, as well as the introduction of cationic lipids by hybridization of EVs with lipid nanoparticles. Protein engineering was applied to introduce HALO protein at the surface of EVs for the introduction of targeting ligands. GalNac triantenna was incorporated through HALO and these modified EVs displayed improved binding to primary human hepatocyte spheroids. Intravenous injection of engineered EVs carrying Cas9 mRNA and sgRNA resulted in editing of the PCSK9 target gene in liver tissue. These novel protein engineering approaches will be valuable for development of EVs therapeutics.

ABS#44

Poster session, July 15

A residue-based pharmacophore approach to study and manipulate protein interfaces

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We describe a novel computational method, Protein Ligand Interface Design (ProtLID), to study protein interfaces, including: identifying cognate ligands from a subproteome for a given target receptor protein and redesigning known interfaces for selectivity. ProtLID designs an optimal protein interface for a given receptor by running extensive molecular dynamics simulations of single residue probes. The type and location of residue preferences establish a residue-based pharmacophore. Here, we highlight two protein design applications with PROTLID. The first application concerned with programmed cell death-1 (PD-1). Blockade of this inhibitory checkpoint pathway has shown therapeutic importance by rescuing T cells from their exhausted state. Cognate ligands of the PD-1 receptor include the tissue-specific PD-L1 and PD-L2 proteins. Engineering a human PD-1 interface specific for PD-L1 or PD-L2 can provide a specific reagent and therapeutic advantage for tissue-specific disruption of the PD-1 pathway. We custom-designed a human PD-1 interface specific to human PD-L1 without any significant affinity to PD-L2. In subsequent cell assay experiments, half of all single-point mutant designs proved to introduce a statistically significant selectivity, with nine of these maintaining a close to wild-type affinity to PD-L1. The second application focused on Herpes virus entry mediator (HVEM), which interacts with three ligands from two different superfamilies using two different binding interfaces and generates functionally distinct bidirectional signaling pathways for controlling both inflammatory and inhibitory responses. The engagement with ligands CD160 and BTLA is associated with inhibitory signals, whereas inflammatory responses are regulated through the interaction with LIGHT from the TNF superfamily. We computationally redesigned the HVEM recognition interfaces using ProtLID and achieved a switchable binding specificity. In subsequent cell-based binding assays the new interfaces, designed with only single or double mutations, exhibited selective binding to only one or two out of the three cognate ligands.

ABS#45

Aggregates, Amyloids, or Condensates? (July 16, AM)

Super-resolution imaging of transcription in living cells

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We will discuss the latest efforts in our laboratory to develop highly sensitive methods ofmicroscopy, to go directly inside living cells and uncover the behavior of single biomoleculesas they effect their function in transcription. Transcription is the first step in gene expressionregulation, during which genetic information on DNA is decoded into RNA transcripts. Methodologically, the so-called live cell single molecule and super-resolution techniques –that break the optical diffraction limit– are revealing with unprecedented spatial andtemporal resolutions, novel emergent phenomena inside the living cells. We will discuss ourrecent discoveries on highly dynamic biomolecular clustering, and phase transitions in vivo.These discoveries are challenging the 'textbook view' on how our genome (DNA) is decodedin living cells.

ABS#46

Poster session, July 13

A mild and chemoselective CALB biocatalysed synthesis of sulfoxides exploiting the dual role of AcOEt as solvent and reagent

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Sulfoxides are an important class of organic compounds that often are seen used in organic synthesis as chiral auxiliaries, synthons for C–C bond forming reactions, directing groups in C–H bond functionalisation and can partake in numerous other functionalisation reactions. Moreover, sulfoxides are widely found in pharmaceutically active ingredients such as the blockbuster drug omeprazole. Sulfoxides are commonly obtained through the oxidation of the corresponding sulfide precursor.1 However, the current methodologies adopted for their synthesis rely on the use of harsh chemicals such as nitric acid, hypohalites, peroxides and oxone, all of which present limited industrial use as can be shock sensitive and explosive, hence unsuitable for large scale production.2

Following our interest in the development of new and industrially applicable green methodologies for the synthesis of sulfur-containing drugs and drug-like synthons, herein we report a facile, chemoselective and scalable biocatalytic protocol for the synthesis of sulfoxides using immobilised Candida antarctica lipase B (CALB), a very robust enzyme which retains its activity in both aqueous and organic solvents, and AcOEt with a dual role of more environmentally friendly reaction solvent and enzyme substrate.3 A series of 27 sulfides that included alkyl, aryl, carbonyl and alkene bearing compounds as well as omeprazole were successfully oxidised in high yields and with excellent E-factors to the corresponding sulfoxides with little to no overoxidation by-products. In addition, a large-scale experiment starting from 3 g of sulfide substrate afforded the corresponding sulfoxide in excellent yield. Finally, a series of enzyme recyclability experiments were carried out to further confirm the industrial potentiality of the methodology.

This method proves to be cost effective, robust and selective with few side-reactions. Furthermore, we show that the use of AcOEt as solvent and CALB substrate improves the industrial sustainability of the method, providing an overall greener methodology.

ABS#50

Poster session, July 14

Identifying the Role of Conformational Entropy in Integral Membrane Protein Folding and Function

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Molecular recognition is at the heart of complex biochemistry: it is directly or indirectly related to the function of biological macromolecules and informs drug design. Therefore, we must understand the principles that govern these dynamic reactions. Several years ago, the Wand Lab developed a strategy to employ advanced NMR relaxation techniques to measure protein side chain motion as changes in conformational entropy. This approach, termed the "entropy meter," revealed that proteins retain considerable conformational entropy in the folded state and that these changes in entropy can be a major contributor to the change in Gibbs free energy.

The vast majority of experimental insight into protein motion, and the entropy that it represents, has been derived from soluble proteins. Very little is known about the internal motion of integral membrane proteins (IMPs), despite their abundance in biology. The first such study on IMPs was recently reported by our group and the results indicate, as might be anticipated, that IMPs are qualitatively dynamically distinct from their soluble proteins. Two proteins with quite different topologies were examined: Sensory rhodopsin II (pSRII), a seven transmembrane α-helical bundle, and outer membrane protein W (OmpW), an 8-stranded β-barrel. Both were found to be unusually dynamic. Indeed, these two IMPs are more dynamic (and therefore entropic) than any soluble protein examined by these methods [1]. These findings raise several fundamental questions such as: Are IMPs generally this dynamic? If, so, how does this residual conformational entropy contribute to thermodynamics of their function? A high conformational entropy could explain, for example, the stability of IMPs in the membrane bilayer in the absence of the "hydrophobic effect." Preliminary data suggests that the IMP VDAC1 also exhibits fast dynamics in nanodiscs. This project centers on understanding the role of conformational entropy on the stability and function of IMPs.

ABS#51

Poster session, July 14

In Silico Studies Suggest Structural Basis for Amyloid-Apolipoprotein Co-deposition

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In Alzheimer's disease (AD), insoluble amyloid-beta (Aβ) fibrils deposit in the brain, contributing to pathology. Apolipoprotein E (apoE) is a major genetic causative risk factor for AD. Amyloid fibrils in AD and other amyloidoses co-deposit with apolipoproteins, yet the structural basis for apolipoprotein-amyloid interactions is unclear. We created potential models of these interactions using protein-protein docking. NMR structures of lipid-free human apoE4 or micelle-bound human apoC-III were docked to high-resolution cryo-EM structures of patient-derived Aβ1-40 and Aβ1-42 fibrils. Docking of intact apolipoproteins showed no specific binding. Since apolipoproteins contain amphipathic α-helices connected by flexible linkers, we omitted flexible linkers and docked α-helical fragments. Fragment docking showed that hydrophobic faces of apoE4 α-helices bind to hydrophobic surfaces on the sides or ends of Aβ fibrils, while basic residues flanking these apoE hydrophobic helical faces interacted with acidic or aromatic arrays in some Aβ fibrils. Similar results for apoC-III fragment docking suggests this binding mode applies to other apolipoprotein-amyloid interactions. Combining these fragment docking poses into contiguous, full-length models of apoE4 or apoC-III consistently showed specific binding to hydrophobic surfaces along the fibril axis or ends. Apolipoproteins can adopt this open conformation via domain movement around flexible linkers between amphipathic α-helices. This movement closely resembles apolipoprotein-lipid interactions. Molecular dynamics simulations showed that open apoE4 models docked to Aβ1-42 fibrils remained stably bound to the fibril and increased fibril rigidity. We propose that apolipoprotein binding to hydrophobic arrays along the fibril axis stabilizes fibrils and interferes with secondary nucleation and fragmentation, while apolipoprotein binding at fibril ends halts elongation and dissolution. This mechanism helps reconcile conflicting reports regarding apoE's effects on Ab aggregation and is supported by extensive prior experimental evidence. ApoE domain opening and direct involvement of Arg/Cys158 in amyloid binding may contribute to apoE isoform-specific effects in AD.

ABS#52

Poster session, July 13

Characterization of the Dynamics of Interleukin-1 Receptor Antagonist by NMR Spectroscopy

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The interleukin-1 (IL-1) system plays an important role in the immune system, having a great impact in the development of different diseases when it becomes dysregulated. Interleukin-1 receptor antagonist (IL-1Ra), a low molecular weight cytokine has been historically used as a protein therapeutic for its ability to serve as IL-1 signaling cascade blockade. However, its short in vivo half-life time has largely limited its use in the clinic. We would like to improve its interaction with the interleukin-1 receptor (IL-1R) by increasing their affinity. It has been recently shown that detailed knowledge of the fast-internal motion of proteins provides insight into the influence of conformational entropy in protein-ligand interactions. Here we are interested in the thermodynamic origins of the interaction of IL-1Ra with the IL-1 receptor with a particular emphasis on the role of conformational entropy. Nuclear magnetic resonance (NMR) relaxation experiments are being used to characterize the dynamic disorder of amino acid side chains of IL-1Ra in its free state and while bound to IL-1R. This information identifies regions of IL-1Ra that become more ordered or less ordered upon binding IL-1R and therefore contribute an unfavorable or favorable change in conformational entropy to the free energy of binding, respectively. Assignments of the backbone and side-chain residues of IL-1Ra have been obtained and will be presented together with NMR studies of IL-1Ra in its free form. Supported by the NIH and Texas A&M University.

ABS#53

Poster session, July 13

Allosteric Regulation of Coregulator Recruitment in FXR

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The farnesoid x receptor (FXRα, NR1H4) is a bile acid-regulated transcription factor that plays a role in bile acid, carbohydrate, and lipid metabolism. Coregulators are an important class of proteins that are recruited to transcriptional complexes and promote the up/down-regulation of target genes. While it has been shown that coregulator recruitment to FXR is modulated in a ligand-specific manner, how minor modifications in bile acid structure drive differences in coregulator recruitment and, more broadly, diverse transcriptional outcomes is not understood. Here, we use cell reporter assays, molecular dynamics simulations, and binding experiments to determine how small differences in ligand structure give rise to preferences in coregulator recruitment. We observe that obeticholic acid (OCA), a potent synthetic bile acid analog, allosterically modulates helix 5 of FXR to achieve enhanced activation compared to endogenous bile acids. We examine the mechanisms underlying this allosteric regulation and the role of coregulator recruitment in the increased activity of OCA. These studies provide insight into strategies for the design of potent FXR ligands.

ABS#55

Poster session, July 15

An in vitro Mimic of Protein and RNA Folding in Cells

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In the last few decades, it has become evident that the complex cellular environment is challenging to reproduce in vitro. Inside cells, both folding and dynamics of proteins and RNA are affected due to steric crowding and non-steric chemical interactions. The varying effect of the cytosol on biomacromolecules is not easy to predict. For example, inside cells, phosphoglycerate kinase (PGK) is stabilized and variable major protein-like sequence expressed (VlsE) is destabilized. Here, we have developed an in vitro cytomimetic that reproduces the in-cell stability trend of biomacromolecules, ranging from small peptides and short RNA hairpins to large globular proteins. Our approach uses a mixture of polyethylene glycol (PEG) or Ficoll to mimic macromolecular crowding and Mammalian Protein Extraction Reagent (M-PER) or Pierce IP lysis buffer to mimic the in-cell chemical interactions. We find that a combination of 150 mg/ml Ficoll and 60% Pierce IP lysis buffer can mimic the opposing in-cell folding behavior of the globular proteins, PGK and VlsE. Moreover, a similar mixture of 150 mg/ml Ficoll and 20% M-PER reproduces the in-cell stabilization of a small λ-repressor peptide, λ6-85, and short fourU RNA hairpin. Our results suggest that 150 mg/ml of Ficoll and 20% M-PER can be broadly used as an in vitro cytomimetic to predict in-cell peptide, protein, and RNA folding stability and kinetics.

ABS#56

Poster session, July 14

Investigating the Sensing Mechanism of VanS: A Histidine Kinase Essential for Vancomycin-Resistant Enterococci

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Vancomycin-resistant Enterococci (VRE) are classified by the CDC as a "serious threat" in antibiotic resistance, accounting for almost 55,000 infections in 2017. Because vancomycin has been used as a last-resort antibiotic, resistance towards this drug leaves few other treatment options available. It is imperative to understand the mechanism of resistance to develop new ways to fight VRE. Vancomycin binds and sequesters lipid II, an intermediate in cell-wall synthesis, resulting in cell death. VRE evade cell death via remodeling of lipid II to decrease vancomycin's binding affinity. This resistance phenotype is regulated by a two-component system, VanRS. VanS is a membrane-bound histidine kinase that senses vancomycin and, in response, phosphorylates VanR. VanR is a transcription factor that, when phosphorylated, directs transcription of several lipid II-

remodeling genes, activating resistance. However, the mechanistic and structural details behind VanS sensing and signal transduction remain unknown. Furthermore, various VRE strains potentially have different mechanisms of activation. With our focus on the two most clinically prevalent VRE strains, types A and B, we hypothesize that VanS activation is modulated by interaction with lipid II, since this intermediate accumulates in cells in response to vancomycin treatment. Here, we use in vitro methods to reconstitute VanS in nanodiscs, a natural membrane environment, and determine the role of lipid II in VanS activation. We directly compare the activities of VanS from type A (VanSA) and type B (VanSB) VRE in the presence and absence of lipid II. These studies will elucidate how VRE are able to sense vancomycin and activate resistance at the molecular level. In understanding this, we can develop new approaches (i.e., inhibitors) to specifically target and inhibit VanS activation and therefore inhibit VRE.

ABS#58

2023 Emil Thomas Kaiser

High Throughput Methods for Studying Protein Stability

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There is a need for inexpensive, accurate and high throughput methods for measuring protein stability. Differential Scanning Fluorimetry (DSF) is popular technique which reports protein thermal stability (Tma). This method relies on the selective recognition of unfolded states by fluorogenic dyes. However, DSF applications remain limited by protein incompatibilities with existing DSF dyes. We recently overcame this obstacle through the development of a protein-adaptive DSF platform (paDSF), which combines a novel dye library "Aurora" with a streamlined procedure to identify protein-dye pairs. We find that paDSF can successfully return accurate Tma values for 94% (66 of 70) of proteins, tripling the previous compatibility. Excitingly, we also find that paDSF can be used to monitor biological processes that were previously inaccessible, such as the interdomain allostery of O-GlcNAc Transferase (OGT). Here, an update on this method and its most recent uses will be discussed. Based on these observations, we suggest that paDSF is a generalizable tool to interrogate protein stability, dynamics and ligand binding.

ABS#59

Aggregates, Amyloids, or Condensates? (July 16, AM)

Small Molecule Properties Define Partitioning into Biomolecular Condensates

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Biomolecular condensates regulate cellular function by compartmentalizing molecules without a surrounding membrane. Condensate functions arise from specific exclusion or enrichment of molecules. Thus, understanding the principles governing condensate composition is critical to characterizing condensate function. While principles defining macromolecular composition have been described, understanding of small molecule composition remains limited. Here we quantified partitioning of ~1700 biologically relevant small molecules into condensates composed of different macromolecules. Partitioning varied nearly a million-fold across compounds but was strongly correlated among condensates, indicating disparate condensates are physically similar. Enriched compounds did not generally bind macromolecules with high affinity under conditions where condensates do not form, suggesting partitioning is not governed by site-specific interactions. Correspondingly, a machine learning model accurately predicts partitioning using only computed physicochemical features of the compounds, chiefly those relating to hydrophobicity. These results suggest that a hydrophobic environment emerges upon condensate formation, dictating the enrichment and exclusion of small molecules.

ABS#60

Structures of Mega-Complexes (July 13, PM)

Bacterial production of recombinant contraceptive vaccine antigen from CatSper displayed on a human papilloma virus-like particle

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CatSper is a calcium ion dependent channel present in the principal piece of sperm tail. It plays a crucial role in sperm hyperactive motility and so in fertilization. Extracellular loops of mouse sperm CatSper were used to develop a vaccine to achieve protection from pregnancy. These loops were inserted at one of the three hypervariable regions of Human Papilloma Virus (HPV) capsid protein (L1). Recombinant vaccines were expressed in E. coli as inclusion body (IB), purified, refolded and assembled into virus-like particles (VLP) in vitro, and adsorbed on alum. Four vaccine candidates were tested in Balb/C mice. All of the constructs proved immunogenic, one showed contraceptive efficacy. This recombinant contraceptive vaccine is a non-hormonal intervention and is expected to give long-acting protection from undesired pregnancies.

ABS#61

Membrane Proteins: From Natural to Designed (July 14, PM)

Cry(o)ing over Multidrug Resistance: Structural Analysis of the Anaerobic Efflux Pump MdtF

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Multidrug efflux pumps are inextricably linked to the development of antimicrobial resistant bacteria; inhibiting these pumps could therefore potentially recapitulate the activities of previously ineffective antibiotics. The membrane-spanning tripartite efflux pump MdtEF-TolC is upregulated in Escherichia coli within acidic and anaerobic conditions, akin to the mammalian gut or bacterial biofilms, where it provides a fitness advantage and confers drug resistance. Currently, no high-resolution structural information for MdtEF-TolC exists. Here, we report the cryo-EM structure of the inner membrane protein, MdtF, in both wildtype and V610F (a mutant which elicits an altered multidrug resistance phenotype) forms to a 3.35 and 3.18 Å resolution, respectively. Crucially, these structures were determined within styrene maleic acid lipid particles (SMALPs) which permits the capture of a membrane protein within its native-like lipid milieu, enabling us to resolve bound structural lipids. By combining conformational readouts from H/D exchange mass spectrometry with our structural and lipid information, essential substrate-protein and lipid-protein interactions necessary in regulating key functional conformations could be elucidated. Collectively, these studies reveal new insights into the structure-function of an anaerobic multidrug efflux pump which plays key roles in detoxification of noxious substances and antibiotic resistance within anaerobically grown Escherichia coli.

ABS#62

Poster session, July 15

The GCE4All Research Center: Promoting your ability to use Genetic Code Expansion in your research

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Genetic Code Expansion (GCE) is an extremely powerful, but highly underutilized way to site-specifically place useful chemical groups into proteins. GCE alters translation, allowing site-specific incorporation of non-canonical amino acids (ncAAs) at genetically encoded sites during protein synthesis. The GCE4ALL Research Center is funded by Oregon State University and the NIGMS Biomedical Technology Development and Dissemination program. Its mission is to improve GCE tools so that researchers around the world can easily use GCE technologies to generate designed forms of proteins for probing and visualizing how life works, and for developing diagnostics and therapeutics. Our research is organized around two Technology Development Projects focused, respectively, on optimizing GCE tools to incorporate ncAAs for (i) bioorthogonal ligations, and (ii) biochemical probes and posttranslational modifications. All developed GCE technologies are collaboratively "road-tested" in the context of authentic, challenging biomedical research problems and then made readily available to the broader research community. Recent advances include

(i) new tetrazine-containing ncAAs for rapid and quantitative labeling of proteins both in vitro and in mammalian cells, (ii) various Fluorine-containing ncAAs probes, (iii) a cell line for high efficiency site-specific incorporation of phosphoserine into E. coli produced proteins, (iv) a new "PermaPhos" system for high-efficiency incorporation of a non-hydrolyzable phosphoserine-mimic into E. coli produced proteins, and (v) a tRNA synthetase library and protocol so any lab can evolve a new tRNA-synthetase for an ncAA they want to incorporate into proteins. Our presentation will provide an overview of the GCE4All Research Center structure, feature these new developments. We look forward to speaking with anyone interested in adopting GCE technologies into their research and to share about exciting upcoming GCE training opportunities via our hosted workshops and conferences. As you have interest in using GCE in your research, please explore our resources at https://gce4all.oregonstate.edu/ or contact us at gce4all-center@oregonstate.edu.

ABS#63

Poster session, July 15

Solution-state NMR investigations of the Salmonella typhimurium tryptophan synthase 144 kDa heterotetramer

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The tryptophan synthase (TS) system is a well-studied model system for understanding protein-protein communication and allosteric regulation, due to the high level of synchronization exhibited between the alpha subunit (αTS) and beta subunit (βTS) to channel the indole intermediate. Our previous work on E.coli TS indicated the importance of conformational dynamics and allosteric networks on function, although most of these studies focused on αTS in the absence of βTS. Here, we successfully adapted a growth protocol to generate 13C-isotopically labeled S. typhimurium (St) TS. We generated TS samples in two manners – by addition of separately expressed/purified αTS and βTS subunits, and expression/purification of the entire 144 kDa TS complex. Surprisingly, there were differences in the d1-13CH3 Ile NMR spectra of these two samples. Resonances in StTS were also responsive to a variety of ligands, confirming that structural/dynamic changes in one subunit or the other are capable of propagating through the entire complex. We were also able to collect methyl triple quantum relaxation violated coherence transfer data on StTS to determine a limited set of order parameters, which report on picosecond-nanosecond timescale conformational dynamics. These studies indicate that StTS is dynamic on this timescale, consistent with past work on E. coli αTS. These results also indicate that solution-state NMR can provide insight into the full StTS complex, which complements previous work using solid-state NMR.

ABS#65

Poster session, July 13

High-throughput nanobody technologies in biomedical research

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Camelid VHH single-chain antibodies (nanobodies) have recently emerged as a compelling class of antibody fragments for biomedical research and therapeutics. However, it remains challenging to identify high-affinity and multi-epitope targeting nanobodies. Here we develop a proteomics strategy that enables in-depth identification, affinity classification, and structural characterization of serum nanobody repertoires. We benchmarked various antigens including disease targets and small motifs that are weakly immunogenetic. An unprecedented large repertoire (up to 9,000) of highly unique and high-affinity nanobody families are confidently identified for each antigen and are classified based on their physicochemical properties. Subsequent expression and biophysical verifications of hundreds of nanobodies confirm over 50% of our nanobodies bind specifically with high-affinity (pM or higher).

To understand the structural landscape of nanobody repertoires, we developed a novel AI-enabled computational pipeline that integrates structural proteomics techniques such as cross-linking/mass spectrometry and mutagenesis experiment for accurate, high-throughput epitope mapping. We modeled >100,000 specific antigen-nanobody

complexes. Our big data revealed a nanobodies target various dominant epitope classes. Even within each class, there are substantial structural diversity likely critical to prevent the antigenic drift. Affinity matured nanobodies master shape and charge complementarity and their antigen binding can mimic conserved intracellular protein-protein interactions.

To improve the translational potential and clinical applications of nanobodies, we developed "Llamanade," the first in-class software for nanobody humanization. Using sequence as input, Llamanade can rapidly extract sequence features, model structures, and optimize solutions for humanization. Llamanade has been experimentally verified and is freely available as both a standalone software and on a webserver.

Collectively, our technologies will find broad utility in basic and translational research.

ABS#70

RNA-Protein Machines: Ancient Synergies (July 14, AM)

Asymmetric Genomic RNA Egress and Virus Disassembly Driven by Capsid Genome Rearrangements by HDXMS and cryo-EM

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Icosahedral RNA viruses are macromolecular assemblies, consisting of protein capsids encapsulating RNA genomes. Capsids function to shield the viral genome until they encounter favorable host environments where they undergo programmed disassembly to release their genome for replication inside a target host cell. While it is well established that viral particles must undergo programmed disassembly to release their genome for replication, the role of the genome-capsid core in sensing environmental conditions for disassembly remains poorly understood. Our results reveal that genomic RNA tightly binds a subset of viral coat proteins to form a stable RNA-capsid core. The RNA-binding domain of the coat protein showed characteristic bimodal deuterium exchange kinetics. A small (5.7%) fraction of the R domain in the native TCV virus particle with lower exchange (∼6.2 Da) while a majority of the R domain (∼94.3%) showed a higher exchange (∼11.7 Da). HDXMS also reveals a conformational rearrangement of the RNA-capsid core in disassembly intermediates formed by plant cell-like conditions (low Ca2+ and high osmolyte). A cryo-EM structure of the expanded state intermediate particle shows that RNA is asymmetrically concentrated near a single 5-fold axis during disassembly. The two conformations reflect a differential effect of the RNA genome on the R domain. The structure of the virion determined by symmetric model reconstructed at 3.0 A resolution showed a protrusion seen at the 5-X fold axes on the virus' inner surface, and represents the asymmetric egress locus for genomic RNA. In summary, integrative HDXMS with cryo-EM has revealed the organization and dynamics of the RNA-capsid interior of icosahedral RNA viruses, highlighting the active role of genomic RNA in driving asymmetric virus disassembly.

ABS#71

Poster session, July 13

Identifying Hotspot Residues and Assessing the Impact of Binding Site Definition of Proteins Using a Pharmacophore Approach

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Many biomedical applications, such as classification of binding specificities or bioengineering, depend on the accurate definition of protein binding interfaces. Depending on the choice of method used, substantially different sets of residues can be classified as belonging to a protein's interface. A typical approach used to verify these definitions is to mutate residues and measure the impact of these changes on binding affinity. Besides the lack of exhaustive data this approach generates, it also suffers from the fundamental problem that a mutation (either on the interface or outside of it) introduces an unknown amount of alteration for the rest of the interface. In this study, first, we explore the impact of alternative binding site definitions on the ability of a protein to recognize its cognate ligand using a pharmacophore approach, which does not affect the original interface. Our results show that, on average, we can mis-define approximately 20-30% of the true biological interface and still recognize cognate ligands of the receptor with reasonable, albeit lower, confidence than the original starting interface. This observation provides guidance on the minimum expected accuracy of interface definition that is required to capture the biological function of a protein. Second, we provide a new approach to identify critical ("hot spot") residues of an interface. The approach performs a

combinatorial modulation of the pharmacophore description to assess the impact of interface residues of a cognate protein receptor in recognizing their corresponding cognate binding partner.

ABS#72

Poster session, July 13

Optimal Selection of Suitable Templates in Protein Interface Prediction

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Molecular-level classification of protein-protein interfaces can greatly assist in functional characterization and rational drug design. The most accurate protein interface predictions rely on finding homologous proteins with known interfaces since most interfaces are conserved within the same protein family. The accuracy of these template-based prediction approaches depends on the correct choice of suitable templates. Choosing the right templates in the immunoglobulin superfamily (IgSF) is very challenging because its members share low sequence identity and display a wide range of alternative binding sites despite sharing structural homology. Cell surface IgSF proteins are implicated in many diseases and constitute one of the largest protein families in the human proteome. Despite their critical biomedical importance, few IgSF protein interfaces have been thoroughly characterized. In this work, we present a new approach to predict protein interfaces. First, template specific, informative evolutionary profiles are established using a mutual information-based approach. Next, based on the similarity of residue level conservation scores derived from the evolutionary profiles, a query protein is hierarchically clustered with all available template proteins in its superfamily with known interface definitions. Once clustered, a subset of the most closely related templates is selected, and an interface prediction is made. These initial interface predictions are subsequently refined by extensive docking. This method was benchmarked on 51 IgSF proteins and can predict non-trivial interfaces of IgSF proteins with an average and median F-score of 0.64 and 0.78, respectively. We also provide a way to assess the confidence of the results. The average and median F-scores increase to 0.77 and 0.81, respectively, if 31% of low confidence cases are removed. Lastly, we provide residue level interface predictions, protein complexes, and confidence measurements for singletons in the IgSF.

ABS#73

Peptide Modalities: Size Doesn't Matter (July 14, AM)

Tuning GLP-1 and GIP "Dual Agonist" Activation Using N-Terminal Modifications

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Glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are two incretin hormones that regulate glucose homeostasis and fat cell metabolism. These effects are mediated by the binding and downstream signaling events that ensue when GLP-1 and GIP interact with their cognate receptors, GLP-1R and GIPR. We have shown that activation at these receptors is preserved when the N-termini of the native peptides are monoalkylated, a region that is crucial for receptor activation and in a region that is embedded deep into the membrane. These modifications have the additional benefit of rendering the peptides refractory to proteolysis by protease dipeptidyl peptidase-4 (DPP-4), a frontline enzyme that inactivates the ligand within minutes. While variety of N-alkyl modifications on GLP-1 is tolerated, we are now expanding the scope for GIP and exploring the effect of tertiary N-termini on the activity of both peptides. We report here (1) investigation of the limits of available space for alkyl groups in the receptor binding pocket; (2) interrogating intermolecular interactions with hitherto untested chemical groups in the binding pocket and (3) identification of molecular functionality that favors one receptor over the other. The ability of tuning receptor activation by N-terminal alkylation is especially powerful in recently emerging multi agonist therapeutics. GLP-1 and GIP dual agonists such as tirzepatide®, that can activate both GLP-1R and GIPR receptors. We report here, using tirzepatide as the template, the ability to modulate the potency at two

receptors with N-terminal modifications that favor GIP over GLP-1, thereby reducing side effects.

ABS#74

Modern Anti-viral Strategies (July 13, AM)

Whole Virion Simulations Illuminate the Vulnerabilities of Influenza Glycoproteins

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The resurgence of influenza A virus (IAV) during the ongoing COVID-19 pandemic has posed an additional threat to public health. This emphasizes the urgent need for a universal influenza vaccine that remains effective despite the relentless antigenic drift. The main targets are the two principal membrane glycoproteins, hemagglutinin (HA) and neuraminidase (NA), whose functional balance is at the basis of IAV virulence and transmissibility. Here, we have used the 'computational microscope' to provide unseen views of the glycoprotein interplay and dynamics. By integrating experimental structural data with computational approaches, we have built two massive models of the whole H1N1 IAV accounting for different strains and glycosylation profiles. Mesoscale, all-atom molecular dynamics simulations of these systems tallying ~160 million atoms revealed previously unknown insights into the conformational plasticity and interplay of influenza glycoproteins in situ, highlighting vulnerable states where cryptic or occluded epitopes on the HA and NA surface become transiently accessible to the host immune system. Specifically, we observed peculiar "breathing" and "tilting" motions of HA, which allowed us to characterize their kinetics and determine their antigenic implications for vaccine design. We also observed a previously unappreciated "head-tilting" motion of NA, resulting in the exposure of a novel conserved epitope on the underside of its head. By means of negative-stain electron microscopy experiments, we reveal how this NA's "dark side" epitope is accessed by a human monoclonal antibody that we termed NDS.1. Overall, our study provides a detailed molecular-level understanding of the functional motions and the interplay of influenza glycoproteins in a crowded environment. These findings illuminate the vulnerabilities of HA and NA to the immune system, suggesting new strategies and opportunities for the development of novel vaccines and anti-viral therapeutics.

ABS#77

Poster session, July 13

Discovery, Characterisation and Engineering of Class A3 evasins inhibiting the Inflammatory Chemokines

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Chemokines, the key regulators of leukocyte trafficking, are attractive targets for anti-inflammatory therapy. Evasins are anti-inflammatory, chemokine-binding proteins found in tick saliva, with important therapeutic potential. However, therapeutic application of evasins will require manipulation of their chemokine target selectivity. Here we describe a new family of evasins, class A3 evasins, that is unique to the tick genus Amblyomma and distinguished from "classical" class A1 evasins by an additional disulfide-bonded pair of cysteine residues near the chemokine recognition interface. The class A3 evasin EVA-AAM1001 (EVA-A) bound to CC chemokines and inhibited their receptor activation. However, unlike class A1 evasins, EVA-A did not utilise N- and C-terminal regions to differentiate chemokine targets. Instead, structures of EVA-A bound to four chemokines revealed a deep hydrophobic pocket, unique to class A3 evasins, that interacts with the residue immediately following the CC motif of the chemokine (the "CC +1" residue). The preference of EVA-A for chemokines with aliphatic CC+1 residues results from negative selection against binding of aromatic CC+1 residues into this pocket. Consequently, mutations to alleviate this negative selection yielded broad-spectrum chemokine inhibitors. This study illustrates that class A3

evasins are an excellent platform for engineering proteins with targeted chemokine binding selectivity for applications in research, diagnosis or anti-inflammatory therapy.

ABS#78

Poster session, July 13

A comparison of substrate binding characteristics between the E. coli Hsp70 DnaK and the human Hsp70s HspA1 and Hsc70

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Hsp70 chaperones are key players in maintaining a healthy cellular proteome. Their diverse array of functions, including preventing aggregation and promoting productive folding of nonnative proteins, relies on their ability to bind clients. Research elucidated the remarkable versatility in substrate binding of the Hsp70 E. coli paradigmatic chaperone DnaK. DnaK shows preferred binding to extended, hydrophobic sequences often flanked by positively charged residues. Client binding is promiscuous in that there is no consensus sequence for binding; moreover, substrates can be bound in a 'forward' (N to C) as well as a 'reverse' (C to N) orientation in the Hsp70 binding site. But binding is restricted to substrate side chains that can be optimally fitted in distinct binding pockets lining the chaperone's substrate binding cleft. While the Hsp70 chaperone family is highly conserved among prokaryotes and eukaryotes, the human Hsp70s Hsc70 and HspA1 exhibit distinct characteristics. Peptide arrays covering the full-length sequence of the E. coli alkaline phosphatase precursor proPhoA show differential binding to DnaK, Hsc70 and HspA1. Furthermore, crystal structures of the substrate binding domain of DnaK and HspA1 bound to the NR model peptide (NRLLLTG) show very similar interactions between the chaperone binding cleft and the peptide. However, the binding affinity for NR peptide, ATPase activation upon NR binding and the degree of interdomain docking are greatly diminished in the human chaperones compared to DnaK. These results evoke questions about what determines distinctive substrate binding selectivity in human Hsp70s, and how sequence and structural differences translate into functional divergence.

ABS#79

Poster session, July 14

In vitro construction of enzyme assemblies that enhance enzyme activity

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Enzymatic activity is usually evaluated in dispersed conditions to avoid irreversible aggregation. Based on the results of these studies, predictions of the intracellular functions of enzymes and technological development for industrial applications are progressing. Recently, it has been reported that enzymes can exist as dynamic assemblies in cells, but the formation mechanism of enzyme assemblies and its effect on the enzymatic activity remains elusive. In this study, we aimed to construct the enzyme assembly in vitro and to investigate the factors that affect enzymatic activity.1 L-lactate oxidase (LOX) was used as a model enzyme because it is a well-studied enzyme, and its activation is important for industrial applications such as biofuel cells. We tried to form the enzyme assemblies based on electrostatic interaction by adding a polymer with a pair of charges to the enzyme. Dynamic light scattering analysis and optical microscopy revealed that the negatively charged LOX form the enzyme assemblies with the positively charged poly-L-lysine (PLL). Michaelis-Menten plots show that the formation of enzyme assemblies results in a increases in the kcat and decrease in the Km of LOX when compared with uniformly dispersed state. The size of the enzyme assemblies varied from tens of nanometers to several micrometers depending on the salt concentration. Interestingly, it was observed that the smaller the size of the enzyme assembly, the higher the activation effect of LOX. Furthermore, far-UV CD spectroscopy revealed that the secondary structure of LOX changes under conditions of formation of the enzyme assemblies, which may contribute to its activation. These results suggest that assembly size and conformational changes are factors in LOX activation associated with enzymatic assemblies. This highlights the importance of considering enzyme assembly states in solution to understand and exploit enzyme function.

ABS#80

Poster session, July 13

Suppression of aggregation and amyloid fibril formation of human lysozyme by Luteolin under macromolecular crowding condition

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It is well established that the biological cell has a crowded milieu, significantly impacting how proteins aggregate and interact. Understanding how aggregation and fibrillation of amyloidogenic proteins are affected by macromolecular crowding is crucial for the treatment of many amyloid-related disorders1. Most in vitro studies on protein amyloid formation and its inhibition by small molecules have been carried out in dilute solutions that do not accurately reflect the intricacy of their cellular environment. Human Lysozyme (HuL) has been used as a model protein to identify compounds that can prevent protein aggregation2. In the present work, we have used PEGs to simulate crowded cell-like conditions, then examined the possible inhibitory effect of a selected polyphenol, Luteolin, on human lysozyme aggregation at pH-2. It is a naturally occurring flavone earlier used as an effective inhibitor of amyloid formation for some proteins3. The findings indicate that while Luteolin inhibits HuL aggregation and fibrillation in dilute solution to some extent, its complete inhibition is observed in the presence of PEGs as investigated by ThT, light scattering, and TEM studies. The species formed in the combination of PEG8000 and Luteolin are less hydrophobic, less toxic, and have α-helix-rich structures compared with control samples, which are hydrophobic and contain β-sheet as shown by ANS hydrophobicity assay, MTT assay, and CD spectroscopy, respectively. Fluorescence titration studies indicate a binding constant of 1.9 x 106 M-1 for the interaction of Luteolin with HuL in dilute conditions and 10 fold increase in presence of 5%peg8000. This is suggestive of strong interaction with HuL modulated by PEGs. The results emphasize the importance of macromolecular crowding conditions in protein aggregation and amyloid inhibition studies. Studies using other amyloidogenic proteins would be required to understand the role of macromolecular crowding in small molecule mediated inhibitory effect on protein aggregation and amyloid formation.

ABS#81

Poster session, July 14

Convergent Mechanisms of Nanobody-Mediated Neutralization of the CFTR Inhibitory Factor Cif

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Cif is a dimeric Pseudomonas aeruginosa epoxide hydrolase that inhibits airway clearance and facilitates more damaging airway infections in Cystic Fibrosis (CF) patients [1,2]. By diverting the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) to degradation pathways and destroying a pro-resolving signaling intermediate, Cif exacerbates CF lung pathophysiology while threatening to attenuate the benefit of therapies that restore CFTR-mediated airway homeostasis [2-4]. We are therefore pursuing strategies for the rapid detection and effective inhibition of Cif in therapeutic contexts. Previously, we developed a panel of high-affinity anti-Cif nanobodies and used them to establish an ELISA displacement assay to assess inhibitor potency [5]. Here, we present a mechanistic blueprint for this assay and investigate the structural relationships governing nanobody-mediated neutralization of Cif. We accomplished this by co-crystallizing Cif with a diverse subset of nanobodies and using X-ray crystallography to determine the structures of six different Cif:nanobody complexes as well as a tripartite structure of Cif simultaneously bound by two different nanobodies. A subset of Cif:nanobody structures share a preferred mechanism of neutralization where a bulky hydrophobic sidechain donated by complementarity determining region (CDR) 3 of the nanobody inserts into the Cif active site. Unexpectedly, this phenomenon is recapitulated by two nanobodies using CDR2 instead of CDR3 demonstrating convergent mechanisms of Cif neutralization. Following superposition by least-squares fitting of Cif co-crystallized with inhibitors or nanobodies, we conclude that the binding of inhibitors and nanobodies to Cif creates steric overlap, thereby validating the mechanistic basis for the ELISA displacement assay. Lastly, the tripartite structure highlights a pair of nanobodies that recognize non-overlapping Cif epitopes. This enabled the development of a cost effective and sensitive assay to

quantitate native Cif in clinical samples, and permits exploration of intra- and inter-molecular conformational regulation of Cif activity.

ABS#86

Poster session, July 15

Folding of Prestin's Anion-Binding Site and the Mechanism of Outer Hair Cell Electromotility

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Prestin responds to transmembrane voltage fluctuations by changing its cross-sectional area, a process underlying the electromotility of outer hair cells and cochlear amplification. Prestin's voltage-dependent conformational rearrangements partially depend on the binding of Cl- to an electrostatic gap between the TM3 and TM10 helices. Using hydrogen-deuterium exchange mass spectrometry, we find that prestin displays an unstable anion-binding site, where folding of the amino termini of TM3 and TM10 is coupled to Cl- binding. This event shortens the TM3-TM10 electrostatic gap, thereby reducing prestin's cross-sectional area. These folding events upon anion-binding are absent in SLC26A9, an anion transporter closely related to prestin. We observe helix fraying of prestin's anion-binding site but cooperative unfolding of multiple peripheral helices, with implications to prestin's fast electromechanical rearrangements. These results highlight a novel role of the folding equilibrium of the anion-binding site in defining prestin's unique voltage-sensing mechanism and electromotility.

ABS#87

Protein Folding and Function in Context (July 13, AM)

Understanding efficient endosomal escape: A structural and biochemical approach

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Although next generation protein-based therapeutics and biologics remain the fastest growing segment of modern biopharma, their therapeutic potential is limited by their inability to efficiently access intracellular targets. This limitation arises from the large size and polarity of most proteins, rendering them unable to passively diffuse across the plasma membrane. Rather, proteins are taken up into the endocytic pathway where they are inevitably degraded by lysosomes.
Our lab designed ZF5.3, a single domain C2H2 zinc finger that harbors a unique c-terminal penta-arginine motif. Using a mechanism that is only partially understood, ZF5.3 escapes the endocytic pathway to reach the cytosol of cells with greater than 50% efficiency across multiple cell lines. [1-3] Previous work demonstrated that escape occurs in a HOPS-dependent manner from the membranes and lumen of late endosomes and lysosomes that are characterized by a low pH. Work presented here demonstrates that while ZF5.3 is folded and thermostable at pH 7.5, it undergoes a cooperative change in secondary structure between pH 4 and 5 as judged by circular dichroism. More detailed investigation using NMR revealed that this conformational change is associated with the protonation of a single Zn(II)-binding His side chain whose pKa corresponds almost exactly with the pH of late endosomes/endolysosomes.[3] We hypothesize that ZF5.3 protonation during endosomal maturation catalyzed by the HOPS complex reveals a nascent amphipathic helix in the first step of efficient endosomal escape. [4] Using high resolution NMR experiments, we have solved the pH-dependent structures of ZF5.3 and aim to further define their dynamics within endosomal compartments as they give rise to escape. With these structures in hand, we hope to better define the properties that give rise to the efficient endosomal escape of ZF5.3 and further serve to inform the improved design of protein-based theraputics, capable of reaching their intracellular targets.

ABS#88

Poster session, July 14

Biophysical studies of a novel protein interaction partner of human ribonucleotide reductase

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Genomic instability is a common trait in many human cancers and can be driven by imbalances in deoxyribonucleotide triphosphate (dNTP) pools. A major regulator of the dNTP pools in humans is ribonucleotide reductase (RNR), the sole enzyme capable of de novo generation of deoxyribonucleotides from ribonucleotide substrates via a radical mechanism. Eukaryotic RNRs are composed of two subunits: the catalytic a subunit and the radical-generating b subunit. The two subunits must physically interact to form an a2b2 holocomplex competent for radical transfer to initiate catalysis. Enzymatic activity is controlled by the binding of ATP or dATP to an N-terminal region in the a subunit termed the cone domain. The binding of these effectors results in the formation of morphologically identical, but differentially stable a6 hexamers, where the b subunit only breaks apart a6-ATP, and not a6-dATP. Non-nucleotide effectors of activity, namely the 60 kDa IP3R binding protein released by IP3 (IRBIT), have been identified in metazoans [1]. Previous studies have found that IRBIT binds to human RNR, but specifically in the presence of dATP, enhancing the inhibition induced by dATP [1]. Additionally, the interaction strength appears to be modulated by phosphorylation of IRBIT [1]. To understand the molecular details of this interaction, we use protein expressed in insect cells to carry out mass photometry (MP) and electron microscopy (EM) studies. We find that IRBIT alone is a mixture of dimers and tetramers. We see robust a6-dATP formation using mass photometry, and addition of IRBIT results in the formation of a large species of a molecular weight above 1 MDa. Negative stain EM shows the formation of large particles that are distinct from the a6-dATP particles. Understanding the IRBIT:HsRNR complex would open doors to non-nucleotide regulation of RNR activity and provide a new target for anticancer drugs.

ABS#90

Poster session, July 13

Cryo-EM Structural Analysis and Tool Design of Pump-like Channelrhdopsin ChRmine

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ChRmine, a new type of channelrhodopsin recently discovered, has broadened the application of optogenetics with its large channel conductance, red-shifted action spectrum, and high light sensitivity. ChRmine and its homologs work as potent light-gated ion channels, but they are phylogenetically more similar to archaeal trimeric pump-type rhodopsins (e.g. Bacteriorhodopsin) than to canonical dimeric channelrhodopsins (e.g. ChR2), and the molecular mechanisms for the passive ion conduction by ChRmine was unknown. It was a big question of why ChRmine can function as an ion channel, despite having a similar amino acid sequence to pump-type rhodopsins.

Here, I determined the cryo-electron microscopy (cryo-EM) structure of ChRmine at 2.0 Å resolution[1] (figure1A). The structure reveals unusual architectural features never seen before in channelrhodopsins including the pump-like trimeric assembly, a short transmembrane helix (TM) 3 unwound in the middle of the membrane, a twisting extracellular loop-1 (ECL1), and an outward tilted TM2 (figure1B). These features create the large intracellular and extracellular cavities within the monomer and thereby enabling ChRmine to function as a potent ion channel (figure1C).

Next, by leveraging the structural information, I have engineered two new variants of ChRmine with 1) further red-shifted action spectrum and greatly reduced blue shoulder (named rsChRmine for red-shifted ChRmine) and 2) accelerated on/off channel kinetics (named hsChRmine for high-speed ChRmine). Furthermore, I combined rsChRmine with two different Ca2+ indicators (GCaMP6 and XCaMP-B) and successfully performed a “three-color optogenetics experiment” in which the activities of multiple neuronal populations were optically modulated and monitored simultaneously in living mice using three colors of light (blue, cyan, and red) (figure2).

ABS#93

Poster session, July 15

Understanding the Conductance Behaviour of Engineered Peptide-Assembled Ion Channels from Simulations of Their Docking-Predicted Structures

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We used peptide-peptide docking and molecular dynamics simulations to study the conductive behaviour of engineered cWza ion channels. In single-channel current recordings, these channels show interconversion between low- and high-conductance states, with a collapse to a single high-conductance state upon mutation of Tyrosines with Cysteines without a clear explanation (Figure 1). Our results suggest that computationally predicted structures can distinguish discrete pore conformations into separate conductance states, with their dynamic simulations revealing stable and unstable conductance behaviours. However, not all models were stable, and many models exhibit hyper-conductance. The sampling of trajectories visiting low-conductance states is extremely limited, and further studies are needed to improve the accuracy and stability of docking-predicted structures. Nevertheless, our results show that some residue pairs are particularly important for regulating conductance, such as the salt bridge between Arginine (28-R) and Glutamate acid (25-E), whose disruption by Tyrosine seems a common theme in those channels known to display conductance interconversion (Figure 5). Overall, our study sheds light on the complexity of ion channel conductance and highlights the potential of computational methods in advancing our understanding of these mechanisms in these and maybe other similar transmembrane synthetic ion channels.

ABS#95

Poster session, July 13

The Complex Interaction of the Regulator of G-Protein Signaling 10 (RGS10) and Calmodulin

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G-Protein Coupled Receptors (GPCRs) are of high therapeutic importance, being the target of approximately 35% of all drugs currently in clinical use. Agonist binding to a GPCR activates G-protein signaling by promoting exchange of GDP for GTP on the G-alpha subunit of the heterotrimeric G-protein and subsequent dissociation of G-alpha from the beta-gamma dimer. Signaling is terminated when G-alpha-bound GTP is hydrolyzed to GDP, returning the system to the resting state. RGS (Regulators of G-protein Signaling) proteins are GTPase accelerating proteins (GAP) that stimulate the intrinsic GTP hydrolysis catalytic activity of G-alpha, therefore regulating G-protein signaling. Like GPCRs, proteins that regulate G-protein signaling, including RGS proteins, are considered solid drug targets. Interestingly, the ubiquitous calcium signaling protein calmodulin (CaM) binds to some RGS proteins, suggesting CaM regulates RGS function. However, the nature and consequences of this interaction remain poorly understood. We are focusing on the interaction of CaM with RGS10. RGS10 is the most abundant RGS protein in microglia, playing key roles in neuroinflammation and neurodegeneration. We are studying the affinity and structure of the RGS10-CaM complex. Using intrinsic tryptophan fluorescence measurements, we found that RGS10 binds to CaM with a low micromolar affinity which is Ca2+ and ionic strength dependent. Using NMR spectroscopy, we confirmed this interaction and identified the residues of RGS10 that interact with CaM, based on chemical shift changes. Phe-for-Trp mutants confirmed the NMR results indicating the Trp residues are not essential for CaM binding and established the contributions of the Trp residues to the intrinsic RGS10 fluorescence. Using the individual N- and C-terminal domains of CaM, we determined that RGS10 binds preferentially to the C-terminal domain. Our results are important for an overall understanding of RGS function and regulation of G-protein signaling and suggest a role for CaM in this regulation.

ABS#96

Poster session, July 13

Multiple Conformations of Helix A From the N-Terminal Domain of Cre Connect Those in Monomer/Dimers and in Intasomes

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Cre recombinase is a bacteriophage P1 protein that binds specifically to loxP sites, promoting circularization of the viral genome and ensuring the equitable assortment of viral plasmids during cell division. Cre comprises two domains joined by a flexible linker and it forms a tetramer (intasome) of intercalating active and inactive conformations that cut and rejoin specific sites in loxP[1]. Structural information for Cre is available for intasomes and for a monomer (PDBIDs: 7RHY[2]) and a dimer (PDBIDs: 7RHZ [2]) bound to DNA. Information regarding Cre in solution is missing and there is little information about the isolated domains. The N-terminal domain displays two different conformations when bound as a monomer/dimer compared to the intasome, characterized by the displacement of helix A. As this helix engages in quaternary contacts in the intasome, this switch could be relevant to intasome formation. We characterized the conformational landscape of the isolated N-terminal domain with all-atom molecular dynamics (MD) simulations. The starting structures are an active and an inactive conformation from an intasome (PDBID: 1Q3U[3], chains F and A, respectively), 7RHY, and 7RHZ (chains A and B); for each system we ran four replicas of five microsecond-long MD simulations using Charmm36m[4], TIP3P water, in GROMACS[5]. Helix A samples different states located in a wide basin with small barriers between them, from a monomer-like to an intasome-like conformation and beyond, in the microsecond timescale. The most stable conformation corresponds to that in intasomes. Helix A also unfolds and refolds. Not all states are available for all the runs, pointing to gatekeeping residues locking the helix. Calculated NMR chemical shifts for each conformer suggest specific residues that would have two peaks in 13C-proton HSQC experiments.

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ABS#97

Poster session, July 15

Mechanisms of Voltage Sensing Under an Applied Electric Field

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Voltage-dependent ion channels regulate the opening of their pores by sensing the membrane voltage. This process underlies the propagation of action potentials and other forms of electrical activity in cells. The voltage dependence of these channels is governed by the transmembrane displacement of the positive charged S4 helix within their voltage-sensor domains. The movement of S4 at hyperpolarizing membrane voltages in some channels is thought to directly clamp the pore shut through the S4-S5 linker helix. We use cryo-electron microscopy to visualize this movement in two voltage-dependent potassium (Kv) channels in lipid membrane vesicles with a voltage difference across the membrane.

In the nondomain-swapped channel Eag1 (Kv10.1), multiple structural configurations show that the applied electric field displaces S4 toward the cytoplasm by two helical turns, resulting in an extended interfacial helix near the inner membrane leaflet. The position of S4 in this down conformation is sterically incompatible with an open pore, thus explaining how movement of the voltage sensor at hyperpolarizing membrane voltages locks the pore shut in this kind of channel.

In the domain-swapped channel KCNQ1 (Kv7.1), which is regulated not only by the membrane voltage but also by the signaling lipid PIP2, hyperpolarizing voltages displace S4 to an interfacial position in such a manner as to sterically occlude the PIP2-binding site. Thus, in KCNQ1 the voltage sensors' influence on the channel's gate is indirect by acting primarily as a regulator of PIP2 binding.

These results illustrate how similar voltage sensor movements in Eag1 and KCNQ1 can confer voltage sensitivity through two very different mechanisms, both of which differ from the canonical model for voltage-dependent ion channels.

ABS#98

Proteins in Motion (July 15, AM)

Elevator mechanism dynamics in a sodium-coupled dicarboxylate transporter

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Elevator Mechanism Dynamics in a Sodium-coupled Dicarboxylate Transporter

VcINDY, the sodium-dependent dicarboxylate transporter from Vibrio cholerae, is responsible for C4- and C5-carboxylate uptake into cells. The molecular mechanism of how VcINDY physically moves substrates across the membrane, and does so in an energetically efficient manner, is unclear. Here, we use single-molecule fluorescence resonance energy transfer experiments to directly observe the individual mechanistic steps that VcINDY takes to translocate substrates across a lipid bilayer, and then test key predictions of transport cycle mechanistic models. Our data provide the first direct evidence that VcINDY undergoes stochastic, elevator-type conformational motions that enable substrate translocation. Kinetic analysis suggests that the two protomers of the VcINDY homodimer undergo those motions in a non-cooperative manner, and thus catalyze two independent transport reactions. The relative substrate independence of those motions supports the notion that the VcINDY transport cycle maintains strict co-substrate coupling using a cooperative binding mechanism. Finally, thermodynamic modeling provides insight into how such a cooperative binding mechanism provides a generalized approach to optimizing transport for many secondary active transporters.

ABS#99

Capturing Protein Interactions (July 14, PM)

Structural Basis of Signal Transduction Within Environmental Sensing PAS Regulated Ser/Thr Kinases

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Protein kinases control many cell signaling pathways as part of multidomain proteins, with associated domains regulating kinase activity. This study focuses on the PAS (Per-ARNT-Sim) sensory domains and their regulation of STKs (Ser/Thr Kinases). PAS domains exert regulatory control on the effector domains by binding ligands/cofactors internally (e.g., O2 binding to heme in FixL; flavins within the Light-Oxygen-Voltage (LOV)-type PAS domains). However, the signaling mechanism from the sensory to effector domain, and PPIs (Protein-Protein Interactions) within full-length proteins or complexes, remain elusive. To address these, we investigate the PAS-STK of hPASK, an important regulator of cellular metabolic pathways, and the LOV-STK of AsPhot1, a key player in plant physiology in response to blue light. We use complementary biochemical and biophysical tools, including NMR, HDX-MS, and cryo-EM, to study the signal transduction from PAS/LOV to the STK domain.

Our high-pressure NMR and HDX-MS data and biochemical assays with artificial ligands suggest a potential ligand binding site and identify crucial Protein-Protein Interactions (PPIs) within hPASK. With AsPhot1, our data reveals substantial conformation changes in the dark versus light state upon photoactivation, highlighting key regions that may be necessary for LOV-regulated STK functions. We also observe that kinase activation destabilizes the linker region between LOV-STK, which interacts with the n-lobe of the kinase near the ATP binding site. We speculate that the rearrangement of these linkers could be critical for STK substrate association and downstream functions.

Moreover, using a low-resolution cryo-EM map with computational resources we built a model for the LOV-STK structure which uncovers critical PPI sites within AsPhot1 and provides additional insights into understanding how protein kinases interact with their associated domain via conserved spine residues and subdomains.

Our findings will aid in the development of new optogenetic tools and therapeutics for metabolic diseases (e.g., diabetes and obesity) related to mammalian PAS-regulated kinases.

ABS#100

Protein Evolution: Lessons from the Past (July 13, PM)

Ligand-induced shifts in conformational ensembles that describe transcriptional activation

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Nuclear receptors function as ligand-regulated transcription factors whose ability to regulate diverse physiological processes is closely linked with conformational changes induced upon ligand binding. Understanding how conformational populations of nuclear receptors are shifted by various ligands could illuminate strategies for the design of synthetic modulators to regulate specific transcriptional programs. Here, we investigate ligand-induced conformational changes using a reconstructed, ancestral nuclear receptor. By making substitutions at a key position, we engineer receptor variants with altered ligand specificities. We combine cellular and biophysical experiments to characterize transcriptional activity, as well as elucidate mechanisms underlying altered transcription in receptor variants. We then use atomistic molecular dynamics (MD) simulations with enhanced sampling to generate ensembles of wildtype and engineered receptors in combination with multiple ligands, followed by conformational analysis and correlation of MD-based predictions with functional ligand profiles. We determine that conformational ensembles accurately describe ligand responses based on observed population shifts. These studies provide a platform which will allow structural characterization of physiologically-relevant conformational ensembles, as well as provide the ability to design and predict transcriptional responses in novel ligands.

ABS#102

Poster session, July 15

The importance of proteasome grip depends on substrate stability

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The 26S proteasome is responsible for the unfolding and degradation of intracellular proteins in eukaryotes. A hexameric ring of ATPases (Rpt1-Rpt6) grabs onto substrates and unfolds them by pulling them through a central pore and translocating them into the 20S degradation chamber. A set of pore loops containing a so-called aromatic paddle motif is believed to be important for the proteasome's ability to unfold and translocate substrates. Based on structural and mechanistic experiments, paddles from adjacent Rpt subunits, which are arrayed in a spiral staircase conformation, grip and pull on the substrate in a hand-over-hand type mechanism, disengaging at the bottom of the staircase and re-engaging at the top. We examined the roles of the aromatic paddles by mutating them, singly or in combination, and determining the effects on substrates with differing stabilities, using degradation assays where we can measure unfolding rates. For an easy-to-unfold substrate (a circular permutant of GFP), mutations had little effect on degradation rates. For a substrate with moderate stability (enhanced GFP), there were modest effects of individual mutations on GFP unfolding rates, and alternating aromatic paddle mutants had a much larger detrimental effect on unfolding than sequential mutants. For a more stable substrate (superfolder GFP), unfolding is overall much slower, and multiple simultaneous mutations essentially prevent unfolding. Our results highlight the context-dependent need for grip during unfolding, support the hand-over-hand model for substrate unfolding and translocation, and suggest that for hard to unfold substrates, it is important to have simultaneous strong contacts to the substrate for unfolding to occur. Our results also suggest a kinetic proofreading model, where substrates that cannot be easily unfolded are instead clipped, removing the initiation region and preventing futile unfolding attempts.

ABS#103

Poster session, July 13

Resolving chaperone action on ribosome-bound nascent chains with single-molecule spectroscopy

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Proteins begin to fold during their synthesis by the ribosome. Stretches of unfolded polypeptides that accumulate co-translationally are prone to misfolding and aggregation before the domain can fold into its native structure. Molecular chaperones stabilize unfolded proteins, preventing premature folding into non-native states. They also rescue proteins from misfolded structures and protect folded domains against destabilizing interactions. However, the molecular mechanisms of how trigger factor interacts with nascent chains on the ribosome are not well understood. Trigger factor is the first molecular chaperone encountered by nascent proteins emerging from the ribosome. We have developed a single-molecule approach for directly visualizing the interaction of trigger factor with nascent chains. With nascent chains of the E. coli elongation factor

G protein, we find that trigger factor dynamically engages with unfolded polypeptides on the timescale of seconds. Binding changes with nascent chain length and is dependent on trigger factor recruitment to the ribosome exit tunnel. Our results suggest how interactions of the trigger factor chaperone with its nascent chain clients is tuned to achieve productive folding. Our experimental system sets the stage for exploring molecular mechanisms of chaperone action on nascent proteins.

ABS#105

Engineering Protein Fate and Function (July 16 AM)

A DNA-triggered protein biosensor for point-of-care diagnostics

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The fields of DNA engineering and protein engineering have evolved largely independently. DNA-based devices are relatively straightforward to design by virtue of their predictable folding, but they lack biological activity. Conversely, protein-based devices offer a myriad of biological functions but are much more difficult to design due to their complex folding. We bridge the disciplines of DNA and protein engineering to generate a protein switch that is activated by a specific DNA sequence, by means of the alternate frame folding (AFF) mechanism. A single protein switch, engineered from nanoluciferase and herein called nLuc-AFF, is paired with different DNA technologies to create a biosensor for a DNA or RNA sequence of choice, sensors for serotonin and ATP, and a computational device that processes two DNA inputs. nLuc-AFF is a genetically-encoded, ratiometric, blue/green-luminescent biosensor whose output can be quantified by cell phone camera. The biosensor is not falsely activated by decoy DNA sequences, and it retains full ratiometric readout in 100% serum. The AFF design approach can be applied to other proteins and enzymes to convert them into DNA-activated switches.

ABS#108

Poster session, July 15

Uncovering Molecular Features that Contribute to Tau Spread and Aggregation

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Several neurodegenerative diseases, such as Alzheimer's disease, are characterized by the spread and aggregation of the protein tau. Recently, we identified a cellular receptor, Low-density lipoprotein Receptor-related Protein 1 (LRP1), that regulates the tau spread pathway (Rauch et al., 2020). Knockdown of LRP1 prevents tau spread in human induced pluripotent stem cell-derived neurons and the mouse brain, suggesting that the tau-LRP1 interaction could be a viable drug target for disease intervention. Unfortunately, a detailed understanding of the tau-LRP1 molecular complex is still lacking. Our lab is focused on deciphering the tau-LRP1 structural interface and discerning how tau aggregate structure can influence LRP1 processing. Further, we have taken a detailed look at how distinct post-translational modifications (PTMs) to tau's structure can influence tau uptake and spread. We have developed protocols to express and purify tau and LRP1 proteins, and we are mapping the tau-LRP1 interface using covalent-labeling mass spectrometry. We have established cellular platforms to model tau propagation and have shown that this process can be influenced by tau aggregate structure and tau PTMs. We are specifically focused on understanding how modifications like phosphorylation and acetylation can impact the tau-LRP1 interaction and influence tau aggregation in cells. We propose that our work will provide the foundational groundwork for the development and evaluation of potential AD therapeutics.

ABS#109

Poster session, July 14

Generation of Site-specific Antibodies to a Membrane Protein by Using a Scaffold Protein

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Monoclonal antibodies (mAbs) as therapeutics represent the fastest growing class of drugs on the market[1]. The high level of specificity and affinity to the target molecule

achieves a high level of efficacy and fewer adverse events, and their ability to target diverse molecules and the modes of action of the mAbs allow them to be applied to a wide range of therapeutic targets[2]. Multi-spanning membrane proteins, which are embedded in the cell surface lipid bilayer, represent a large potential target class for therapeutic drug discovery[3]. There are, however, relatively limited numbers of marketed biologics targeting multi-spanning membrane proteins. This reflects the technical challenges involved in isolating mAb against these proteins. The problems arise due to the difficulties in purifying and stabilizing multi-spanning membrane proteins. Furthermore, the extracellular region is typically small and accessibility of epitopes to antibodies is restricted. Therefore, suitable antigen formats for immunization and screening should be prepared. Here, we designed a new antigen format using a scaffold protein, and generated antibodies to the extracellular region of a membrane protein Glut1 using the designed antigen for immunization and screening.

In the antigen design, the extracellular helical region of Glut1 as an epitope was grafted into one of the loops of a scaffold protein Adhiron (Fig.). First, the possible structure of the designed antigen was verified by circular dichroism (CD) measurements and molecular dynamics (MD) calculations. Next, we immunized an alpaca with the designed antigens and obtained VHH antibodies by phage display method. The binding of these VHH antibodies to a recombinant Glut1 protein was evaluated by surface plasmon resonance (SPR), and their binding to Glut1 on cells was further evaluated by flow cytometry (FCM). We succeeded in generating antibodies to the extracellular region of Glut1 by using a new antigen format we designed using a scaffold protein.

ABS#112

Poster session, July 13

Evolutionary paths that link orthogonal pairs of binding proteins

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Some binding pairs exhibit extreme specificities that functionally insulate them from homologs. Such binding pairs evolve mostly by accumulating single-point mutations, and mutants are selected if their affinity exceeds the threshold required for function. Thus, homologous and high-specificity binding pairs bring to light an evolutionary conundrum: how does a new specificity evolve while maintaining the required affinity in each intermediate? Until now, a fully functional single-mutation path that connects two orthogonal pairs has only been described where the pairs were mutationally close enabling experimental enumeration of all intermediates. We present an atomistic and graph-theoretical framework for discovering low molecular strain single-mutation paths that connect two extant pairs and apply it to two orthogonal bacterial colicin endonuclease-immunity pairs separated by 17 interface mutations. We were not able to find a strain-free and functional path in the sequence space defined by the two extant pairs. By including mutations that bridge amino acids that cannot be exchanged through single-nucleotide mutations, we found a strain-free 19-mutation trajectory that is completely functional in vivo. Despite the long mutational trajectory, the specificity switch is remarkably abrupt, resulting from only one radical mutation on each partner. Each of the critical specificity-switch mutations increases fitness, demonstrating that functional divergence could be driven by positive Darwinian selection. These results reveal how even radical functional changes in an epistatic fitness landscape may evolve.

ABS#113

Poster session, July 14

Rhodoquinone Biosynthesis enzyme A (RquA): A Methyltransferase Homologue Catalyzing Amino Transfer

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Rhodoquinone is a lipophilic electron-transport cofactor that is essential for the bioenergetics of a variety of bacteria and selected eukaryotic organisms that survive in low-oxygen environments. It allows the electron transport chain to function with fumarate as a final electron acceptor instead of oxygen. Rhodoquinone biosynthesis enzyme A (RquA), homologous to S-adenosyl-

L-methionine (SAM)-dependent methyltransferases, has been shown to be required for rhodoquinone biosynthesis in bacteria [1]. Although ubiquinone, which is structurally similar to rhodoquinone, is a known substrate for rhodoquinone biosynthesis, the mechanism of ubiquinone to rhodoquinone conversion was not known [2]. In this study we characterized the in vitro mechanism of rhodoquinone biosynthesis [3]. RquA from Rhodospirillum rubrum was recombinantly overexpressed in Escherichia coli and purified by affinity and size-exclusion chromatography. An in vitro functional assay was developed where RquA activity was measured by mass spectrometry or high-performance liquid chromatography. We observed that RquA only requires SAM and Mn2+ for the conversion of ubiquinone to rhodoquinone. Using 15N-SAM in the assay produced rhodoquinone with a 15N-amino group, confirming SAM as the amino donor. Unlike known aminotransferases, RquA does not use pyridoxal 5'-phosphate (PLP) as a coenzyme to transfer amino group from SAM. Furthermore, isotopically labeled ubiquinone and SAM were used in the functional assay which was monitored by nuclear magnetic resonance spectroscopy to identify methanol, methylthioadenosine, bicarbonate, and an aldehyde hydrate as the additional products of RquA reaction. Future studies aim to characterize the structure of RquA bound with substrates and identify the catalytic steps required to produce rhodoquinone. Overall, these findings provide initial characterization of RquA enzyme and reveal RquA as an example of a non-canonical SAM-dependent enzyme that does not catalyze methyl transfer, but instead catalyzes amino transfer.

ABS#114

Poster session, July 14

Exploring Inhibition within Protein-Membrane Interfaces Using Membrane-Mimicking Reverse Micelles

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Membrane proteins not only are fundamental to cellular function, but they act as the gateway to cells. One class of membrane proteins, peripheral membrane protein (PMPs), reside in aqueous compartments of the cell until they are recruited to the membrane to perform their function. Despite their importance, nearly all target-based inhibitor screens are conducted against PMPs in their water-soluble state. Major limitations for PMP inhibitor discovery originates from technical challenges associated with inhibitor screening against the membrane-embedded state. While fragment-based inhibitor design has arisen as a powerful technology, the application to the functional, membrane-bound PMPs has yet to emerge. Commonly used membrane models (micelles, bicelles, nanodiscs, liposomes, etc.) are either too large for biophysical fragment screening methods or are incompatible with fragments. To circumvent this issue and screen PMPs in their most functionally relevant state, we have developed a novel screening method that leverages membrane-mimicking reverse micelles (mmRM) and allows identification of small molecule fragments with the ability to bind to PMPs while membrane bound. A commercial fragment library was utilized to test our new method against glutathione peroxidase 4 (GPx4), a lipid hydroperoxidase implicated in the prevention and induction of ferroptosis. This screening method allowed for the successful identification of at least four confirmed hits for the protein at its membrane-bound residues. Kd values were extracted using NMR titrations and revealed high-quality fragment binding. Proton NOSEY experiments uncovered interesting and varying partitioning properties of the fragments that bind within the protein-membrane interface. Moving forward, fragment hits can be used as building blocks of inhibitors or activators of GPx4. Additionally, this method can be applied to fragment screening of other membrane interacting proteins to help overcome barriers surrounding screening of membrane proteins.

ABS#115

Poster session, July 14

Revealing the Mechanism of Action of Lipid Chaperone Proteins Using Reverse Micelle Confinement

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Revealing the Mechanism of Action of Lipid Chaperone Proteins Using Reverse Micelle Confinement.
Fatty acid localization plays an important role in cellular processes such as: signaling, membrane synthesis, gene transcription regulation, lipid storage and lipid trafficking. However, due to the poor solubility and hydrophobic

nature of lipids. Cells use specialized lipid chaperones such as Fatty Acid-Binding Proteins (FABPs) to deliver these molecules to their target locations.

One example is FABP4 which has become an attractive therapeutic target due to its implications in diseases such as diabetes, obesity, atherosclerosis, and insulin resistance. Studying the structure and function of FABP4 is key to elucidating and understanding lipid trafficking within the cell as well as treating related diseases. Despite intense interest, the precise mechanism of lipid transfer between protein and membrane remains unclear. A major barrier is that the protein-membrane interaction central to lipid transfer is weak and fleeting, making direct, structural studies impossible. Our group has designed a novel method to confine this type of protein to its membrane-bound state using membrane mimicking reverse micelles (mmRMs) in conjunction with NMR spectroscopy to elucidate and understand this process. Preliminary structural data are promising and suggest that the mmRM based approach will enable structural characterization of FABP4 bound to a membrane. Thanks to these powerful tools we are one step closer to answering questions like: What is the mechanism of action that FABP4 uses to transport fatty acid? Which are the key residues involved in the conformational change of FABP4 upon lipid binding? How can we design effective inhibitors for this type of protein?

ABS#117

Poster session, July 13

Uncovering the Molecular Mechanisms of Tau's Degradation in Microglia

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The microtubule associated protein Tau is an intrinsically disordered protein that is known to aggregate in multiple neurodegenerative diseases1. Aggregates of Tau spread from cell to cell in a prion-like manner with a mechanism that is not fully understood2,3. Tau is primarily expressed by neurons within the central nervous system (CNS), but Tau pathology has also been observed in glial cell populations such as microglia4. Recent studies have suggested microglia can actively contribute to Tau spread5,6, however the molecular mechanism driving this observation is unknown. In this study, we have developed a quantitative platform that allows us to monitor Tau uptake and endosomal trafficking within microglia. Our platform utilizes a split-luciferase system that can accurately quantify Tau levels over five orders of magnitude. We have used our system to measure the degradation kinetics of monomeric vs. fibrillar Tau in a variety of cell types, including human iPSC-derived microglia. We have measured how cell stressors and post-translational modifications impact Tau degradation. Additionally, we have quantified the rate of Tau endolysosomal escape and have begun to dissect the molecular pathways that contribute to microglial Tau processing. Our results shed light on potential mechanisms that may underlie disease phenotype in patients and ultimately could be leveraged to develop novel therapeutic strategies.

ABS#119

RNA-Protein Machines: Ancient Synergies (July 14, AM)

Context-dependent RNA localization and organization in nuclear speckles

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Eukaryotic cells are highly compartmentalized. In addition to membrane-bound organelles, multivalent interactions among many RNA and protein species often drive the formation of membraneless condensates or granules. Dynamic RNA localization to these phase-separated granules profoundly impacts gene expression and other vital cellular activities, provides a key mechanism for stress response and adaptation, and has tight connections to human diseases. Nuclear speckles represent one type of such membraneless granules in the nucleus of higher eukaryotes, enriched in snRNP species, splicing factors, polyadenylated RNAs and certain long noncoding RNAs. Nuclear speckles are implied to play roles in several mRNA processing steps, including transcription enhancement, splicing quality control and RNA export. However, direct evidence for involvement of nuclear speckles in mRNA metabolism remains a frontier challenge due to its compositional complexity. Using fluorescence microscopy and super-resolution imaging, our recent results demonstrate that the RNA substrates exhibit sequence context-dependent localization to nuclear speckles. In addition, the RNAs also show a preferential organization within nuclear speckle. Transcriptomic analysis further suggest that nuclear speckles might play a role in kinetic coordination of splicing.

ABS#120

Poster session, July 13

Near-attack Conformations of the Active-site Residues Modulate the Catalytic Function of Spl DnaX Intein Enzyme

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Intein enzymes catalyze the splicing of their flanking polypeptide chains and have found tremendous biotechnological applications. More than 600 intein genes have been reported, but only a few have been thoroughly characterized. The goal is to understand the structural and dynamic basis of their catalysis to engineer intein enzymes with novel applications.

The structure of a 136-residue DnaX intein enzyme, derived from Spirulina platensis (abbreviated as Spl DnaX intein), was determined by solution NMR spectroscopy (PDB entry: 7CFV). Since the terminal residues of the enzyme form the catalytic core, the neighboring N- and C-terminal residues influence the catalytic rate. The influence of all 20 amino acids was determined at these sites in the Spl DnaX intein and significant variation was observed for spliced product formation as well as N- and C-terminus cleavage reactions. To investigate the dependence of these reactions on the extein residues, molecular dynamics (MD) simulations were performed on 8 extein variants, each for a duration of 1.5μs using GROMACS. This revealed that the conformational sampling of the active-site residues differed among these extein variants. We found that the extein variants that sample higher population of near-attack conformers (NACs) of the active-site residues undergo higher product formation in the activity assays. Very good correlation between the NAC populations from the MD simulations and the corresponding product formation from the assays enabled us to elucidate the mechanistic roles of several conserved active-site residues in the splicing reaction.

Overall, this study shows the catalytic power of Spl DnaX intein enzyme, which is found to be highly tolerant to most of the extein residues that regulate the catalytic function by restricting the near-attack conformations of the active-site residues. This work provides important insights into this enzyme and should help in the engineering of similar promiscuous intein enzymes for novel applications with less dependence on extein residues.

ABS#122

Poster session, July 15

Combining AI-based Modeling and Flexible Docking for Efficient Refinement of Protein-RNA Complexes using PaCS-MD

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A new technique for refining protein-RNA complexes has been proposed, which combines AI-based modeling and flexible docking. The method involves using an enhanced sampling method called PaCS-MD to accelerate the conformational sampling of flexible RNA regions, which enables the construction of plausible models for protein-RNA complexes. PaCS-MD was extended to include flexible docking, which allowed the construction of protein-RNA complexes from those obtained by AI-based modeling. The technique was demonstrated by constructing several protein-RNA complexes of the RNA-binding Musashi-1 (MSI1) family of proteins, which were validated by comparing key RNA-binding residues with experimental complexes. The results suggest that PaCS-MD improves the quality of complex modeling compared to the standard protocol based on template-based modeling (Phyre2). Additionally, PaCS-MD may also be useful for constructing complexes of non-native RNA-binding to proteins.

ABS#123

Poster session, July 13

Proposal for the model of aHUS pathogenesis caused by anti-CFH autoantibodies based on the functional analysis of novel antibodies

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Atypical hemolytic uremic syndrome (aHUS) is a disease associated with dysregulation of the immune complement system, especially of the alternative pathway (AP). Complement factor H (CFH), consisting of 20 domains called complement control protein (CCP1-20), downregulates the AP as a cofactor for mediating C3 inactivation by complement factor I by binding to C3 and sialic acid (SA) on erythrocytes [1]. However, anomalies related to CFH are known to cause excessive complement activation and cytotoxicity. In aHUS, mutations and the presence of anti-CFH autoantibodies (AAbs) have been reported as plausible causes of CFH dysfunction, and it is known that CFH-related aHUS carries a high probability of end-stage renal disease [2, 3]. Elucidating the detailed functions of CFH at the molecular level will help to understand aHUS pathogenesis. In this research, we used biophysical data to reveal that a heavy-chain antibody fragment, termed VHH4, recognized CFH with high affinity. Hemolytic assays also indicated that VHH4 disrupted the protective function of CFH on sheep erythrocytes. Furthermore, X-ray crystallography revealed that VHH4 recognized the Leu1181–Leu1189 loop in CCP20, a known anti-CFH AAbs epitope. We next analyzed the dynamics of the C-terminal region of CFH using molecular dynamic simulations and showed that the epitopes recognized by anti-CFH AAbs and VHH4 were the most flexible regions in CCP18-20. Finally, we conducted mutation analyses to elucidate the mechanism of VHH4 recognition of CFH and revealed that VHH4 inserts the Trp1183 in CCP20, which is important for the binding of CFH to sialic acid (SA), into the pocket formed by the complementary determining region 3 loop. These results suggested that anti-CFH AAbs may adopt a similar molecular mechanism to recognize the flexible loop of Leu1181- Leu1189 in CCP20, leading to aHUS pathogenesis.

ABS#124

Membrane Proteins: From Natural to Designed (July 14, PM)

Exploration of lipid-membrane protein interactions a study of an evolutionary deprived in silico designed protein

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Membrane proteins (MPs) play important roles in cellular function and survival, making them prime targets for drug discovery. However, understanding their interaction with lipids can be challenging for most biophysical methods. The membrane-spanning regions of MPs are embedded in the lipid bilayer, and it is becoming increasingly clear that lipids can mediate changes to their structure, function, and stability. To address this challenge, we used an in silico designed protein called TMHC4_R (Lu, et al., 2018, Science) as a scaffold for mutational studies. We employed artificial intelligence predictions and coarse-grained molecular dynamics simulations to study the connection between specific lipid recognition and conformational stabilization. Our aim was to identify sequence features that contribute to specific lipid recognition, and ultimately to connect lipid recognition with conformational stabilization. Using native mass spectrometry (MS) combined with ion mobility spectroscopy (IM), we monitored lipid binding and how it affects the stability of TMHC4_R mutants. Through our approach, we were able to identify a specific binding site for cardiolipin (CDL) (Corey, et al., 2021, Sci. Adv.) and a lipid-activated salt bridge switch that increased the stability of the TMHC4_R protomer (see Figure 1). These findings provide a foundation for targeted mutational studies that can further connect CDL recognition and conformational stabilization. By identifying first principles of lipid interactions, we hope to open new avenues for studying the interplay between lipids and MPs in drug discovery. In conclusion, our work demonstrates the importance of understanding the complex interactions between MPs and lipids and by building on our results, researchers can gain a deeper understanding of how lipids mediate changes to the structure, function, and stability of MPs, and ultimately improve the design of drugs that target these important proteins.

ABS#125

Modern Anti-viral Strategies (July 13, AM)

Experimental and Computational Characterization of Enterovirus 68 3C Protease for the Development of Robust, Direct-Acting Antiviral Inhibitors

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Certain viruses in the picornaviridae family, specifically enterovirus-D68 (EV68), have emerged as global health concerns over the last decade with severe symptomatic infections with EV68 able to result in long lasting neurological deficits and death. There are currently no US Food and Drug Administration approved drugs for any non-polio enterovirus, highlighting the need to develop strategies against these lethal enteroviral strains. One particularly attractive class of potential drugs are small molecules inhibitors, which can act as direct-acting antiviral (DAA) inhibitors towards the conserved active site of EV68 3C protease, which is essential for viral propagation. DAAs designed to target 3C proteases can potentially achieve robust inhibition across enterovirus species. However, as drug resistance in viruses can be prevalent, it is paramount to design inhibitors less susceptible to resistance mutations. We aim to determine the cocrystal structures of EV68 3C protease bound to viral substrates as well as generate homology models of viral substrates validated by molecular dynamic simulations. I will then use these data to elucidate the substrate specificity for EV68 3C protease and calculate the substrate envelope. I will design novel DAAs that target EV68 3C protease, after characterizing previous inhibitors of 3C and 3C-like to establish a starting compound based on potency. Analogs based on the starting compound will be computationally ranked to determine the best compounds to synthesize and tested in a FRET-based enzyme inhibition assay. Cocrystal structures of novel compounds with EV68 3C protease and characterization within the substrate envelope will assess inhibitors' susceptibility to drug resistance mutations. Overall, this study aims to develop a robust, novel compounds with resistance-thwarting protease inhibition against the emerging pathogen that is EV68.

ABS#126

Poster session, July 15

NorthEastern Collaborative Access Team (NE-CAT) Crystallography Beamlines for Challenging Structural Biology Research

Igor Kourinov (1); Malcolm Capel (1); Ali Kaya (1); Ed Lynch (1); Frank Murphy (1); David Neau (1); Kay Perry (1); Cynthia Salbego (1); Jonathan Schuermann (1); Narayanasami Sukumar (1); James Withrow (1); Steve Ealick (1)

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The NorthEastern Collaborative Access Team (NE-CAT) focuses on the design and operation of synchrotron X-ray beamlines for the solution of technically challenging structural biology problems and provides an important resource for the national and international research community. Currently NE-CAT operates two undulator beamlines: a 6-22 keV tunable energy beamline 24ID-C and a 12.66keV fixed energy beamline 24ID-E (optimized for Se SAD experiments).

Both beamlines are equipped with state-of-the-art instrumentation. MD2 micro-diffractometers, installed at both beamlines, provide very clean beams down to 5 microns in diameter and are capable of visualizing micron-sized crystals. Large area pixel array detectors (including EIGER2-16M) provide very fast noiseless data collection and make possible to resolve large unit cells. Both beamlines are equipped with custom-built ALS-style robotic sample automounters with dewars capable of holding 14 pucks. To improve the diffraction from low-resolution crystals, NE-CAT has installed a humidity controlled device in the 24ID-E hutch, and "room-temperature" data collection is also supported. Both beamlines are supporting remote access through a NE-CAT developed web based user interface. Locally developed software suite RAPD provides data collection strategies, quasi-real time data integration and scaling and simple automated MR/SAD pipelines through 384 core computing cluster. Users of the beamlines are supported by experienced resident crystallographers.

To meet the needs of technically challenging crystallographic projects, cutting-edge hardware and software ideas are implemented. A summary of beamline capabilities, technology, scientific highlights, many future developments during APS upgrade will be presented.

Funding for NECAT is provided through a P30 grant (GM124165) from the NIGMS and from the NE-CAT member institutions. The NE-CAT facility is open to the whole crystallography community via APS General User Program. Detailed descriptions of the beamlines can be found at http://necat.chem.cornell.edu.

ABS#128

Proteins in Motion (July 15, AM)

Research at a PUI: Elucidating the Mechanism of DNA Pol Theta in Cancer

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It is thought that many diseases, including cancer, begin at the DNA level. DNA is constantly being bombarded by endogenous and exogenous factors. The cell's mechanism of defense is a class of enzymes known as DNA polymerases which repair DNA damage and are regarded as guardians of the genome. Of these DNA polymerases, DNA Polymerase Theta (POLQ or Polθ) has been shown to repair UV damage including the common lesions cis-syn cyclobutane–pyrimidine dimer (CPD) and a (6-4) photoproduct (PP). Despite this role, Polθ introduces mutations during this type of repair, and it is unclear if it protects the genome or contributes to genomic instability. In addition, POLQ has been shown to be upregulated in several types of cancer and is associated with poor survival rates. There are limited research studies exploring an aberrant mutant POLQ with a link cancer. Through a collaboration with the Yale SPORE in Skin Cancer, we have identified several melanoma derived POLQ somatic mutations.Our objective is to determine the biochemical mechanism by which wild-type (WT) and aberrant Polθ correctly select and incorporate a single nucleotide during polymerization in order to elucidate the functional differences between WT Polθ and the cancer associated variants. Our undergraduate researchers at Rhode Island College have played an integral role in this project by generating, expressing, and purifying Polθ and its variants. The students performed a variety of biochemical assays including pre-steady state and single turnover kinetic primer extension assays to determine polymerization rates (kpol) and nucleotide binding preferences (Kd (dNTP)).Our preliminary data suggest these variants experience a decreased rate of DNA polymerization compared to WT, suggesting aberrant Polθ has altered DNA repair function, which may contribute to cancer.

ABS#129

Aggregates, Amyloids, or Condensates? (July 16, AM)

Understanding Tau's Interactome: microtubules, lipid membranes, and anionic co-factors

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The protein tau associates and stabilizes microtubules (MT) to maintain neuronal health. Post-translational modifications lead to tau aggregation in brains with neurodegenerative diseases. To understand the mechanism of the early misfolding events in AD, we are using magic-angle-spinning (MAS) solid-state NMR spectroscopy to investigate the structure and dynamics of tau bound to microtubules [1], lipid bilayers, as well as amyloid fibrils formed with anionic cofactors.

When complexed with MTs, tau exhibits well-resolved dipolar 2D correlation spectra that can be assigned to a 45-residue segment spanning the R' and the C-terminal half of the R4 repeat. This result changes the prevailing model that all 5 MT-binding regions are immobilized. Moreover, the NMR assigned immobilized residues of R' dock well into cryoEM densities [2], leading to a revised paradigm of MT-binding.

When complexed to lipid membranes, tau converted from a disordered random coil to an ordered b-sheet. Small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs) cause different equilibrium conformations. SUV-bound tau developed amyloid fibrils while LUV-bound tau does not but has a different b-sheet assembly that contains the R' repeat. Removing cholesterol from the SUV abolished fibril formation. Thus, high membrane curvature and cholesterol are both required for fibril formation.

In the presence of heparin, P2R tau assembles into amyloid fibrils. We assigned the rigid b-sheet core to the R2 and R3 repeats. Unexpectedly, the folds differ between 24˚C and 12˚C: R2 forms a b-arch at 24˚C but a continuous b-strand at 12˚C, which dimerizes with another protofilament. This indicates that R2 has enhanced conformational plasticity than R3, similarly to in brain-extracted 4R tau aggregates.

These results have implications for the functional and pathological state of tau in diseases. They also demonstrate the power of solid-state NMR to reveal both the structure and dynamics of complex biomolecular assemblies in neurodegenerative diseases.

ABS#130

Poster session, July 15

Structural Basis for Regulation of Actin Polymerization by Palladin

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The cytoskeletal protein palladin is necessary for embryonic development and wound healing, but upregulation

is also associated with metastatic forms of cancer. In addition to binding and bundling F-actin, palladin is also able to compensate for Arp2/3 in actin comet tails and nucleates actin filament assembly in vitro. Palladin interacts with numerous actin-associated proteins and a single immunoglobulin domain (Ig3) is the minimal F-actin binding domain. Yet, the molecular mechanism of binding and implications of these interactions on cell motility are still elusive. Here, we used a combination of NMR, small-angle X-ray scattering, cross-linking mass spectrometry, and other biochemical approaches to generate an integrated structural model of the F-actin:palladin complex. On the basis of our model, we propose that palladin enhances polymerization by bridging actin protomers, regulating nucleation efficiency by stabilizing dimers of actin via its Ig3 domain. We also examined the ubiquitously expressed 90 kDa isoform of palladin which binds tightly and crosslinks F-actin but does not interact significantly with G-actin as opposed to the isolated Ig3 domain. Changes in actin-binding stoichiometry between the isolated Ig3 domain and 90 kDa palladin and a notable decrease in actin polymerization brought about by full-length palladin were also uncovered. Thus, we suggest that 90 kDa palladin may be sterically hindered such that interactions with monomeric actin are prevented, whereas Ig3 interactions with G-actin promote nucleation and spontaneous polymerization. Our findings suggest a novel role of palladin in actin-based motility and advance our mechanistic understanding of palladin's role in metastasis.

ABS#132

Poster session, July 13

Understanding Protein-Lipid Interactions in a Native Reverse Micelle System

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Structure, function, and dynamics of Peripheral Membrane Proteins (PMPs) are affected by the composition membrane to which they are bound. In order to better understand the interactions between these proteins and lipids, a membrane-mimicking reverse micelle (mmRM) system was recently developed as an improved membrane model for PMP studies using NMR. The objective of this study is to further develop mmRMs as a membrane model using naturally derived lipids. Our newest development, native RMs (nRMs), well simulate the complexity and heterogeneity found within the lipid fraction of cellular membranes. The nRMs were constructed from natural sources and optimized to successfully encapsulate a multitude of PMPs, including glutathione peroxidase 4 (GPx4), phosphatidylethanolamine binding protein 1 (PEBP1), and fatty acid binding protein 4 (FABP4), yielding high-quality NMR spectra. Next, we are pursuing specificity of lipid-protein interactions within the context of a natural-like lipid environment. Current measurements are of relative lipid affinities using methods such as surface plasmon resonance or lipid overlay assays with single lipids in a homogeneous lipid background, typically using phosphocholine-based surfactants. Our nRMs allow measurements of lipid specificities within a natural, lipid background, reflecting the heterogeneity found in cellular membranes. The general strategy is to use a paramagnetic label, attached to a mutated cysteine residue, which will increase the rate of relaxation for those lipids binding to the protein. This will lend itself to describing if the protein has a certain affinity for any lipids above others. Initial results with GPx4 match the known substrate preferences for anionic lipids; measurements with other proteins are ongoing. The novel method of encapsulating proteins in naturally derived nRMs will not only reveal more physiologically relevant substrate specificities but will provide a platform for a variety of PMP structure, function, and dynamics investigations.

ABS#133

Poster session, July 14

The Biomolecular cryo-EM Facility at UC Santa Cruz: What Should You Expect?

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Cryo-EM has become a highly effective tool in the field of structural biology. In 2013, the ‘resolution revolution’, initiated by a faster, more sensitive detector, drastically changed the field and pushed the visible details of molecules to atomic resolution. This cutting-edge approach offers the unique possibility of investigating biological molecules in their native state and revealing how these cellular machines function.

The Biomolecular cryo-EM facility at the University of California, Santa Cruz, is a leading provider of cutting-edge structural biology solutions using transmission electron microscopy. We provide access and guidance to a complete workflow from sample analysis and optimization to grid preparation, automated high-resolution imaging, data analysis, and tridimensional reconstruction, which allows structure determination via model construction and validation.

Our Glacios 200 kV is coupled with a Gatan K2 Summit direct detector, which allows sample preparation quality to be checked using our screening time blocks. Low-magnification maps are initially collected using SerialEM, allowing fast selection of the top grids based on the overall ice quality. Pre-screened grids can be imaged and auto-processed to obtain high-resolution 3D reconstructions. Using SerialEM coupled to cryoSPARC-Live, our team is capable of obtaining near-atomic resolution within ~16 hours of unsupervised data collection and processing. Our workflow allows preferential orientation and image and particle quality analysis, increasing the data quality and resulting in an initial 3D reconstruction in-live.

For data processing, our facility manages and grants access to one of our six GPU-based workstations and storage. We have all of the most commonly used software packages for single-particle and subtomogram averaging analysis. Multiple levels of service and access are available and correspond with the type of analysis desired. On-site individual training is provided for users from different levels, with no geographical restrictions. We currently have 30+ ongoing projects, including collaborators from four different countries and companies.

ABS#134

Protein Folding and Function in Context (July 13, AM)

FRET as a High-Throughput Reporter for Fold Switching in the NusG Family

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With their ability to adopt different secondary structure configurations, fold-switching proteins challenge the idea that proteins exist in a single functionally relevant fold. Recent work from the Porter group suggests that fold switching is not an uncommon phenomenon; for example, fold switching is predicted for 24% of the ~15,000 members of the universally conserved NusG family of transcription factors. Here we develop a high-throughput assay that can test fold-switch predictions in the NusG family. E. coli RfaH is a representative fold switcher from this family, assuming a compact ground state and switching to a NusG-like extended state upon binding to an ops-paused transcription elongation complex. We use Förster resonance energy transfer (FRET) as a tool for distinguishing compact and extended ground-state folds, as a proxy for fold-switching and single folding, respectively. We applied our approach to screen 15 members of the NusG family for fold switching ability in U2OS cells. The donor to acceptor fluorescence intensity ratio (D/A) is a measure of the relative compactness of the protein, with lower D/A values indicating a more compact fold switch conformation. In 83% of cases, the results of our screen are consistent with those determined by in vitro experimental methods. The remaining cases are comprised of one false negative and two false positive results. This work demonstrates that FRET is a promising high-throughput reporter for fold switching ability in the NusG protein family and could help address the need to test thousands of sequences for fold switching.

ABS#141

Poster session, July 14

ApoE lipid transport by ABCA7 genetic risk variants

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Alzheimer's disease (AD), the most common cause of dementia in older adults, disproportionally affects African Americans with an incidence rate as much as three times higher, compared to other racial/ethnic groups. Multiple factors contribute to this racial disparity however, an in-depth understanding of the biological or genetic contributions does not exist. Compelling evidence indicate that genetic variants of the lipid transport protein, ABCA7, is more strongly associated with AD in African Americans. To understand how ABCA7 contributes to AD on the molecular level, we used a combination of structural and cell biology techniques. We have found that the ABCA7 T319A variant, which confers risk in

African Americans, is expressed and localizes to the plasma membrane and has reduced ATPase activity when expressed in human cell lines. Proteomic studies indicate reduced levels of the phospholipase C eta (PLCH1) protein in cells that expressed ABCA7 T319A compared to wild-type. PLCH1 is involved in the metabolism of phosphoinositol bisphosphate PIP2. Our results suggest that this variant may contribute to AD by reducing the levels of PIP2, a phospholipid reported to be decreased in the AD brain. These results provide a framework for targeting mechanisms that can increase PIP2 levels as an effective strategy mitigating AD disparities.

ABS#142

Poster session, July 14

Mode of Substrate Binding for Ketohexokinase Across Isozymes and Species Implies an Induced-Fit Mechanism

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Ketohexokinase (KHK) is an adenosine triphosphate (ATP)-dependent enzyme that catalyzes the first reaction in fructose metabolism. The liver isozyme, KHK-C, has become a target for pharmacological development against fatty liver and metabolic syndrome. Recent evidence has implied that the more ubiquitous isozyme, KHK-A, functions as a protein kinase after it is phosphorylated by AMP-dependent protein kinase, an activity absent in KHK-C. This property leads to downstream effects ameliorating reactive-oxygen species (ROS) and the expression of genes required for cell adhesion, without which metastasis is promoted. The KHK isozymes differ by alternative splicing of exon 3, which encodes 45 out of 298 amino acids. Both isozymes exist as homodimers interlocked with a β-clasp domain. The structure of mouse KHK-A in its unliganded form was determined using macromolecular X-ray crystallography and refined to 1.85 Å resolution. The structure revealed that mKHK-A could indeed adopt a different conformation than the human KHK-A structures when substrates are bound, contrary to the previous proposal (Trinh et al., 2009). Furthermore, the conformational change observed in the mouse KHK-A structure is conserved with that of human KHK-A. When compared to other unliganded KHK-A structures, mouse KHK-A structure adopts a β-clasp conformation similar to other unliganded KHK-A structures, whereas KHK-C unliganded structures adopt a wider range of rotational/hinge motion of the β-clasp. Adenosine kinase and ribokinase, which belong to the same PfkB family of carbohydrate kinases, exhibit a similar mode of conformational change upon ribose/nucleoside binding, which has been proposed as an induced-fit mechanism for ATP binding. This suggests that the KHK isozymes across different species operate in a similar mechanism of induced-fit binding during catalysis.

ABS#145

Poster session, July 15

Sequence independent activity of a predicted long disordered segment of the human papillomavirus L2 capsid protein during virus entry

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The human papillomaviruses (HPVs) are small non-enveloped viruses that trigger approximately 5% of human cancers. The virus particle contains multiple copies of two viral proteins, L1, which forms the rigid outer shell of the capsid and binds to the cell surface, and L2, which directs the viral DNA to the nucleus during virus entry. After HPV is internalized into the endosome lumen during entry, the L2 capsid protein protrudes through the endosome membrane into the cytoplasm to bind cellular factors such as retromer required for intracellular virus trafficking. Membrane protrusion of L2 is driven by a cationic cell-penetrating peptide (CPP) adjacent to the retromer binding site near the C terminus of L2. AlphaFold modeling and sequence analysis predicts that the L2 proteins of diverse human and animal papillomaviruses contain an ∼110-residue disordered segment immediately N-terminal to the CPP and the retromer binding site. Large deletions in this segment inhibit cytoplasmic protrusion of HPV16 L2, association with retromer, virus trafficking, and infectivity, but this segment can tolerate small in-frame insertions and deletions. The infectivity of mutants with small deletions directly correlates with the size of the segment. Strikingly, the activity of the large deletion mutants can be restored by inserting protein segments with diverse compositions and chemical

properties into this region, including randomly scrambled sequences, a tandem array of a short sequence, and segments of unrelated proteins including the intrinsically disordered region of a cellular protein. These results indicate that the length of the disordered segment, not its sequence or composition, determines its activity during virus entry. We propose that the length dependence of this protein segment reflects the requirement for L2 to protrude through the membrane far enough to bind retromer. Sequence independent but length dependent activity has important implications for protein function and evolution.

ABS#147

Poster session, July 14

Investigation of Metal-Induced Protein Precipitation Reactions Across Multiple Proteomes

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Cu-induced protein aggregation has long been related to cellular dysfunction and cellular Cu-toxicity. The biophysical basis behind metal-induced protein aggregation and the relative susceptibility of cellular proteins to this effect remains poorly understood. Reported here is the susceptibility of proteins to precipitate upon addition of Cu, probed using a Metal-induced Protein Precipitation (MiPP) methodology. MiPP allows for the determination of Cm values for each identified protein (i.e., the concentration of Cu at which half the protein is precipitated). This ultimately enables a quantitative means to assess the sensitivity of proteins across proteomes to Cu precipitation. Subsequent analyses of the proteins assayed by MiPP reveals biophysical properties that correlate with MiPP tolerant and sensitive proteins.

As part of this work proteins in an E. coli cell lysate were exposed to a range of Cu concentrations, insoluble protein aggregates were precipitated by centrifugation, and the remaining soluble protein in the samples at each Cu concentration was quantified using quantitative bottom-up proteomics experiment with isobaric mass tags and an LC-MS/MS readout. Subsequent bioinformatics analyses were performed to elucidate biophysical properties, such as amino acid content and secondary structure, tied to a protein's sensitivity or tolerance to Cu-induced protein precipitation. In E. coli, unstructured regions were found to be more prevalent in MiPP tolerant proteins, while α-helical regions were more prevalent in sensitive proteins. Our analyses also showed that His and Cys had higher percentages in sensitive proteins compared to tolerant proteins. Ongoing work is focused on using the MiPP methodology established to study the behavior of proteins in yeast and mammalian cell lysates to help determine overarching properties responsible for MiPP sensitivity or tolerance (e.g., is MiPP sensitivity/tolerance an conserved property of proteins or is it driven by amino acid composition and/or secondary structure as observed in our E. coli results?).

ABS#149

Undergraduate Research Session

Defining the Interface between Palladin and F-actin using Crosslinking Mass Spectrometry (XL-MS)

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In 2000, the protein palladin was discovered and has been shown to play a critical role in actin polymerization and is specifically upregulated in metastatic cancer. The longest isoform consists of five immunoglobulin domains and the third immunoglobulin domain (Ig3) of palladin has been identified as the portion that directly binds to F-actin. The direct involvement of Ig domains F-actin binding is a novel finding and while several lysine amino acids have been shown to be responsible for this interaction, the specific amino acids on the surface of F-actin that participate in the interaction with palladin remain unknown. To gain further insight into this complex, we have conducted chemical crosslinking coupled to high-resolution mass spectrometry (XL-MS) experiments to identify the interface residues between palladin and F-actin. In these experiments, actin was first polymerized, followed by the addition of crosslinking reagents that form covalent bonds between specific chemical groups on the surface of palladin and F-actin. We identified six consistent interprotein crosslinks using DMTMM. We next used previously determined structures and intersubunit crosslinks to dock palladin and F-actin. Our integrative structural model reveals that the Ig3 domain of palladin binds along the side and between two adjacent

actin monomers. Future experiments will involve expressing actin in Pichia pastoris and using site-directed mutagenesis of the identified actin residues to confirm precisely which residues are responsible for interaction with palladin.

ABS#150

Poster session, July 15

Applying Protein Systems for Controllable Biomineralization of Semiconductor Quantum Dots

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Light harvesting devices, such as photovoltaics and photocatalysts, have achieved high efficiencies in recent years; however, such efficiencies require specialized inorganic materials synthesized at high temperatures and pressures with toxic precursors or solvents. Here, I demonstrate an alternative approach to synthesize functional materials in a scalable and sustainable way that uses proteins found in biological systems. In the first part of my talk, I will demonstrate the synthesis of semiconductor quantum dots using a protein found in nature. Originally identified in the bacteria Stenotrophomonas maltophila, the biomineralization process was found to rely on a single enzyme, cystathionine gamma-lyase, which catalyzes nanocrystal growth by producing reactive sulfur from the amino acid L-cysteine. Biomineralization was used to generate many types of semiconductor quantum dots including cadmium sulfide, lead sulfide, copper indium sulfide and the first reported fully biomineralized lead sulfide/cadmium sulfide core/shell quantum dots. In the second part of my talk, I will demonstrate an alternative biomineralization approach that uses the artificially designed de novo protein, Construct K (ConK), to produce CdS quantum dots. ConK was not designed for function but found to catalyze production of reactive sulfur from L-cysteine precursors, enabling biomineralization of metal sulfide nanocrystals. Because de novo proteins are created by design to fold into stable structures, they are highly tolerant to mutations in their amino acid sequence, making them ideal candidates for future addition of new functionalities and properties. For both systems, I will present results that include nanocrystal characterization (absorbance and fluorescence spectroscopy, TEM) and in-depth protein characterization, including identification of the active site.

ABS#152

Poster session, July 15

Effects of Heparin and pH on the Solution Conformation and Fibril Formation of Serum Amyloid A

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Serum amyloid A (SAA) is an intrinsically disordered lipid transport protein that deposits extracellularly in AA amyloidosis, a life-threatening complication of chronic inflammation. SAA interactions with glycosaminoglycans are a major driver of AA amyloidosis and its therapeutic target. Like many other amyloidoses, AA has a lyzosomal origin wherein acidic pH is crucial to amyloid formation. Binding to lipids, glycosaminoglycans, and acidic pH critically influence SAA fibrillogenesis. We explored combined effects of heparin and pH on the solution conformation and fibrillation kinetics of lipid-free SAA.

Recombinant murine SAA was incubated with heparin (10:1 wt/wt) at 37oC, pH7.4-5.0. Histidines were blocked by diethyl phenyl carbonate to test their role in heparin binding and pH-induced conformational changes. Amyloid fibrils were formed upon 48h incubation of SAA+/--heparin at 37oC with stirring. Fibrils were harvested by centrifugation and the pellets were re-suspended in 10mM PB, pH7.4 for structural studies.

Far-UV CD and FT-IR spectra revealed a heparin-induced conversion from the predominantly disordered to ordered conformation. At neutral pH, heparin-bound SAA showed ~35% alpha-helix but little beta-sheet. At acidic pH the beta-sheet content increased, reaching ~50% at pH5.5; these structural changes were reversed upon pH increase from 5.5 to 7. His-blocked SAA bound heparin and showed heparin-induced alpha-helix at pH7 but no beta-sheet at pH5.5. Fibrillation kinetics monitored by ThT fluorescence showed that heparin dramatically accelerated SAA fibrillation and eliminated the lag phase at plasma but not lysosomal pH. Electron micrographs of SAA+heparin showed fibrils at any pH explored.

In summary, heparin induces pH-dependent conformational changes in native lipid-free SAA and strongly promotes fibrillation at neutral but not acidic pH. Histidine is critical to heparin-induced structural changes in SAA at plasma but not lysosomal pH. The results suggests different potential routes of SAA fibrillogenesis, a heparin-independent lysosomal pathway and a heparin-induced extracellular pathway.

ABS#153

Poster session, July 15

Role of Complementarity-Determining Regions 1 and 3 in Pathologic Amyloid Formation by Human Immunoglobulin κ1 Light Chains

Daniele Peterle (1); Elena Klimtchuk (2); Esther Bullitt (3); Lawreen Connors (2); John Engen (1); Olga Gursky (2)
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Immunoglobulin light chain (LC) amyloidosis is a life-threating and intrinsically variable disease that poses a challenge for both treatment and comprehension due to the large number of patient-specific mutations. To investigate the molecular origins of this disease, we studied 14 proteins related to the germline genes IGKVLD-33*01 and IGKVLD-39*01, which belong to the κ1-family. We used hydrogen-deuterium exchange monitored by mass spectrometry (HDX MS) to analyze the conformational dynamics of full-length recombinant LCs and their fragments, in combination with various other analytical tools, such as thermal stability, proteolytic susceptibility, amyloid formation, and also experimental and bioinformatic tools to assess sequence-dependent amyloidogenicity. Our findings were then mapped onto the atomic structures of native and fibrillary proteins. Unexpected differences between the two κ1 subfamilies were observed. Amyloid LC related to IGKVLD-33*01 was found to be less stable and formed amyloid faster than its germline counterparts, whereas amyloid LC related to IGKVLD-39*01 had similar stability and formed amyloid slower. These and other differences suggest that various factors influence amyloid formation. For the 33*01-related amyloid LC, these factors involved mutation-induced destabilization of the native structure and likely stabilization of amyloid. The atypical behavior of 39*01-related amyloid LC was attributed to increased dynamics/exposure of amyloidogenic segments in C'V and EV β-strands that could initiate aggregation, along with decreased dynamics/exposure near the Cys23-Cys88 disulfide, whose rearrangement is rate-limiting to amyloidogenesis. Our findings suggest that closely related LCs have distinct amyloidogenic pathways and that the antigen-binding regions CDR1 and CDR3, linked via the conserved internal disulfide, are crucial factors for LC amyloidogenicity.

ABS#155

Poster session, July 14

Protein Dimer Stability in Concentrated Sugar Polymer Solutions

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Synthetic polymer solutions at concentrations of hundreds of grams per liter are used to stabilize proteins and mimic the crowded intracellular environment (1). Several theories have been developed to explain the stabilizing effect of crowding, but almost all assume only contributions from hard (steric) contacts, ignoring soft (enthalpic) chemical interactions. We quantified the effects of the synthetic sugar-polymers, dextran and Ficoll™, as well as their monomers (sucrose and glucose, respectively), on two homodimer variants of the B1 binding domain of streptococcal protein G (2,3). One dimer forms via domain swapping, while the other involves simple side-by-side dimerization. The dimers are fluorine labeled to facilitate detection using F-19 nuclear magnetic resonance spectroscopy. We measured the amounts of dimer and monomer as a function of temperature, cosolute size and cosolute concentration, allowing quantification of the stability (i.e., free energy), enthalpy, and entropy of dissociation. The cosolutes increase dimer stability and affect the enthalpy. We analyze the data using a recently developed, more inclusive protein crowding model (4).

ABS#156

Poster session, July 13

A Spectroscopic Approach to Understand Protein Flexibility Using Red-Edge Excitation Shift

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Local flexibility is important for protein function since flexibility plays a role in molecular recognition and turnover. However, directly determining local flexibility in a protein is challenging. Methods exist to measure protein flexibility, such as NMR spectroscopy and small angle x-ray scattering, but they are technically demanding. An informative and under-utilized approach is red-edge excitation shift (REES) spectroscopy, which is sensitive to the local environment around a fluorophore. REES measures the red shift in the emission intensity maximum that arises when a fluorophore is excited near its low-energy excitation limit. The degree of red-shift correlates to the amount of conformational states the fluorophore can sample, with residues located in highly flexible regions having the smallest degree of REES. In the present study, we are using REES effect experiments to analyze fluctuations in chlorogenic acid (CGA) esterase, an enzyme that may be useful in the food and biomass industry for CGA removal. Single tryptophan (Trp) mutants were generated, with each Trp residue serving as a reporter for its surroundings. Results show that the degree of REES correlates well with solvation, as buried Trp residues display a larger effect than residues located in mobile regions of the protein. The REES signatures of a Trp residue placed near the CGA esterase active site indicate that this region possesses conformational flexibility, but less than would be expected based on B-factor analysis, suggesting that the active site may be more rigid than previously thought. Overall, this study demonstrates that REES is a promising method for measuring local protein flexibility.

ABS#157

Poster session, July 14

Functional Divergence of LXR and FXR from Their Common Ancester

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Liver X receptor (LXR) and farnesoid X receptor (FXR) are intracellular sensors for sterols and bile acids respectively. LXR and FXR belong to the nuclear receptor superfamily of transcription factors and in response to specific ligand binding, they maintain a finely tuned regulation of bile acid and cholesterol metabolism. Belonging to the same subfamily (NR1H), these receptors evolved from a common ancestor to diverge into these two "yin and yang" receptors that work together in opposite directions for the maintenance of total body fat and cholesterol homeostasis. Despite their structural and mechanistic similarities, both receptors have different DNA binding preferences and cognate ligands that confer opposing downstream functions. To understand how these paralogous transcription factors evolved divergent functions and DNA specificities, we have reconstructed an ancestral receptor for functional and structural characterization. This project aims to understand the evolutionary paths between the ancestor and both FXR and LXR lineages. To achieve this aim, we are employing a series of DNA binding assays, transcriptional activity assays, mutational analysis and molecular dynamics simulations.

ABS#159

Poster session, July 13

Transporter or ion channel? Structure and dynamics of AM2-mimicking BM2 viroporin from solid-state NMR spectroscopy

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The transmembrane (TM) domain of the M2 viroporin of influenza A and B viruses conducts protons to acidify the virion for uncoating. Biophysical and structural data to date suggest that the mechanism of proton conduction is not identical between the two proteins. AM2 undergoes significant backbone rearrangement between high pH (closed state) and low pH (open state), hence it is proposed to behave like a transporter [1]. In comparison, BM2 undergoes a symmetric scissor-like motion between high and low pH, and hence has been proposed to behave like a channel (Fig. 1a) [2]. To understand the amino acid

sequence basis for this mechanistic difference, we have designed a triple mutant of BM2, GDR-BM2, which shares the proton conduction properties of AM2. These mutations introduce two key structure elements of AM2 into BM2: a glycine "kink" in the middle of the TM domain, and an aspartate-arginine "electrostatic lock" near the tryptophan gate. We employ solid-state NMR to investigate the conformation and dynamics of this AM2-mimetic GDR-BM2. Uniformly 13C, 15N-labeled GDR-BM2 is reconstituted into POPC/POPG bilayers at pH 4.5. 13C and 15N chemical shifts are measured from 2D and 3D 13C-13C and 15N-13C correlation spectra (Fig. 1b), and indicate that GDR-BM2 has a similar a-helical TM conformation as WT BM2. Water-edited experiments indicate that the open GDR-BM2 channel is less hydrated than WT BM2. 13C-1H and 15N-1H DIP-SHIFT measurements indicate that the mutant undergoes fast uniaxial rotational diffusion in the liquid-crystalline membrane. Under these conditions, we measured 15N-1H dipolar couplings site-specifically using 2D 15N-13C correlation experiments to obtain information about the helix orientation of GDR-BM2. Our data show that the AM2-mimicking mutations change the orientation and hydration of BM2, giving insight into the sequence determinants of the proton conduction mechanism of this canonical proton channel.

ABS#160

Poster session, July 14

Molecular Mechanism of Holliday Junction Branch Migration by an Assymetric RuvB Hexamer

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The Holliday junction (HJ) is a universal DNA intermediate of homologous recombination that is involved in many fundamental physiological processes. In bacteria, RuvB, a motor protein of the AAA+ ATPase superfamily, drives branch migration of the Holliday junction with a mechanism that had yet to be elucidated. Here, we report two cryo-EM structures of RuvB in complex with DNA and nucleotides, providing a comprehensive understanding of HJ branch migration. Six RuvB protomers assemble into a spiral staircase, in the shape of a ring, with DNA in the central pore. Four protomers of RuvB hexamer interact with the backbone of the DNA substrate, suggesting a pulling-and-revolving mechanism of DNA translocation with a basic step size of 2 nucleotides. Moreover, the variation of nucleotide-binding states in our RuvB hexamer supports a sequential model for ATP hydrolysis, ADP release, and ATP reloading, which occur at specific positions on the RuvB hexamer. Furthermore, the asymmetric assembly of RuvB also explains the 6:4 stoichiometry between RuvB and RuvA, which assembles into a complex to coordinate HJ migration in cells. Taken together, we provide a comprehensive framework for the mechanistic understanding of HJ branch migration facilitated by RuvB motor protein, which may be universally shared in both prokaryotic and eukaryotic organisms.

ABS#162

Poster session, July 13

Linker Length and Composition Influence Actin Binding and Bundling by Palladin

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Palladin is an actin binding protein that helps to regulate cell shape, cell motility, and function of the actin cytoskeleton. It has previously been shown that the Ig3 domain of palladin is the minimal actin binding domain and binds to negatively charged actin filaments through lysine residues on the Ig3 surface. Yet, despite the fact that the Ig4 domain shows no actin binding affinity, the tandem Ig3-4 construct shows increased actin binding and bundling ability compared to Ig3 alone. While the structures of these two individual domains are known, information about their relative orientation and flexibility remains limited. The linker region between these two Ig domains is predicted to be intrinsically disordered and is of particular interest due to its extended length as compared to other protein's tandem Ig domain linker regions. The palladin Ig3-4 linker consists of 41 amino acids whereas other proteins containing tandem Ig domains tend to have much shorter linkers such as titin with 0-3 residue linkers or myotilin with a 7 residue linker. Furthermore, almost 25% of the Ig3-4 linker is arginine residues which could contribute to the increased actin affinity of the tandem construct. Therefore, we set out to

characterize the Ig3-4 domain pair of palladin with emphasis on its molecular structure, dynamics and actin binding interactions. This study investigates this linker region through a series of mutations that assess the impact of linker length, sequence, and charge as well as the effect of swapping Ig4 with another Ig domain. These mutants were analyzed by actin cosedimentation assays, chemical crosslinking, NMR, and SAXS. We found evidence of interactions between Ig3 and Ig4 and the linker region mutations produced varied results indicating that multiple factors influence the function of this region.

ABS#163

Poster session, July 13

Structural Study of a Non-Canonical ATP-Binding Cassette (ABC) Importer, the E. coli Ribose Transporter

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The ribose ABC importer (RbsABC) is an inducible, high affinity ribose permease. Canonical substrate-binding protein-dependent ABC importers are divided into type I and type II ABC importers and have two active ATPase sites. Based on biochemical studies, E. coli RbsABC is a non-canonical substrate-binding protein-dependent ABC importer: (1) the fused nucleotide-binding domain (NBD) of RbsABC has only one active ATPase site and undergoes asymmetric ATP hydrolysis in its transport cycle and (2) RbsABC possess a 'hybrid' structural profile that consists of features from both type I and II ABC importers. Elucidating how these unique features confer mechanistic differences to the non-canonical RbsABC will contribute to a general understanding of how ABC importers achieve substrate transport, especially in the enigma of how ATP hydrolysis couples to substrate transport. In this study, we solved the structure of RbsABC using X-ray crystallography and further elucidated the transport mechanism using cryo-electron microscopy. The RbsABC transmembrane domain possess a BtuC-like fold. An unusual channel-like substrate translocation pathway in RbsC that was not observed in other ABC importers was observed in the beryllofluoride-trapped crystal structure. Negatively charged residues line the periplasmic opening and hydrophobic moieties line the rest of the pathway to the cytoplasmic opening, suggesting a non-measurable binding affinity towards ribose to aid in its release on the cytoplasmic side In the vanadate-trapped RbsABC cryo-EM structure, Leu255 of TMH8 acts as a periplasmic gate to prevent rebinding of ribose to the RbsB. On the other hand, Phe127 of TMH5 is the gating residue for the cytoplasmic gate, possibly to prevent reflux of ribose into the translocation pathway. Our structural study showed that RbsABC may belong to a non-canonical class of ABC importer. Not all importers are equal, as RbsABC provides evidence that an ABC importer can achieve unidirectional transport with asymmetric ATP hydrolysis.

ABS#164

Poster session, July 13

Structure of Yeast ALA Synthase Reveals Divergent Mechanisms of Enzyme Autoregulation Governing Heme Biosynthesis

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Heme is an essential cofactor involved in numerous biological processes, ranging from oxygen transport to cellular differentiation (1). Biosynthesis universally begins with the rate-limiting production of aminolevulinic acid (ALA), which is catalyzed by the pyridoxal phosphate (PLP)-dependent enzyme ALA synthase (ALAS) in α-proteobacteria and non-plant eukaryotes (2). ALAS homologs feature a highly conserved homodimeric core, but across eukaryotes, a divergent C-terminal extension (Ct-ext) has emerged with differential regulatory functions (3). Previous work has shown that in Saccharomyces cerevisiae ALAS (Hem1), the Ct-ext uniquely interacts with conserved active-site elements, and Ct-ext truncation leads to a 25% loss of function. However, the mechanism by which the Hem1 C-terminus controls enzyme activity was unknown (4).

We developed a mutant enzyme lacking a portion of the C-terminus (Hem1 ΔCT) to elucidate how this divergent extension modulates enzyme function (5). We determined the Hem1 ΔCT crystal structure to 2.1 Å

resolution (PDB ID 8EIM), which revealed increased disorder and altered PLP binding compared to wildtype. Using hydrogen-deuterium exchange mass spectrometry, we confirmed that these disordered regions exhibit increased solvent accessibility and correspond to conserved motifs critical for activity of all ALAS enzymes. This greater flexibility leads to a decrease in substrate binding and cooperativity that significantly lowers catalytic efficiency. Further, our data point toward a novel role of the Hem1 Ct-ext in mediating functional asymmetry within the homodimer. Collectively, these findings demonstrate that the Hem1 Ct-ext directly stabilizes protein structure to maintain functionality, representing a new means of eukaryotic ALAS autoregulation (5).

ABS#165

Poster session, July 13

Multifaceted Membrane Interactions of Human Atg3 Promote LC3-Phosphatidylethanolamine Conjugation during Autophagy

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Macroautophagy is a cellular process that involves the degradation and recycling of cellular components including damaged organelles, invasive microbes and cytosolic proteins. Autophagosome formation is an essential step in this process and requires the covalent conjugation of LC3 proteins to the amino headgroup of PE (phosphatidylethanolamine) lipids. Atg3 acts as a catalyst by transferring LC3 from an LC3-Atg3 intermediate to PEs in autophagic membranes. Atg3's function requires the presence of an N-terminal amphipathic helix (NAH) that selectively interacts with membranes that contain lipid-packing defects, such as the leading edge of the growing phagophore where membranes are highly curved. We previously have shown that the membrane association of the NAH from human Atg3 (hAtg3) is necessary, but not sufficient, to catalyze LC3-PE conjugation, and a conserved region following the hAtg3 NAH tightly coordinates its membrane geometry-sensitive interaction with its catalytic activity. However, the mechanism by which the C-terminal located catalytic center is directed to the membrane surface for effective LC3-PE conjugation remained obscure.

Our current studies of hAtg3 reveal that its C-terminal catalytically important regions are conformationally dynamic and directly interact with the membrane, concurrent with its N-terminal membrane curvature-sensitive amphipathic helix. Highly curved phagophoric rims function not solely as a geometric cue to recruit hAtg3 as previously demonstrated, but moreover their interaction with hAtg3 promotes LC3-PE conjugation by rearranging its catalytic center and bringing substrates into proximity. We confirm the functional relevance of these newly discovered interactions by in vitro lipidation and in vivo cellular assays. Our studies advance the notion that autophagosome biogenesis is directly guided by the spatial interactions of Atg3 with highly curved phagophoric rims.

ABS#167

Poster session, July 14

Influences of Agonists, Coactivators, and RXR Dimerization on FXR Allostery

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The farnesoid X receptor (FXR) is the main target receptor of drug developers working on treatments for non-alcoholic fatty liver disease. It is widely known that FXR exhibits ligand-dependent activation, but the mechanism of activation or features of ligands that correlate with their ability to activate FXR remains unclear. My research seeks to elucidate the patterns of FXR behavior; to accomplish this goal, I am performing 4 sets of triplicate 1 μs classical molecular dynamics simulations on the FXR ligand-binding domain, varying the presence of the SRC-1 coactivator and the retinoid X receptor (RXR) heterodimer. Each set contains 28 complexes, each with a different bile acid or bile acid homolog docked in the FXR ligand-binding pocket. From the resulting data, we will identify the physical properties of ligands that correlate with high transcriptional activity. In addition, these simulations may provide insight into how the ligand-binding pocket of FXR undergoes conformational changes in response to the binding of active versus inactive ligands.

ABS#168

Poster session, July 13

Discovery of Potential Inhibitors of New Delhi Metallo-beta-lactamase 1 in Klebsiella pneumoniae by High-throughput Virtual Screening

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The New Delhi Metallo-beta-lactamase 1 (NDM-1) enzyme was first detected in a patient who contracted a urinary tract infection caused by the bacterium Klebsiella pneumoniae. NDM-1 is a carbapenemase that hydrolyzes the amide bond in beta-lactams, rendering them ineffective. Due to the robust antibiotic resistance it confers, there exists a worldwide concern about bacteria acquiring this enzyme, even more now due to the discovery of 33 clinical variants. Bacteria harboring NDM-1 and its variants have been regarded as superbugs, thus making this protein an excellent target for small-molecule drug discovery.

In pursuit of identifying potential inhibitors of NDM-1, high-throughput virtual screening of over 100,000 chemical compounds was conducted via GOLD (CCDC), a molecular docking software, using a crystal structure of NDM-1 (PDB ID: 5ZGE). Positive and negative controls, selected from previous literature and the PDB database were screened, with the highest-scoring positive control being 88.97. Here, we found that multiple large libraries yielded two potential inhibitors, Chembridge identifiers 6xx913 and 5xx00, which scored significantly higher than other screened compounds and controls, scoring 105.37 and 101.68 respectively.

These top-scoring ligands were then purchased to be tested in a wet-lab setting to validate virtual screening results. Recombinant NDM-1 protein lacking the periplasmic localization sequence was expressed via autoinduction in a pET vector, and purified via nickel affinity chromatography. Differential scanning fluorimetry (DSF) was used as a binding assay with the top-scoring compounds to detect ligand binding through shifts in the melting temperature.

We anticipate using these preliminary results as data to show that ligands 6xx913 and 5xx00 are potential novel inhibitors of NDM-1 and could lead to new molecular scaffolds to fight the resistance to beta-lactam drugs brought about by this enzyme and its clinical variants.

ABS#171

Poster session, July 15

A Proteome-wide Identification of Hsp70 Binding Sites in E. coli

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Protein homeostasis is at the heart of cellular viability. The E. coli chaperone system consists predominantly of Trigger factor (Tif), Hsp70 system and GroEL/ES system. Tif acts at the level of translation and GroEL/ES system acts post-translation, whereas Hsp70 functions at the interface. Hsp70 in E. coli consists of DnaK and the co-chaperone DnaJ. The goal of our study is to identify the proteome-wide binding-sites for DnaK. We transiently unfolded the E. coli proteome by heat shock to let the DnaK bind. DnaK famously has two conformations namely, open, ATP-bound and closed, ADP-bound conformation. After binding to disordered regions in a protein, DnaK goes from the ATP-bound state to the ADP-bound state, and the latter has a very high affinity for the protein. The strategy used to trap the bound state, was the depletion of ATP (by the addition of apyrase) such that the ADP-bound state prevails and DnaK remains in the bound state. The DnaK-bound state was then studied by limited proteolysis (Lip) using protinaseK and then probed by mass spectrometry. This allowed us to identify all the binding-sites in the E. coli proteome and thus understand the function of Hsp70 system in depth. To the best of our knowledge, our study is the first of its kind to identify a proteome-wide DnaK binding-site.

ABS#172

Poster session, July 14

Domain swapping of CaMKII holoenzyme in the context of linker-dependent calcium sensitivity modulation

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Many physiological processes such as memory formation and cardiac function rely on Ca2+ signaling. A central

machinery in cells responsible for decoding the Ca2+ oscillation is Ca2+/calmodulin-dependent protein kinase II (CaMKII) whose structure highlights many interesting features that make CaMKII activation sensitive to the frequency of the incoming Ca2+ waves. In this study, we aim to characterize the structure of CaMKII with the hope of providing molecular basis for CaMKII activation. The N-terminal kinase domain of CaMKII is kept autoinhibited at basal state and gets activated by Ca2+/CaM in a cooperative manner. However, it is still unclear the molecular basis for the cooperative activation of CaMKII. Meanwhile, CaMKII protein appears as holoenzymes consisting of six or seven vertical dimers that oligomerize via the C-terminus hub domain (aka the association domain). Previous crystallographic study of CaMKII holoenzyme suggests the kinase domain can dock on the hub domain of the same polypeptide chain, which can support our recent observation showing the hub can regulate the sensitivity of CaMKII holoenzyme to Ca2+/CaM, but not the cooperative activation. We present here a new crystallographic structure of CaMKII holoenzyme showing a domain-swap conformation of the dimer subunit in the holoenzyme, which we hypothesize to contribute to the cooperativity of CaMKII activation. The sensitivity to Ca2+/CaM can also be tuned by the linker region that connects the kinase and the hub domain and is subjected to extensive alternative splicing. We show that not only the length, but also the overall net charge of the linker region can affect the overall conformation and the sensitivity to Ca2+/CaM of the holoenzyme. Our study suggests a mechanism by which the domain-swap conformation confers the cooperativity and allows for the diversity of CaMKII via alternative splicing, all which are important for its Ca2+ frequency response.

ABS#173

Poster session, July 13

Regulation of Connexin43 Gap Junction Formation by Alternative Translation

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Cardiovascular disease remains the leading cause of death in the United States, claiming over 800,000 lives annually. The heart comprises billions of individual cardiomyocytes which are electrically and mechanically coupled by gap junction structures. Connexin 43 (Cx43, GJA1) is the primary gap junction protein of the heart with reduced localization of Cx43 to intercellular junctions resulting in electrical disturbances and the arrhythmias of sudden cardiac death. The majority of research into Cx43 regulation and gap junction loss in disease, has focused upon transcriptional and pot-translational pathways. Cx43 mRNA was recently found, however, to undergo alternative translation initiation yielding N-terminally truncated protein isoforms, of which the 20 kDa, GJA1-20k, is most abundantly expressed. GJA1-20k promotes gap junction formation through facilitation of Cx43 oligomerization into gap junction channels but where this occurs in the cell and how this is regulated remain unknown. With loss of GJA1-20k occurring during stress, concomitant with arrhythmogenic cardiac gap junction disruption, alternative translation is therefore a powerful new avenue for therapeutic intervention once such mechanisms have been elucidated. Here we investigated the subcellular localization of ectopically expressed GJA1-20k on both the micro and nanoscale to identify in which compartment GJA1-20k and Cx43 interact. Utilizing confocal- and stochastic optical reconstruction-microscopy (STORM) we find GJA1-20k is enriched within the endoplasmic reticulum and Golgi apparatus of the vesicular transport pathway. Moreover, N- and C-terminally tagged GJA1-20k with varying domain lengths were used to identify that GJA1-20k complexes with Cx43 via C-terminal interactions. Results are complemented and confirmed with biochemical assays including co-immunoprecipitation and subcellular fractionation. Unraveling the localization and trafficking behavior of Cx43 via GJA1-20k modulation provides insight into harnessing this post-transcriptional process, paving a way for pharmacological targeting of global alternative translation.

ABS#174

Poster session, July 15

Structure of the Non-Helical Fibril of the Alzheimer's Disease Tau Core

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The microtubule-associated protein tau aggregates into amyloid fibrils with different molecular structures in different neurodegenerative diseases, but the mechanism

for forming these diverse structures is unknown. In Alzheimer's disease (AD), the tau fibrils are twisted and incorporate less than 20% of the full-length protein into the rigid core (residues 306–378) [1]. Recently, cryo-electron microscopy (cryo-EM) data showed that a protease-resistant fragment of tau (residues 297-391) (Fig. 1a) self-assembles in vitro in the presence of divalent cations to form twisted fibrils that have a similar molecular conformation as the AD paired helical filament (PHF) tau [2]. To investigate whether this tau construct is uniquely predisposed to this molecular structure and fibril morphology, we fibrillized this protein under very similar conditions, monitored the fibril morphology using transmission electron microscopy (Fig. 1b), and determined the rigid core structure using 2D and 3D solid-state NMR (ssNMR) spectroscopy (Fig. 1c). Unexpectedly, we found that tau (297-391) assembled predominantly into non-twisting ribbons. A small fraction of fibrils were initially twisted but converted to flat ribbons over several days. The ssNMR structure shows a rigid core spanning residues 305–357, which forms a β-arch with a turn at 322CGS324. Two protofilaments assemble into an antiparallel dimer whose interface spans residues G323 to I354. The four-layered dimeric β-sheet core shows stabilizing sidechain interactions that resemble those found in neurodegenerative brains such as corticobasal degeneration (CBD) and Pick's disease. This molecular structure shows that subtly different pH and ionic strengths can lead to distinct conformations for this protease-resistant core of tau. Our study also demonstrates that the structures of non-twisting amyloid fibrils, which are difficult to solve using cryo-EM, can be readily determined using ssNMR.

ABS#175

Poster session, July 14

Characterization of Mycobacterial Phosphoarginine Modification and their Interactions with ClpC1 and ClpC2

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Mycobacterium tuberculosis (Mtb) is an ancient bacterial pathogen that has plagued human life for millennia, and still causes greater global mortality than any other bacterial infection. Increased resistance of Mtb strains to existing antibacterial drugs drives an urgent need for the development of novel therapeutics. The Clp family of ATP-dependent proteases is one promising group of Mtb drug targets. Clp protease are essential for viability in Mtb, and compounds that disrupt their activity have therapeutic potential against drug-resistant Mtb [1][2]. These enzymes consist of two major components: a ring-shaped unfoldase (ClpC1 or ClpX) and a barrel-shaped serine peptidase (ClpP1P2). Clp unfoldases selectively recognize substrate proteins, unfold them using energy from ATP hydrolysis, and pass them into the associated peptidase for destruction. Interestingly, it was recently shown that the N-terminal domain (NTD) of the distantly related ClpC in Bacillus subtilis (Bsu) recognizes post-translational phosphoarginine (pArg) modifications as markers for degradation [3]. With the use of phosphoproteomics in Mycolicibacterium smegmatis, a non-infectious surrogate of Mtb, we show that these modifications exist in mycobacteria [4]. Based on strong sequence and structural homology between BsuClpC and MtbClpC1 NTDs, we hypothesize that pArg modifications in mycobacteria act with similar function as markers for degradation of misfolded proteins. Additionally, our crystallographic and biophysical data suggests that pArg binds and disrupts the oligomeric state of ClpC2, a small stress-associated protein with strong homology to the NTD of ClpC1. ClpC2 also tightly binds ClpC1-targeting antibiotics and plays a role in transcriptional regulation [5]. Providing a possible mechanism of pArg binding to perturb mycobacterial persistence. Our work provides the first direct evidence of pArg in mycobacteria and point towards a role for these modifications in stress pathways.

ABS#177

Poster session, July 13

Bacterial Endochitinase Inhibits Breast Cancer Cell Proliferation and Migration

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Chitinase enzyme belongs to the glycosyl hydrolase family 18 and is widely expressed in prokaryotes and eukaryotes, including mammals. Several chitinases and chitinase-like proteins have been reported in mammals, despite the absence of endogenous chitin. However, the

studies on their physiological functions are obscure. Hence, this study aims to investigate the effect of chitinase and chitinase-like proteins on cell proliferation and migration. The endochitinase gene 'chiC' and exochitinase gene "chiB' from Serratia marcescens as well as human chitinase 3-like 1 glycoprotein, CHI3L1 from Homo sapiens were cloned in a bacterial expression vector. The recombinant proteins were expressed, and purified by affinity chromatography. Different concentrations of the purified proteins were used to study the cell viability of the breast cancer cell line (MCF-7 cells). Cell viability was measured by MTT and WST1 assay, and the effect of chitinase and chitinase-like protein on cell proliferation was studied by clonogenic assay. The migrational study was analyzed by wound healing assay, trans-well migration, and invasion assays. Further, the expression of certain EMT marker genes at mRNA level was analyzed by qRT-PCR. Cell cycle analysis of PI-stained cells was also done along with a few proliferation markers such as p-ERK ½, p-AKT, and SMP 30. It was observed that bacterial endochitinase (chiC) reduced cell viability, cell migration, and invasion significantly. These observations along with our in silico molecular docking analysis suggest that chiC probably reduced cell migration by cleaving the glycosyl residues of glycosylated surface proteins required for cell adherence and migration owing to the structural similarities between the chitin and the hyaluronic acid present on the mammalian cell surface.

Keywords: chitinase, cancer cell migration, cell proliferation

ABS#179

Poster session, July 15

Chemigenetic Fluorescent Indicators for Metal Ions: Engineering Protein Functions Using Synthetic Ligands

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Fluorescent indicators are molecules that change their fluorescence properties (intensity, wavelength, etc.) upon binding or reaction with a substance of interest. They are widely used in biology because they enable visualization of the dynamic behavior of targets, such as calcium ion, in living cells and tissues with high sensitivity. To date, genetically encoded biosensors based on a (green) fluorescent protein are the most popular indicators, but they can be created only when there is a known protein-based binding domain for the target. Recently, attempts have been also made to develop "chemigenetic" indicators, which are composed of both a protein component and a synthetic molecule component. For example, there are several reports where an environmentally sensitive synthetic fluorescent dye has been covalently bound to a HaloTag protein [1], genetically fused with a target-binding protein [2]. We reasoned that, by covalently attaching synthetic ligands for target substances to fluorescent proteins, we may be able to create indicators that are specific to targets for which there is no known protein-based binding domain. Accordingly, we developed a new design of chemigenetic calcium and sodium ion indicators consisting of a small molecule chelator and a fluorescent protein, connected by HaloTag [3]. After extensive protein engineering, the new calcium ion indicator showed large fluorescence response and could be applied for live cell imaging. In a related project, we also developed high-performance chemigenetic potassium ion indicators based on a synthetic dye and potassium binding peptides, through iterative directed evolution of the protein. By further optimization, these indicators will provide biologist end-users with new tools and capabilities.

ABS#182

Structure Prediction and Design (July 15, AM)

Structure of a beta-lactamase variant from an evolution-informed design strategy

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TEM-1 beta-lactamase is generated by bacteria to hydrolyze the beta-lactam ring of target compunds to confer antibiotic resistance. Since its discovery in the 1960s numerous variants have been identified, revealing a remarkable ability to adapt to challenge by penicillins and other drugs. The enzyme has been widely used as a model for understanding the nature of protein evolution. In vitro mutagenesis and the presence of naturally evolving variants have shown that a small of number of changes can have a profound impact on the stability and function of the enzyme. Here, we describe the crystal structure of an E.coli TEM-1 variant that retains its catalytic ability in spite of 84 amino acid differences over

262 residues. The variant was designed using a computational strategy that exploited sequence co-variation during evolution to predict fitness and retain fold/function. The residue changes are dispersed thoughout the 3-D structure of the enzyme and comprise both conserved and highly divergent changes in side chain chemistry. Interestingly, novel Arg/Trp stacking in the variant suggest that alternative local interactions may contribute to the overall preservation of enzyme structure and function.

ABS#185

Poster session, July 14

Structure of the cytosolic domain of the lysosome associated protein TMEM55B

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TMEM55B is a 284-residue membrane protein that localizes to lysosomes in eukaryotic cells. In association with JIP4, TMEM55B has been shown to regulate lysosomal positioning under various stress conditions. In addition, the protein is suggested to be a lipid phosphatase that catalyzes the production of phosphatidylinositol 5-phosphate. The 3-D structure of the protein has not been experimentally determined, and it lacks similarities to known proteins in databases. Topologically, 3-D predictions suggest that the N-terminus up to Arg206 is situated in the cytosol, followed by two transmembrane helices and a short 10-residue cytosolic tail. Here we report the crystal structure of an N-terminal cytosolic domain of TMEM55B comprising residues 80-166 at 1.8 Å resolution. The structure was determined successfully using the AlphaFOLD prediction as the search model for molecular replacement. It consists of two tandem β-sandwich motifs that pack together to form an extended and relatively flat globular domain. Each motif is stabilized by two Zn2+ atoms coordinated by cysteine residues with tetrahedral geometry. A DALI search of related folds identified the lysine transporter protein LysW from Thermus thermophilus is the closest structural relative with an RMSD of 2.3 Å over 41 residues for a single β-sheet motif. There are no significant sequence relations between LysW and TMEM55B apart from the cysteine residues that coordinate Zn2+.

ABS#186

Poster session, July 14

Relating Turnover Dynamics to Catalytic Efficiency Identifies Enzyme Mutants with Increased Activity

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Enzymes represent a versatile and efficient class of catalysts, critical for the biochemical reactions on which life depends and useful in many industrial and therapeutic applications. However, challenges persist in redesigning enzymes' catalytic functions, particularly when targeting increased activity. In this regime, most structure-based computational enzyme redesign approaches focus on the energetic difference between the enzyme-bound ground state and transition state. This framework, couched in transition state theory, typically neglects the structural and dynamical behaviors occurring inside the reactant well and along the path from reactant to product. We postulated that additionally treating the dynamics of complete turnover events could uncover structural characteristics affecting catalytic efficiency and guide the rational identification of mutants with improved catalytic properties. To test this idea, we redesigned ketol-acid reductoisomerase (KARI) for increased activity on one of its native substrates.

We modified and applied path sampling methods to gather hundreds of thousands of QM/MM atomistic simulations of attempted substrate turnover by KARI, and we used these simulations to compare productive (reactive) attempts with unproductive (non-reactive) attempts by training machine learning models to distinguish between their reactant well enzyme-substrate conformations, which established a relationship between reactant well dynamics and catalytic conversion. These models were then used to guide a structure-based protein redesign algorithm to predict mutations that stabilized productive structures relative to unproductive ones, and candidate mutants were screened computationally to eliminate ones that could impair activity in other ways. So far, this approach has identified eight enzyme mutants with a significant improvement in

calculated activity relative to wild type, with the best variant's improvement expected to be multiple orders of magnitude. Taken together, these results point to a relationship between reactant well enzyme-substrate dynamics and the efficiency of substrate turnover, which can be leveraged to inform redesign for increased activity.

ABS#187

Poster session, July 14

Engineering transketolase to react with pyruvate and aromatic aldehydes as substrates to produce intermediates for high value products

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Transketolase (TK, EC 2.2.1.1) is a vital rate-limiting enzyme for carbohydrate transformation of the pentose phosphate pathway (PPP) non-oxidative branch. TK is a homodimeric thiamine diphosphate (ThDP) and Mg2+-dependent enzyme which links glycolysis and PPP through the reversible transfer of a two-carbon ketol unit from D-xylulose-5-phosphate to either D-ribose-5-phosphate or D-erythrose-4-phosphate [1].

TK has valuable biocatalytic potential in the chemical industries as it has the ability to form C-C bonds stereospecifically, for the synthesis of complex carbohydrates and other high-value compounds. TK has been extensively engineered to accept aliphatic and aromatic aldehydes, and also to use pyruvate as the ketol donor. These reactions open up access to a diverse range of products such as analogues of phenyl acetyl carbinol (PAC), an important pharmaceutical intermediate [2]. In my present study, TK was engineered to accept both pyruvate, as a novel donor substrate, and unnatural acceptor aromatic aldehydes 3-formylbenzoic acid, 4-formylbenzoic acid (FBA), and 3-hydroxybenzoic acid (HBA). A small library of 8 TK variants were engineered carrying 7-8 mutations. Screening of this library identified the best variant with a 40-fold improvement in the yield towards pyruvate and FBAs, relative to wild-type (WT) using reverse phase HPLC analysis. The best variant was able to convert 53% of the substrate into product within 24h, whereas no conversion was observed for WT. The synthesis of 3-(1-hydroxy-2-oxopropyl) benzoic acid was confirmed by NMR and Mass Spectrometric analysis. Molecular Docking experiments suggested cooperation between the mutations responsible for donor and acceptor recognition, which would promote the activity towards both the acceptor and donor. The variants have the potential to be used for developing catalytic pathways to a diverse range of high-value products such as important pharmaceutical intermediates in the synthesis of drugs [3].

ABS#191

Poster session, July 13

Crystal Violet as a Fluorescent Indicator Dye for Amyloid-β Oligomers

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Misfolding of amyloid proteins and their subsequent assembly into aggregates are associated with a class of diseases called amyloidosis, most prominently among them Alzheimer's disease. Alzheimer's disease is a fatal neurodegenerative disorder that affects one in ten seniors over the age of 65 in the US. It has no established cure, and no clinical tests for its definitive early diagnosis. Scientific evidence implicates a type of amyloid aggregates called globular oligomers as one of the biomarkers of and clinical contributors to the disease [1-3]. Yet, there is a lack of experimental techniques to detect, quantify and differentiate oligomers from other amyloid aggregates, thereby impeding the validation of the oligomer hypothesis. Currently, oligomer detection is limited to immunostaining with structure-specific antibodies. However, immunostaining cannot be easily modified for brain imaging of oligomers in vivo.

We are using a kinetics-based screening assay to investigate whether some fluorescent dyes display oligomer-specific responses [4]. Such selectivity would facilitate the detection of globular oligomers over their monomeric precursors or over fibril plaques. Our kinetic assay identified the triarylmethane dye Crystal Violet as selective indicator for Aβ42 oligomers grown under physiological conditions in vitro. This selectivity persisted when staining post-mortem brain tissues of APP/PS1 mice and of AD patients. Therefore, our kinetic screen represents a robust approach for identifying small dye molecules as potential oligomer-selective dyes (OSDs). Such OSDs, in

turn, provide promising starting points for the development of PET probes for pre-mortem and in situ imaging of amyloid oligomer deposits in humans.

ABS#192

Poster session, July 15

Alzheimer's Epitope Phospho-mimetic Full-length Tau Forms a Specific Fibril Fold without Cofactors as revealed by Solid-State NMR and CryoEM

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Alzheimer's Disease (AD) and many other neurodegenerative diseases are characterized by pathological fibrillar aggregates of the protein tau that act like prions: each disease has a specific fibril fold that spreads through the brain and that is well correlated with neurodegeneration [1]. Yet the chemical mechanisms by which this highly soluble protein aggregates into disease-specific structures is unknown, hampering development of therapeutics. Tau undergoes many post-translational modifications in vivo: specifically, the Alzheimer's PHF1 epitope[2] is used to identify pathological Alzheimer's tau fibrils and stage disease progression. As early tau aggregates are found to be positive for PHF1, we hypothesized that this phosphorylation at the PHF1-sites may drive tau towards the AD fold.

To elucidate how specific post-translational modifications in vivo may predispose tau to aggregate into specific folds, here we produced recombinant full-length tau with a phospho-mimetic mutation (Fig. 1a) to recapitulate the chemical properties of the PHF1 epitope. Surprisingly, this full-length tau formed amyloid fibrils without the use of polyanionic cofactors, unlike unmodified full-length tau. We are investigating the structure and dynamics of these fibrils by combining high-resolution MAS solid-state NMR (Fig. 1b) and cryoEM (Fig. 1c). Together, these techniques reveal that this tau fibril forms a three-layered core structure, more similar to the disease-specific ex vivo three-layered folds in progressive supranuclear palsy than the AD fold or the previously studied cofactor-derived in vitro tau. These results represent the first cofactor-free reproducible fibrillization of full-length tau. This work sheds light onto the chemical mechanisms that drive tau into specific prion folds, and on the complementarity of cryoEM and solid-state NMR for studying ordered and disordered protein domains.

ABS#193

Poster session, July 15

DAQ-Score Database: Deep-learning Based Quality Estimation of Cryo-EM Derived Protein Models

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An increasing number of protein structures are determined by cryo-electron microscopy (cryo-EM) as cryo-EM has become one of the most important methods to determine structures. On the other hand, it has been noticed that errors occur in the model building process from cryo-EM maps, probably more frequently than one might think, particularly when the map resolution is not very high. Thus, establishing quality assessment methods has become a crucial and urgent task for biomolecular structure determination with cryo-EM.

We have recently developed a quality assessment method to detect protein structural model outliers using machine learning techniques. Our method, called DAQ (Deep-learning-based Amino acid-wise model Quality) score, uses deep neural network to capture local density features of amino acids and atoms in proteins and assesses the likelihood that modeled residues in a structural model is correct [1]. DAQ is also able to detect not only errors in conformations but also shifts in sequence assignment to otherwise correct main-chain conformations, which is often not easy to detect by checking density fitting.

Here, we performed a PDB-scale model analysis by DAQ. We applied DAQ to around 10,000 protein structure models in PDB that were derived from cryo-EM maps deposited in Electron Microscopy Data Bank (EMDB). We report the tendency of common errors made in the models through the large-scale analysis. When authors deposited updated structure models to PDB over an initial model, we see clear improvement of DAQ score in the updated version of the model. A common type of errors observed include sequence shifts along alpha helices. Model assessment results with DAQ are made available in a database (https://daqdb.kiharalab.org) [2]. The top page and an example of an entry in the database is shown in Figure. The DAQ score can be computed on the Google Colab site (https://bit.ly/daq-score) or local machine (https://github.com/kiharalab/DAQ).

ABS#195

Poster session, July 13

Amyloid fibril self-assembly into 3-D gel clusters vs 2-D sheets

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The deposition of dense amyloid fibril plaques is a pathological hallmark of many human disorders, including many neurodegenerative diseases. Protein aggregation into nonbranching fibrils with a common cross-beta sheet structure characterizes these diseases. Amyloid fibrils also serve biological functions, such as the storage of peptide hormones or bacterial curli fibrils involved in biofilm formation. Due to the tendency of individual fibrils to self-assemble into large structures with unique structural properties, amyloid fibrils are also considered promising candidates for substrates or scaffolds of functional biomaterials. Yet, while the self-assembly of individual amyloid fibrils and associated oligomers from monomers has been extensively studied, the mechanisms causing these fibrils to assemble into larger supramolecular structures remain largely unknown. This will shed light on the processes regulating macroscopic plaque formation observed in disease and elucidate what suprastructures are emerging during fibril self-assembly into functional biomaterials.
In our study, we used pre-formed amyloid fibrils of lysozyme to investigate their self-assembly into supramolecular fibril networks as a function of solution pH or charge screening. We used preformed, isolated fibrils to separate the complex mechanisms regulating amyloid fibril growth from the intrinsic self-assembly behavior of amyloid fibrils into different types of fibrillar suprastructures. Our findings indicate that fibrils assemble into either disordered 3-D gel clusters or ordered 2-D fibril sheets. The latter exhibited the characteristic optical birefringence of amyloid plaques observed in vivo. Upon reversing solution conditions to those supporting isolated fibrils, we found plaques to be significantly more stable than gel clusters. We propose that the reduction in charge repulsion upon changes in solution pH and ionic strength allows short-range anisotropic fibril-fibril interactions to emerge. These, in turn, drive the transition from kinetically trapped three-dimensional gels toward two-dimensional plaques [1].

ABS#196

RNA-Protein Machines: Ancient Synergies (July 14, AM)

Elucidating the Mechanism of Ebolavirus Matrix Protein Dimer Stability and Oligomerization using Peptidomimetics

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Ebolavirus is a filamentous negative-strand RNA virus that can cause severe hemorrhagic fever in humans with high fatality rates. The matrix protein (VP40) of ebolavirus is a structural protein required for viral assembly and budding. VP40 can perform multiple functions during the viral lifecycle with different structures that form through VP40 oligomerization mechanisms. VP40 forms a stable dimer required for filamentous viral particle formation and an RNA-binding ring octamer to regulate viral transcription. We aim to understand the equilibrium between different VP40 oligomeric forms using peptides that probe the dimer interface. VP40 dimerizes by two alpha helices in the N-terminal domain of each monomer. A library of compounds mimicking the VP40 dimer interface with different chemical moieties and position of staples, to maintain the secondary structure, was synthesized (Fig1a). Peptides were screened using a thermal shift assay for changes induced to VP40 protein stability (Fig1b). Peptides that significantly decreased the melting temperature of VP40 were selected and the binding affinity of the peptides to the VP40 dimer was determined using isothermal titration calorimetry. A peptide with a benzene staple at the C-terminus exhibited the highest binding affinity of 55 μM with VP40 dimer (Fig1c). This peptide was then incubated with VP40 dimer and subject to size exclusion chromatography. We observed a peak corresponding to monomeric VP40 in addition to dimer in the presence of peptide (Fig1d). This indicates that the peptide is shifting the equilibrium towards the formation of VP40 monomer. The selected peptide was also able to bind the VP40 octamer as the dimer interface is exposed in the octamer structure. Further structural studies to understand the binding mode of peptide to the VP40 dimer interface is underway along with cellular studies to assess the effect of peptide on filamentous virus particle formation. This study shows that a stapled peptide mimicking the dimer interface binds to VP40 and disrupts the

dimer interface causing a shift in equilibrium toward VP40 monomer formation.

ABS#197

Engineering Protein Fate and Function (July 16 AM)

Engineering of a Highly Efficient Polyethylene Terephthalate Hydrolase via High-throughput Enzyme Evolution and Screening

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Engineering of a Highly Efficient Polyethylene Terephthalate Hydrolase via High-throughput Enzyme Evolution and Screening
Plastics are staples of modern life, seemingly present in all sectors of society and industry. However, pollution of this manmade plastic has become a global crisis. The majority of plastic produced is fated for landfills and the environment, which threatens the health of ecosystems and communities worldwide. Further, even when recycled, conventional recycling methods struggle with mixed waste streams, and the plastic is usually "down-cycled" to lesser valued materials. This presents an impetus for improving the methods to degrade and recycle plastic waste. Recent studies have discovered microbial enzymes that are able to catalyze the breakdown of plastics. In particular, many of these enzymes are active on the plastic polyethylene terephthalate (PET), commonly used in food/beverage containers and textiles. However, these enzymes are not yet suitable for industrial recycling processes, as they lack the stability, activity, and productibility for these processes to be efficient and cost-effective. Towards this end, we developed a novel, high-throughput screening assay for engineering PET hydrolase enzymes, which is able to evaluate unprecedented sizes of enzyme libraries for activity and expression simultaneously. We then applied this novel screening method to a recently-reported PET hydrolase, the goal of which was to engineer this enzyme for improved, efficient degradation of PET plastic waste. Leveraging rational design and directed evolution approaches, we were able to discover enzyme mutants with significantly increased degradation activity toward various PET substrates, in addition to increased stability and expression levels. This work has demonstrated the utility of our novel screening assay for PET hydrolases, which will greatly accelerate the discovery of further improved enzyme mutants, as well as provide highly-active enzyme mutants that can be deployed in bio-recycling processes to "close the loop" on the PET plastics economy and mitigate environmental and landfilled plastic waste.

ABS#198

Capturing Protein Interactions (July 14, PM)

Rewired interaction specificity biases ligand-dependent activation in a mutant tyrosine phosphatase

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Cell signaling proteins are tightly regulated switches that are allosterically activated or inhibited in response to specific cues, such as protein-protein interactions or post-translational modifications. Many disease-associated mutations in signaling proteins disrupt these regulatory mechanisms by shifting the conformational equilibria of these proteins toward less signal-responsive active or inactive states. In this presentation, I will describe our efforts to characterize disease-associated mutations in the tyrosine phosphatase SHP2 that do not operate by directly disrupting allosteric regulation. SHP2 is canonically regulated through auto-inhibitory interactions between its phosphatase domain and its two phosphotyrosine-recognition (SH2) domains. Binding of phosphoproteins to the SH2 domains drives a structural rearrangement that unleashes phosphatase activity in a signal-responsive manner. While most disease-relevant mutations lie at auto-inhibitory interdomain interfaces and hyperactivate SHP2, some mutations lie in the ligand-binding pockets of SH2 domains and have no impact on auto-inhibition. I will discuss one such mutation that we have characterized using a combination of high-throughput biochemical screens, biophysical measurements, molecular dynamics simulations, and cellular assays. Our analyses reveal anomalous, mutation-dependent remodeling of the SH2 domain ligand-binding pocket, which leads to altered phospho-peptide sequence preferences. The functional consequence of this altered specificity is that the mutant SHP2 is sensitized to some activating ligands, but not others, leading to a potential

rewiring of cell signaling. Our study points to a nuanced but potentially prevalent mechanism of action for disease-associated mutations, characterized by changes in protein-protein interaction specificity.

ABS#199

Poster session, July 15

Interaction of Kif7 and Gli2-ZF probed by hydrogen deuterium exchange mass spectrometry

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A conserved feature of Hedgehog signaling is localization of the main effector proteins, Gli (glioma associated protein) transcription factors to microtubules in the primary cilium. The non-motile kinesin-4 protein Kif7 mediates the recruitment of Gli to microtubules. To investigate the structural basis of Kif7-Gli interaction and gain insights into how this modulates Kif7-microtubule binding we performed Hydrogen Deuterium Exchange mass spectrometry (Waters Synapt G2Si HDMSE) on wild type and mutant Kif7-Gli complexes.

WT-Kif7 was compared with a mutant [E502A-Kif7, a dimer but defective in Gli binding], using two different lengths of Kif7 construct: the coiled-coil region only (CC-short) and the coiled-coil with the neck and the motor domain, (CC-MD-Long). There were no measurable differences in HDX between CC-short constructs of WT-Kif7 and E502A-Kif7. Both proteins were more dynamic at the coiled-coil region. However, the comparison between CC-MD-Long of WT and E502A showed the central region of the coiled-coil of the E502A mutant to be more deprotected. We hypothesize that the neck and the motor domains of Kif7 communicate with the coiled-coil region, either through direct quaternary contacts or through allosteric interactions.

The increased protection in the Kif7 coiled-coil region when Gli2-ZF bound to WT-Kif7-CC-short and the Kif7-CC-MD-Long is consistent with biochemical measurements showing, the coiled-coil region forms one of the strongest binding sites for the Gli2-ZF. The mutation at E502A-Kif-7 which is away from the binding site of the Gli2-ZF showed comparatively less protection at the coiled-coil region when alone. In context with the reported biochemical data, the HDX-MS data provided new insight into the allosteric crosstalk due to E502A mutation, leading to the formation of an unstable complex with the Gli2-ZF with low binding affinity. We conclude that HDX-MS of ciliary kinesin Kif7 describes a role for long-range structural interactions in regulating kinesin-microtubule binding by a transcription factor.

ABS#200

Poster session, July 15

Mechanistic and Structural Studies of the Membrane-associated RhoGEF P-Rex and its Modes of Regulation

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Signaling scaffolds can assemble at the cell membrane and form hubs where signaling pathways converge to fine-tune signal transduction. Misregulation of these pathways frequently leads to disease states. We are focused on understanding regulation of Rho guanine-nucleotide exchange factors (RhoGEFs) implicated in cancer metastasis and how these molecules transition from the unactivated to fully active states. Phosphatidylinositol 3,4,5-trisphosphate (PIP3)-dependent Rac exchanger (P-Rex) is a RhoGEF that regulates cell motility through its activation of small GTPases such as Rac1. P-Rex is synergistically recruited to the cell membrane and activated by PIP3 and Gβγ subunits and is a critical regulator of cell migration. Aberrant upregulation of P-Rex is strongly associated with cancer metastasis, and as such it has become an attractive therapeutic target. Our objective is to define the molecular basis for the regulation of P-Rex with the aim of identifying its important regulatory surfaces and mechanisms of activation. We have taken a structural biology approach to address this goal. We generated a 4.1 Å resolution cryo-electron microscopy structure of full-length P-Rex1 in a proposed autoinhibited state. Here, the catalytic core makes unexpected contacts with the C-terminal domain, resulting in a condensed, "wrapped-up" conformation of P-Rex1, occluding the substrate binding site. This is supported by hydrogen/deuterium exchange mass spectrometry data, and further experiments show that P-Rex1 becomes

"unwrapped" when bound to liposomes containing PIP3, exposing the substrate binding site. Furthermore, we have generated a preliminary, low-resolution map for the unactivated state of the closely related RhoGEF P-Rex2 that suggests P-Rex2 may have a different domain architecture from P-Rex1, implying that P-Rex2 may be regulated differently. Our data are consistent with a multifaceted mechanism of activation for P-Rex. By investigating P-Rex structure and regulation through a variety of complementary approaches, we hope to guide the development of therapeutic molecules.

ABS#201

Poster session, July 13

Determining Structural Details of P-Rex2 Inhibition through PTEN

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Rho guanine-nucleotide exchange factors (RhoGEFs) accelerate the function of small GTPases to drive cell motility and proliferation. Phosphatidylinositol 3,4,5-triphosphate (PIP3)-dependent Rac exchanger 2 (P-Rex2) is a RhoGEF frequently mutated in a number of cancers. These mutations allow the protein to escape normal routes of inhibition through unknown mechanisms to drive cancer metastasis. Previous studies on P-Rex2 and its close homolog P-Rex1 show that they maintain low basal activity through autoinhibition by domains outside of the catalytic core which is composed of the Dbl homology and pleckstrin homology (PH) domain tandem. Unlike P-Rex1, P-Rex2 is additionally inhibited through binding PTEN, a canonical tumor suppressor, through unclear contacts. The paucity of P-Rex2 structural information prevents us from detailing these inhibitory mechanisms and exploring P-Rex2 as a potential therapeutic target. We hypothesize that P-Rex2 autoinhibition occurs through interdomain contacts between the PH domain and the IP4P domain and that binding PTEN strengthens this interaction. To test this hypothesis, we are using cryo-electron microscopy (cryo-EM) to resolve the full-length P-Rex2 structure in the absence of activators and in complex with PTEN. We have generated a preliminary, low-resolution cryo-EM map of full-length P-Rex2 that suggests P-Rex2 domain architecture may diverge from P-Rex1. This difference may contribute to why PTEN can regulate P-Rex2, but not P-Rex1. Furthermore, we are investigating the P-Rex2 domains that bind PTEN through characterizing P-Rex2 chimeras containing single domains swapped from P-Rex1. Our preliminary characterization of the chimeras shows changes to P-Rex2 basal activity. Overall, defining the structural details of P-Rex2 regulation will open doors for rational drug design to target P-Rex2 therapeutically.

ABS#203

Poster session, July 14

Investigating Dynamics and Function of Non-Canonical Sensor Histidine Kinases

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Background: One way bacteria sense and adapt to the environment is with two component systems (TCSs), canonically comprised of a sensor histidine kinase (SHK) and cognate response regulator (RR). Signal input to the SHK sensor domain, such as the blue light sensing light-oxygen-voltage (LOV) or small molecule binding Per-ARNT-Sim (PAS), modulates catalytic domain autophosphorylation and transfer of the phosphoryl group to the RR. However, the standard SHK model seems to fail to describe the lesser-studied HWE/HisKA2 family, which are often not dimeric and are known to participate in the general stress response (GSR) networks of Alphaproteobacteria. Methods: Size exclusion chromatography multi-angle light scattering (SEC-MALS) to assess protein oligomeric state and conformation. Hydrogen deuterium exchange mass spectrometry (HDX-MS), NMR, and limited proteolysis to investigate protein dynamics. Measured 32P incorporation to assay autophosphorylation rates. Results: Three novel HWE/HisKA2 family SHKs exhibit non-canonical features. The LOV-HK RT349 autophosphorylates at a higher rate in the dark state, making it the only known naturally occurring LOV-HK with inverted signaling logic. In the lit state, HDX-MS shows increased deuterium exchange, NMR shows increased chemical shift dispersion, and limited proteolysis shows increased susceptibility to digestion, highlighting the complexity of this system. The PAS-HKs RE356 & MS367 are in equilibria between monomeric and dimeric states with different levels of activity. NMR for RE356 shows global conformational shifts upon AMP-PNP binding. Efforts are currently underway to determine RR partners of the three SHKs— this will help to elucidate their

roles in the GSR. Conclusion: These results contribute to mounting evidence that HWE/HisKA2 family members are unique in their oligomeric states and functional activity, offering new perspectives on SHK function and dynamics.

ABS#206

Poster session, July 13

Investigating the evolutionary trajectory of PROM-1 through conservation analysis in the context of sterol biosynthesis

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Prominin-1/CD133 (Prom1) is an integral membrane protein with five transmembrane helices that scaffolds protrusions in the plasma membrane. Prom1 is highly conserved among metazoans and widely expressed in human tissues, with clinical significance in retinal degeneration, stem cell biology, and many types of cancer.
Initial investigations show that mammalian Prom1 stably binds cholesterol, bends the plasma membrane, forms microvesicles, binds partner proteins, and oligomerizes. The cholesterol binding of Prom1 may regulate its membrane bending and scaffolding functions (TAB, unpublished)(1).
There is a progressive evolutionary shift in fungi from cholesterol to ergosterol as the dominant sterol through a diverse set of structurally intermediate sterols (2). Our goal is to investigate the evolutionary trajectory of Prom1 in the context of this transition and test how that affects its functions.
Our phylogenetic analysis suggests that Prom1 homologs can be found in excavata, archaea, and fungi. We used sequence alignment and targeted conservation analysis to test Prom1 residue conservation in opisthokonts, the smallest supergroup containing animals and fungi. Our findings show a tryptophan residue in the transmembrane region of Prom1 that is highly conserved among metazoans and was identified as the site of a pathogenic mutation linked to cone and cone-rod dystrophy(3). Based on structure models, we found an external leucine as well as several other external-facing residues in the transmembrane region that are highly conserved across opisthokonts. Since they are not predicted to be involved in a dimerization interface, we hypothesize that they might constitute a binding site for other proteins.
We will mutate the residues conserved among all opisthokonts and only among metazoans and test if they correlate with established Prom1 functions. These assays will inform the molecular mechanisms of Prom1 opisthokont- and metazoan-specific functions in the evolutionary context of the shift between cholesterol and other sterols.

ABS#208

Poster session, July 14

Optimizing Protein Crystal Stability Using a Network of Disulfide Bonds

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A periplasmic polyisoprenoid binding protein from Campylobacter jejuni(CJ) forms highly porous protein crystals under the right conditions. The resulting array of 13-nm diameter solvent nanopores allows these crystals to act as a scaffold material, with a multitude of downstream applications. However, protein crystals are typically fragile and vulnerable to slight changes in their environment, such as variations in pH, ionic strength, or temperature. Disulfide bonds can stabilize protein structures by linking distant regions of the protein. Similarly, we aim to stabilize a specific protein crystal assembly by introducing cysteines at key locations across protein-protein interfaces. Subsequent covalent bonds formed between two interfacial cysteine residues have the potential to stabilize the crystal matrix and successful disulfide bond formation will lead to the formation of protein polymers, templated by the crystal lattice. In fact, due to the P622 symmetry of the CJ crystals composed of domain swapped dimers, our intended polymers would form a 6-fold braid along the z-axis of the CJ crystals. Using this strategy, we have grown variant crystals dubbed "CJ-Braid," which showed increased thermal and chemical stability compared to the wild-type protein, without the need for third-party crosslinking agents. This strategy has the potential to eliminate the time-consuming crosslinking process, with potential benefits to the repeatable product crystal production as well as eliminating potential carry-over of reactive reagents in the final product. Thus, full validation of the new CJ-Braid variant crystals via x-ray diffraction will improve the practical prospects for deploying CJ crystals for applications in structural biology and biotechnology.

ABS#209

Poster session, July 14

AF2BIND: Prediction of protein-peptide and protein-ligand binding sites using AlphaFold

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The accurate prediction of binding sites in proteins remains an outstanding challenge, despite its potential to accelerate drug discovery and inform on natural protein function. Here, we train a simple MLP neural network, AF2BIND, using embedding features from a protein structure prediction model, AlphaFold2, to accurately predict the binding epitopes of proteins from single structures. AF2BIND effectively captures binding signatures of small-molecule- and peptide-binding sites, without knowledge of the true ligand, achieving state-of-the-art performance compared with other neural-network predictors such as dMaSIF. We envision AF2BIND can be used to improve molecular docking and rapidly identify the most ligandable sites in proteins, needing only the protein sequence as input.

ABS#210

Engineering Protein Fate and Function (July 16 AM)

Rheostats, Toggles, and Neutrals, Oh My!

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Advances in personalized medicine, pathogen evolution, and protein engineering require accurately predicting the effects of amino acid substitutions. To that end, decades of mutation experiments and analyses of protein families have illuminated several widely-used textbook "rules" upon which algorithms are built. However, before ~2012, most studies were biased to evolutionarily-conserved positions; most changes at these positions are catastrophic and "toggle off" function. Changes at non-conserved positions were largely overlooked, despite their key roles in paralog evolution. Post-2012, most studies of nonconserved positions used techniques that agglomerate effects on protein expression with effects on function, which thwarts predictions from first principles. Our detailed studies of nonconserved positions identified a special class of "rheostat" positions that have striking abilities to modulate function when substituted. Rheostat positions have been found in globular soluble, integral membrane, and intrinsically disordered proteins; within a protein, the density of rheostat positions can vary from >40% to near zero. Surprisingly, functional outcomes are not always explained by side chain similarities. Additionally: (1) The rheostat character of a position ranges between the all-or-none substitution behaviors of "toggle" positions and the mutational insensitivity of neutral positions. (2) Some rheostat positions simultaneously modulate multiple functional parameters, e.g., Kd and allosteric coupling; some alter ligand specificity; some alter protein expression and/or stability. (3) The locations of functional rheostat positions show intriguing overlap with positions involved in allosteric regulation. (4) Structural studies show only local perturbations. (5) Dynamic coupling calculations for rheostat substitutions show promising correlations with measured functional changes. Combined, results suggest that emergent properties of coupled amino acid networks could produce the complex outcomes observed for rheostat substitutions.

ABS#211

Poster session, July 15

The Effects of Kinetic Stabilizers on the Structural Dynamics of Amyloidogenic Immunoglobulin Light Chain Using Hydrogen Deuterium Exchange Mass Spectrometry

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AL amyloidosis is a disease caused by the deposition of immunoglobulin light chain (LC) fibrils in several tissues. Different patient-to-patient amino acid LC sequences pose a significant challenge in designing a single therapeutic approach that works for all patients. Recent

research led to potential broad-spectrum small molecule kinetic stabilizers designed to bind to the most conserved amino acid residues and reduce the conformational flexibility of LCs. Here, we evaluated six kinetic stabilizers [1] and their effects on the conformational dynamics of various amyloidogenic (AL, H3, H6, H7, H9, H16, MCG) and non-amyloidogenic LCs (GL, MM). LCs were overexpressed in E coli and purified with affinity chromatography. Deuterium incorporation (HDX) was measured by mass spectrometry (MS) for free and compound-bound forms. Compound M83 had the most significant impact in reducing LC flexibility, consistent with its status as the most potent stabilizer in a fluorescence polarization assay. Increased protection was seen in all LC isoforms tested, with the most significant effects observed in segments corresponding to CDR3, at the dimeric interface. M83 was more effective in providing protection to the non-disulfide tethered dimer and more flexible mutant AL C214S, but it had little to no effect on the isolated variable domain, indicating that the compound stabilizes the entire LC instead of just the isolated variable domains. The degree of protection varied among mutants, and was lower for non-amyloidogenic LCs, reflecting the different intrinsic stability of each isoform. Our findings demonstrate the utility of HDX MS in determining the impact of kinetic stabilizers on LC conformational dynamics and how kinetic stabilizers could be refined for the treatment of AL amyloidosis.

ABS#213

Poster session, July 15

High-Throughput Measurement and Computational Prediction of Dominant-Negative Protein Fragments

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High-Throughput Measurement and Computational Prediction of Dominant-Negative Protein Fragments
Massively-parallel measurements of growth inhibition by protein fragments have been used to discover peptide inhibitors and map protein interaction sites. However, the underlying principles governing fragment-based inhibition have thus far remained unclear. Here, we adapt a high-throughput inhibitory fragment assay for use in E. coli, applying it to a set of ten essential proteins. This approach yielded single amino acid resolution maps of inhibitory activity, with peaks localized to functionally important interaction sites, including oligomerization interfaces and folding contacts. Leveraging these data, we perform a systematic analysis to uncover principles of fragment-based inhibition. We determine a robust negative correlation between susceptibility to inhibition and cellular protein concentration, demonstrating that inhibitory fragments likely act primarily by titrating native protein interactions. We also characterize a series of tradeoffs related to fragment length, showing that shorter peptides allow higher-resolution mapping but suffer from lower activity. We employ an unsupervised statistical analysis to show that the inhibitory activities of protein fragments are largely driven not by generic properties such as hydrophobicity, but by the more specific characteristics of their bespoke macromolecular interactions. Furthermore, high-throughput AlphaFold modeling of fragment-protein binding reveals that the inhibitory activity of protein fragments is strongly associated with their predicted ability to form native-like interactions, suggesting a path to extract design principles for inhibitory fragments. We show that our computational approach is highly effective at predicting protein interaction-inhibitory fragments across diverse proteins of origin. Overall, this work demonstrates fundamental characteristics of inhibitory protein fragment function and provides a foundation for understanding and controlling protein interactions in vivo.

ABS#214

Poster session, July 13

Uncovering the Mechanisms of Novel PAS Domain Chemosensory Transcriptional Regulators

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One-component signal transduction comprises most of the cellular communication in prokaryotes. Within this interesting class of signaling proteins lies the Per-ARNT-Sim (PAS) domain, a superfamily of domains that sense and respond to environmental stimuli such as light,

oxygen, metals, fatty acids, or temperature by binding ligands within their cavity. When found within helix-turn-helix (HTH) transcriptional activators, these PAS-HTH proteins can become powerful tools for control over gene expression. Our lab has previously engineered one of these proteins into a successful blue light-driven optogenetic system, leading us to wonder if we can similarly develop PAS-HTHs into chemosensory transcriptional regulators that can be activated via small molecule recognition. Using a combination of bioinformatic and computational methods, we have discovered PAS-HTHs that have the potential for unique cofactor binding abilities. By combining NMR spectroscopy, mass spectrometry, differential scanning fluorimetry, and X-ray crystallography we aim to better understand the structure-function relationship of three novel PAS-HTH bacterial transcription factors with a varying range of ligand preference and dynamics. Uncovering critical information regarding multimerization state will lend insights into requirements for activation, like we have seen with our blue-light driven system, EL222, activation triggers changes in intramolecular domain interactions allowing for DNA binding. This work will lead to a deeper understanding of one-component PAS domain signal transduction and allow for the development of tools to function as inducible gene expression systems and reporter assays through bioengineering.

ABS#215

Poster session, July 15

Contributions from ClpS surface residues in modulating N-terminal peptide binding and their implications for NAAB development

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Numerous technologies are currently in development for use in next-generation protein sequencing platforms. A notable published approach employs fluorescently-tagged binding proteins to identity the N-terminus of immobilized peptides, in-between rounds of digestion. This approach makes use of N-terminal amino acid binder (NAAB) proteins, which would identify amino acids by chemical and shape complementarity. One source of NAABs is the ClpS protein family, which serve to recruit proteins to bacterial proteosomes based on the identity of the N-terminal amino acid. In this study, a Thermosynechococcus vestitus (also known as Thermosynechococcus elongatus) ClpS2 protein was used as the starting point for direct evolution of a NAAB with affinity and specificity for N-terminal leucine. Enriched variants were analyzed and shown to improve the interaction between the ClpS surface and the peptide chain, without increasing promiscuity. Interestingly, interactions were found that were unanticipated which favor different charged residues located at position 5 from the N-terminus of a target peptide.

ABS#216

Undergraduate Research Session

K63-linked polyubiquitinated substrates induce phase separation of ubiquitin shuttle proteins

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Ubiquitin shuttle proteins can facilitate protein degradation by delivering ubiquitinated substrates to proteasomes. They may also protect substrates from degradation under certain cellular conditions. Shuttle proteins simultaneously bind ubiquitinated substrates via C-terminal ubiquitin associated domains and proteasomal ubiquitin receptors via N-terminal ubiquitin-like domains. STI1-like domains in central intrinsically disordered regions allow for self-interaction of some shuttle proteins like human ubiquilin 2 (UBQLN2). Conditions promoting UBQLN2 oligomerization have been characterized, whereby the presence of K63-linked polyubiquitin chains promotes liquid-liquid phase separation in vitro. However, less is known about the way in which shuttle proteins interact with ubiquitinated substrates. Using sedimentation assays, we examined how ubiquitinated substrates impact the phase separation behavior of UBQLN2 and human Rad23B as well as their yeast homologs Dsk2 and Rad23. In all cases, K63-linked polyubiquitinated substrates promoted phase separation of shuttle proteins in a concentration-dependent manner. UBQLN2 phase separated more readily than the other shuttle proteins, and the 26S proteasome also could co-

sediment with UBQLN2 and K63-linked substrates. In contrast, K48-linked substrates did not significantly induce phase separation for any of these shuttle proteins. Interestingly, K63-linked substrates did not always phase separate with the shuttle proteins. Under phase separation conditions, K63-linked substrates robustly co-sedimented with UBQLN2 but not Rad23B, Dsk2, or Rad23. These findings suggest that although K63-linked ubiquitin controls phase separation of multiple shuttle proteins, different phase separation mechanisms are at play.

ABS#218

Poster session, July 14

Escherichia coli Proteome Adaptation Upon ClpP Protease Inhibition

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Bortezomib (BTZ) is a proteasome inhibitor approved by the FDA for treatment of multiple myeloma. Although its main method of action is binding to the 26S proteasome, it also has off-target effects on serine proteases, including Caseinolytic protease subunit P (ClpP). Recently, BTZ has been used to increase the yield of heterologous protein expression in E. coli with minimal effects on cell culture growth, despite disrupting essential proteases. How BTZ affects the moderate phenotype of inhibited protein degradation without impacting cell culture growth remains unclear. Our objective is to investigate BTZ's mechanism of action in E. coli by quantifying changes in protein abundance across the proteome. We treated E. coli with increasing amounts of BTZ, up to six-fold higher than the working concentration, and performed bottom-up proteomics. We classify key proteins and pathways significantly affected by BTZ treatment. Surprisingly, 95.4% of the observed proteome was not significantly up- or down-regulated by BTZ treatment, including many common chaperones and proteases; GroES, GroEL, GrpE, DnaK, DnaJ, trigger factor, ClpB, ClpX, and Lon. The upregulated subset of the proteome included many ribosomal proteins, and was significantly enriched for the ribosome assembly, translation, and nucleotide biosynthetic biological processes. The 42 downregulated proteins did not show any significant enrichment of gene ontology biological processes. Overall, these changes describe a shift in the cellular proteostasis network to compensate for inhibited protease activity.

ABS#222

Peptide Modalities: Size Doesn't Matter (July 14, AM)

Mapping the Conformational Change that Accompanies Functional Interaction of the E. coli Hsp70, DnaK, with its Nucleotide Exchange Factor, GrpE

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The 70-kDa heat shock proteins (Hsp70s) assist in protein folding and rescue proteins from aggregation using a mechanism that relies on allosteric communication between their nucleotide-binding-domain (NBD) and a substrate-binding domain (SBD) , which is mediated by their interdomain linker. The Hsp70 allosteric cycle is controlled by nucleotide binding/hydrolysis and is modulated by two co-chaperones: J-domain proteins, which help deliver substrates and stimulate ATP hydrolysis, and nucleotide exchange factors (NEFs), which promote the exchange of Hsp70-bound ADP by ATP. A structure of the complex between the NBD domain of E. coliHsp70, DnaK, carrying a point mutation G122D and its NEF, GrpE, in the absence of nucleotide was solved by x-ray crystallography and has served as the accepted model for their interaction (Harrison et al., Science [1997]). However, the facilitation of nucleotide exchange mediated by interaction between this Hsp70 and its NEF requires conformational change (Liu et al., PLoS Comp. Biol. [2010]). Moreover, data in the literature and preliminary observations from our lab have suggested that the structure of the GrpE/NBD apo-complex is not the same in solution as seen in the published crystal structure. Here, we report by NMR analysis that the DnaK NBD/GrpE/ADP complex in solution is consistent with the crystallized complex. By contrast, we observe that the structure in solution in the absence of nucleotide differs significantly from the crystal structure. We are validating the model for the apo-complex emerging from our NMR analysis by designing mutations and assessing their impact on the

binding affinity of the DnaK NBD to GrpE. The resulting structural picture offers a compelling case for the mechanism of nucleotide exchange rate enhancement triggered by GrpE binding to the DnaK NBD. The nucleotide-free complex also reveals a conformational change that can be tracked from the NBD/GrpE interaction interfaces to the interdomain linker, supporting an earlier hypothesis that there is allosteric communication from the NEF to the SBD (Melero et al., J. Biol. Chem. [2019]). [Supported by NIH grant GM18161 to LMG]

ABS#224

Poster session, July 15

Conformations of the HIV-1 Vpu protein in solution and lipids membranes: Insights from a multi-technique approach

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We study the structure-function relationship of the important for HIV-1 accessory protein Vpu. Currently, Vpu is considered as a primarily single-pass transmembrane protein existing in both monomeric and oligomeric states. However, recently we identified in vitro conditions under which Vpu forms stable soluble oligomers. We used protein engineering, size exclusion chromatography (SEC), cryo-EM and EPR spectroscopy to characterize these oligomers. We engineered chimera constructs of MBP or BRIL proteins fused to the N-terminus of Vpu (MBP-Vpu and BRIL-Vpu constructs) to increase the protein size, making it amenable to cryo-EM. SEC confirmed that both MBP-Vpu and BRIL-Vpu form oligomers in solution affirming that the observed oligomerization is Vpu-driven. We further found that Vpu self-associates via its transmembrane domain (Helix 1, H1) and the subsequent protein region plays an oligomer-stabilizing role. The cryo-EM analyses of soluble MBP-Vpu suggest hexamer or pentamer as plausible oligomeric states. The results from continuous wave (CW) EPR on the spin-labeled single cysteine mutants Q36C (numbering in Vpu sequence) in the Vpu C-terminal domain showed that the residue Q36C, which is in close proximity to the oligomer-forming H1, possesses restricted motional dynamics suggesting specific structure of this protein region and confirming the oligomer-stabilizing role. Differently, the observed narrow-lineshape CW EPR spectrum for the residue N55C suggests high conformational dynamics of the distant Vpu C-terminal. These results are strongly supported by DEER distance measurements on MBP-Vpu oligomers. The well-defined distances for Q36C reinforced the oligomeric structure of soluble Vpu. The DEER results for N55C revealed very long and difficult to estimate distances due to high conformational heterogeneity. Altogether our results report on an interesting property of a membrane protein, but could also help to uncover a physiological role of soluble Vpu. We will further discuss conformations of Vpu upon binding to membrane and crystallization of soluble BRIL-Vpu.

ABS#226

Peptide Modalities: Size Doesn't Matter (July 14, AM)

Gonococcal Peptide Mimetic Vaccine Antigen Forms a Beta-hairpin Structure and Binds to a Chimeric Antibody Primarily through Hydrophobic Interactions

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Neisseria gonorrhoeae (Ng) is the etiologic agent of the sexually transmitted infection, gonorrhea, which causes local genital infection and disseminated disease. A mouse monoclonal antibody (MAb), 2C7, recognizes a widely expressed glycan epitope in Ng lipooligosaccharide (LOS) and shows complement-dependent bactericidal activity. These findings supported development of the LOS epitope as a vaccine candidate. Previously, we identified a cyclic peptide mimic of the LOS epitope, CP2, by peptide phage display. Formulated as a multimer, CP2 protected mice against vaginal colonization by Ng. To determine

the structural basis for antibody protection by the mimetic peptide, we performed structural analyses of the human-mouse chimeric 2C7 antibody by X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and molecular dynamics (MD) simulations. We solved and refined the crystalline structures of the Fragment antigen-binding (Fab) 2C7 alone and bound to CP2 to 1.70-Å and 1.65-Å resolution, respectively. The structure of the complex showed that CP2 formed a beta-hairpin and bound the Fab heavy chain primarily through hydrophobic interactions. Comparison with the structure of Fab 2C7 alone indicated that there were no major conformational changes upon binding CP2. Using NMR spectroscopy, we mapped the epitope recognized by the intact chimeric MAb and defined CP2 residues in closest contact with 2C7. Furthermore, transferred nuclear Overhauser effect NMR spectroscopy (trNOESY) experiments, combined with MD simulations, identified the bioactive conformation of CP2 when interacting with 2C7 and, in combination with crystallographic studies, allowed to us to obtain a dynamic, three-dimensional structure of the complex. Collectively, our studies suggest strategies for humanizing MAb 2C7 as a therapeutic against gonococcal infection and for optimizing CP2 as a gonococcal vaccine.

ABS#227

Poster session, July 13

The Structure of Lipid-free Apolipoprotein A-I and the Amyloid-forming Mechanism of Its Variants

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Apolipoprotein A-I (apoA-I) plays an important role in clearing cholesterol and phospholipids from peripheral tissues - a process known as reverse cholesterol transport. This results in the formation of high-density lipoprotein, the 'good cholesterol'. However, the amyloid-forming propensity of apoA-I is also implicated in atherosclerosis and hereditary amyloidosis. This study aims to obtain crystal structure representative of the native lipid-free apoA-I and elucidate the structural mechanism of its amyloid formation – the former has been challenging to researchers for decades. Herein, we obtained crystal of apoA-I in complex with a crystallisation chaperone and solved the structure of lipid-free apoA-I at a resolution of 2.7 Å. The structure reveals that apoA-I is a four-helix bundle at the N-terminal domain (first 184 residues), whereas the C-terminal domain (CTD, residues 185-243) is putatively disordered and unresolved in the structure. This crystal structure was also validated by small angle X-ray scattering. Although the native apoA-I is relatively stable and does not form amyloid fibrils, structural perturbations such as methionine oxidation or various naturally occurring mutations were shown to cause fibril formation in full-length apoA-I within 24 h in vitro under a slightly acidic pH, though the absence of the CTD slowed down fibril formation. Furthermore, limited proteolysis showed that in full-length apoA-I fibrils, only approximately the first 100 residues are rigid and resistant to trypsin digestion. Using hydrogen-deuterium exchange (HDX) coupled with NMR, slowest amide HDX rates were seen in approximately residues 17-47, indicating this region makes up the fibril core. Our structure shows this amyloidogenic region is normally protected by the interactions within the helical bundle, thus destabilisation of the helical bundle conformation may expose this region promoting aberrant interactions which seed fibril formation. In summary, we have structurally characterised apoA-I and experimentally determined its amyloid-prone regions, which may guide design of amyloid-inhibiting agents.

ABS#229

Poster session, July 15

Differential Interaction and Conformational Responses of Isoforms of Disordered Cytoplasmic Domain of NMDA Receptor to Calcium

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N-methyl-D-aspartate (NMDA) ionotropic glutamate receptors are the key player in maintaining the neurotransmission, synaptic plasticity in the neurons.

C-terminal Domain (CTDs) of this receptor has diverse amino acid sequences and they interact with a variety of scaffolding proteins at the post-synapse to fulfill different functions. During calcium influx, the residual calcium ions from the influx modulate the conformations of these CTDs of two isoforms, GluN2A and 2B. The isoforms of the NMDA receptors have different roles in synaptic plasticity and their affinity to the other neighbor proteins. We have shown that GluN2B has 100-fold higher affinity to calcium compare to GluN2A using single molecule forster resonance energy transfer (smFRET), discrete molecular dynamics (DMD) simulation and other biophysical methods. To the best of our knowledge, we are reporting the conformational change and their differential affinities towards calcium for the first time. Furthermore, we have shown that the effect of calcium on the liquid-liquid phase separation that we reconstituted by incorporating post synaptic density 95KDa (PSD95) and synaptic Ras GTPase activating protein (synGAP) in it previously. Hence, the structural study of these disordered proteins and its implication to the LLPS formation in the presence of different intracellular calcium concentration paves new aspect in understanding the role of these tails of NMDA receptors in different signaling pathways.

ABS#231

Poster session, July 15

Ultra-High Affinity Transport Proteins from Ubiquitous Marine Bacteria Reveal Mechanisms and Global Patterns of Nutrient Uptake

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SAR11 bacteria are the most abundant bacteria in the global ocean microbiome and have a broad impact on ocean ecosystems. To thrive in competitive nutrient-poor environments, these bacteria rely on solute-binding proteins (SBPs) that enable nutrient uptake in association with ABC transporters. However, previous studies have relied on homology-based predictions and have therefore been unable to access the molecular mechanisms and functions of these transporters, which are essential for understanding assimilation of dissolved organic matter (DOM) in the ocean. Here, we addressed this problem by performing a comprehensive, genome-wide biochemical study of the SBPs from the prototypical SAR11 bacterium 'Candidatus Pelagibacter ubique' HTCC1062. We show that the transporters have unprecedented binding affinity (Kd ≥30 pM) and unexpectedly high binding specificity, revealing new molecular mechanisms for adaptation to nutrient-poor conditions. We uncover new potential carbon sources for SAR11 bacteria and provide accurate biogeographical maps of nutrient uptake in the ocean. Our results show how functional adaptation at the protein level in ubiquitous marine bacteria impacts global patterns of nutrient assimilation.

ABS#233

Poster session, July 14

Rapid Determination of the Topology of Helical Bundle Membrane Proteins by Water and Lipid-Edited Solid-State NMR Spectroscopy

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Coronavirus envelope (E) proteins belong to the family of viroporins, which oligomerize into ion-conducting alpha-helical bundles that change the ionic permeability of the cell membrane (1). As such, E proteins are antiviral drug targets (2). E proteins are too disordered and small for X-ray crystallography and cryoelectron microscopy. Thus, high-resolution structural information of E in lipid bilayers is so far limited to SARS-CoV-2 E (3), solved using solid-state NMR (ssNMR). The ssNMR approach for structure determination requires multiple 2D and 3D magic-angle-spinning (MAS) experiments that yield resonance assignment, helix orientation, and interhelical distance restraints (4). The large number of experiments requires long signal-averaging times and extensive spectral analysis. To accelerate the structure determination of these alpha-helical bundles, here we develop a novel water-edited and lipid-edited NMR approach, which provide key constraints to the helical bundle topology. Our approach starts with selective excitation of the water or lipid 1H magnetization, which is then transferred to the protein protons and detected in 13C spectra. This allows us to determine which sidechains are in close proximity to the water-filled channel pore versus the non-polar lipid chains. We exploit the high intensities of the methyl 13C signals of Leu, Val and Ile residues to facilitate the structural analysis. Comparisons of cross peak intensities in each 2D spectrum as well as between water- and lipid-

edited spectra give the numbers of water-accessible and lipid-accessible Leu, Val and Ile sidechains.
We demonstrate this approach on the structurally known SARS-CoV-2 E protein (3), then apply it to the E proteins of MERS and human coronavirus (hCoV) NL63. These results show subtle differences in the helical bundle topology of E among SARS, MERS and NL63 viruses, suggesting that these viroporins may have subtle differences in their ion conduction mechanisms.

ABS#235

Modern Anti-viral Strategies (July 13, AM)

Nanobody Technologies Against the Evolving COVID-19 Pandemic

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Recently, fascinated by the exciting biomedical potential of camelid VHH single-chain antibodies(nanobodies), we developed a disruptive proteomics technology for nanobody drug discovery (Xiang et.al Cell Systems 2021). This approach allows us for the first time, to deconvolute an unprecedented large repertoire of matured, circulating, and multi-epitope targeting nanobodies.
Using this approach, we have recently isolated thousands of ultrapotent nanobodies against SARS-CoV-2 including broad-spectrum nanobodies against the full spectrum of SARS-like viruses (Xiang et al Science 2020; Sun et al Nature Communications 2021; Xiang et al Cell Reports 2022; Tubiana et al Cell Reports 2022). In hamsters, we have shown the outstanding preclinical efficacy of an aerosolized nanobody construct for inhalation therapy of viral infection (Nambulli et al Science Advances 2021). The aerosol delivery facilitates deposition throughout the respiratory tract and dose minimization to 0.2 mg/kg. Inhalation treatment quickly reverses animals' weight loss after infection, decreases lung viral titers by 6 logs leading to drastically mitigated lung pathology, and prevents viral pneumonia.
Moreover, we have determined the structures of dozens of antiviral nanobodies targeting a plethora of novel epitopes on the receptor binding domain (RBD). Our efforts also revealed unprecedented antiviral mechanisms by nanobodies can leverage to neutralize drug-resistant pathogens and their escaping variants. Our studies provided new insights into the evolution of immune response towards conserved epitopes.

ABS#238

Poster session, July 13

Structure and Gating of the Hydrophobic Cation Channel of the SARS-CoV-2 Envelope Protein

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The Envelope (E) protein of SARS-CoV-2 is a viroporin essential for the assembly, maturation and egress of new virions. E also disrupts the pH and calcium homeostasis in host cells, leading to severe viral-induced inflammation. In mice, impairment of the E viroporin attenuated the pathogenic effects of the virus, making E an antiviral target for treating COVID-19 [1]. However, the ion-conductive structure and the gating mechanism governing this hydrophobic channel are still unknown, impeding drug development.
Here we use solid-state NMR spectroscopy to determine the structure of E transmembrane domain (ETM) at low pH and in the presence of calcium, which correspond to the conditions for the ion-conductive state [1,2]. Measurement of chemical shifts, interhelical distance, and helix orientations yielded a 0.60 Å resolution structural model. We find that ETM forms a pentameric α-helical barrel with a conical shape and a C-terminal 310-helix. The folding of the channel is predominantly driven by steric hydrophobic packing, and phenylalanines at the helix-helix interface introduce conformational plasticity that may prevent the pore from collapsing. ETM features mobile and hydrated pore openings in the acidic calcium-rich environment, unlike the non-conductive state at neutral pH without calcium [3]. Although the middle of the pore physically allows water passage, this region is de-wetted, suggesting hydrophobic gating regulates ion conduction. These results suggest that increased channel hydration and the C-terminal constriction induced by calcium and acidic pH enhance the hydrophilicity near the hydrophobic gate, facilitating the passage of water and ions.

These results give insights into the channel gating mechanism of SARS-CoV-2 E. The open state structure illustrates how nature uses apolar packing to assemble a hydrophobic channel, and should be useful for designing new E channel inhibitors.

ABS#243

Poster session, July 14

PRO-LSGM: generalizable protein design tool based on a conditional diffusion model

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Deep generative models have shown great capability to learn meaningful representations within protein sequences. Diffusion models drew extensive attention recently due to their powerful generative competence, yet their potential in conditional sequence generation for protein design remains largely unexplored.
Here we present PRO-LSGM, a score-based generative model to design new protein sequences with high fitness in specific properties. A diffusion module was added after an attention based autoencoder, to learn biological representations and generate diverse functional variants. By using the unconditional diffusion model, PRO-LSGM extract features from natural protein sequences as the basis for generating new species. Controllable designs are further implemented for variants with superior functional performance with a conditional diffusion module. The efficacy of this deep learning-based protein design algorithm was evaluated on three fundamental tasks: representation learning, likelihood learning and generation. The results showed that PRO-LSGM can learn physical and chemical properties of amino acids, as well as evolutionary information within the protein sequences. For the prediction task, our model reached high spearman's rank correlation between protein sequence and function. Moreover, the generated sequences are highly similar to the training sequences with high fitness, while the visualization of latent space showed a controllable generative process. The model was demonstrated in several datasets with different functions, including substitutions and indels, showing broad applicability. PRO-LSGM demonstrates the potential of conditionally generating new protein variants in different types, thus providing a generalizable new tool for protein design and functional optimization.

ABS#245

Poster session, July 14

Designing epitope-focused vaccines via antigen reorientation

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A major challenge in vaccine development, especially against rapidly evolving viruses, is the ability to focus the immune response toward evolutionarily conserved antigenic regions to confer broad protection. For example, while many broadly neutralizing antibodies against influenza have been found to target the highly conserved stem region of hemagglutinin (HA-stem), the immune response to seasonal influenza vaccines is predominantly directed to the immunodominant but variable head region (HA-head), leading to narrow-spectrum efficacy. Here, we first introduce an approach to controlling antigen orientation based on the site-specific insertion of short stretches of aspartate residues (oligoD) that facilitates antigen-binding to alum adjuvants. We demonstrate the generalizability of this approach to antigens from the Ebola virus, SARS-CoV-2, and influenza and observe enhanced antibody responses following immunization in all cases. Next, we use this approach to reorient HA in an "upside down" configuration, which we envision increases HA-stem exposure, therefore also improving its immunogenicity compared to HA-head. When applied to HA of H2N2 A/Japan/305/1957, the reoriented H2 HA (reoH2HA) on alum induced a stem-directed antibody response that cross-reacted with both group 1 and 2 influenza A HAs. Our results demonstrate the possibility and benefits of antigen reorientation via oligoD insertion, which represents a generalizable immunofocusing approach readily applicable for designing epitope-focused vaccine candidates.

ABS#246

Poster session, July 14

Characterize the structural and functional nature of interdomain coupling in FXR using Molecular Dynamics simulations

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Farnesoid X receptor is a ligand regulated transcription factor which controls the expression of genes involved in several metabolic processes. When a ligand binds to the ligand binding domain (LBD) of the receptor, conformational changes occur and send an allosteric signal to the DNA binding domain (DBD). Although it has been known for a long time that ligand binding modulates gene expression, the exact mechanisms at the molecular level that faciliate this allosteric communication between LBD and DBD are poorly understood. One major hindrance of this understanding is the absence of experimental structures of full-length FXR. The goal of my research is to use molecular dynamics simulation of full length FXR to understand how ligand binding induces domain-domain communications in FXR and modulates gene expression. In preliminary work, our laboratory built and optimized a model of full length FXR using homology modeling and enhanced sampling. With these inital models, we have commenced accelerated molecular dynamics simulations to enable enhanced conformational sampling of full length FXR in various functional states, including diverse ligand-, DNA- and coactivator-bound states as well as different oligomeric configurations. To characterize the nature of interdomain communication in FXR, we are using sophisticated computational methods to analyze fluctuations, dynamics, essential motions and communication pathways in these FXR complexes. These studies will yield structural insight into domain communication in full-length FXR, as well as reveal the conformational states in FXR that are associated with various functional forms.

ABS#249

Poster session, July 14

Functionalizing De Novo Proteins through Post-Translational Modifications

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Proteins are an incredibly diverse repertoire of biomolecules, made up of different permutations of amino acids. This diversity can be further expanded through alterations such as post-translational modifications, where chemical inputs can modulate the activity of a protein. Recent advancements in the computational tools available to the field of protein engineering have allowed for high confidence designs of de novo protein folds [1]. However, these methods have been limited in their application to designing dynamic proteins with multiple conformational states due to the additional requirement of incorporating this more complex energetic landscape. Tools capable of integrating these multiple conformational states are attractive to fields where inducible changes are desirable such as biological therapeutics [2], biosensors [3], and the development of more complex biologically based synthetic signaling systems where chemical inputs regulate the activity of proteins [4]. Here, we propose a method for the design and selection of proteins whose conformation is driven by the input of a post-translational modification, namely phosphorylation. By coupling rational design with deep learning-based computational tools, we were able to design, express, select, and characterize proteins whose conformational state is driven by the phosphorylation of a specific residue. Proteins of interest were selected via yeast display, characterized by circular dichroism (CD) and Multi Angle Light Scattering (MALS), then validated by mass spectrometry (MS). Overall, we show a proof-of-concept pipeline for designing, selecting, and validating proteins whose conformation is driven by a post-translational modification.

ABS#250

Structure Prediction and Design (July 15, AM)

Flexible Structure-based Design of Small Molecules with Equivariant Diffusion Models

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The computational design of drug-like molecules remains an important challenge, for which we are lacking

efficient tools. We introduce a new computational method for structure-based drug design (SBDD) which produces candidate ligands for pre-determined target proteins. To this end, we leverage recent advances in deep learning for photorealistic image generation, namely denoising diffusion models, for the design of small molecule ligands. By conditioning the generative process on the three-dimensional protein structure, the algorithm cannot only utilize the chemical context but also the geometry of binding pockets for constructing complementary molecular structures together with putative binding poses. Our learning system is based on an SE(3)-equivariant neural network architecture that ensures basic geometric properties and makes the approach particularly data-efficient. It was trained on a large and diverse dataset of available protein-ligand complexes. Our in silico experiments demonstrate that the method effectively generates diverse sets of new molecules with high computational docking scores. Furthermore, minor modifications of the sampling algorithm allow us to apply the same model successfully to a range of other molecule design subtasks, including scaffold hopping, fragment growing, and fragment linking. In several case studies, we fix scaffolds or fragments respectively, and use our framework to complete these substructures. The results show that new, valid chemical compounds are created that have higher docking scores than a control set of de novo generated molecules.

This work provides early evidence that the stunning results obtained with deep learning models for image generation may indeed be transferable to the molecular domain, where they can not only produce novel drug candidates but also help drug design scientists to further optimize these hit molecules through redesign of substructures or optimization of desirable properties. Future work will mainly focus on experimental validation of the computational designs.

ABS#252

Poster session, July 15

Proline Residues Shape Early-Stage Oligomers of Prion-like Polypeptides, Influencing Amyloid-Disrupting Capacity

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Proline residues play a crucial role in regulating the amyloid-disrupting capacity of prion-like polypeptides. However, the molecular mechanism underlying this effect remains elusive. Here, we investigate the impact of proline residues on the early-stage oligomerization of truncated sequences of the TDP-43 C-terminal region (287–322) and their triple proline variants (308PPP310). Using coarse-grained molecular simulation, we study the time evolution of the aggregation process under effective high-concentration conditions (∼25 mM), ensuring long time scales for protein association at laboratory concentrations. We show that the morphological and structural order of early-stage oligomeric species, rather than the average oligomer size, serves as a better indicator of amyloid propensity. Our study reveals that proline variants guide the oligomerization process to form “ordered” oligomer intermediates for shorter truncated sequences (i.e., 307–322) by maintaining their shape-complementarity. However, for longer truncated sequences (i.e., 287–322), proline variants lead to the formation of "disordered" oligomers, suppressing amyloid formation. Our findings suggest that the structure-based kinetic heterogeneity of prion-like sequence fragments drives different aggregation pathways, regulated by the morphological and structural order of early-stage oligomers. This work sheds light on the role of proline residues in regulating amyloid-disrupting capacity by providing a guided morphological restraint for amyloid formation.

Regenerate response

ABS#253

Poster session, July 14

Design and Development of Azapeptide Therapeutics

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The gut-derived incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose insulinotropic polypeptide (GIP) play important physiological roles in maintaining glucose homeostasis. Several stabilized versions of these peptides have been successfully developed as potent therapeutics for treatment of type 2 diabetes (T2D) and obesity. A key consideration for the design and development of drugs based on these hormones is

the mechanism of their protection from the serine protease, dipeptidyl peptidase-4 (DPP-4), a front-line enzyme that catalyzes the truncation of GLP-1 and GIP within minutes abolishing their ability to activate their cognate receptors. Azapeptides contain a semicarbazide backbone modification (amino acid C-alpha to trivalent nitrogen) that subtly perturbs both the backbone and side chain conformations. We report here the design and characterization of several aza-amino acid containing constructs of the GLP-1 and GIP that retain full potency and efficacy at the cognate receptors (GLP-1R and GIPR) that are simultaneously refractory to the hydrolytic deactivation catalyzed by DPP-4.

ABS#254

Poster session, July 13

Assessing the Accuracy of Predicting Interfacial Amino Acid Replacements:A Case Study

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Facilitated by the availability of very large collections of protein structural data for training, the applications of machine-learning algorithms have resulted in unprecedented success in protein structure prediction. However, a similar level of success in predicting the consequences of mutations in proteins has been perceived to lag far behind due to the unavailability (or availability of a handful of protein-specific data) of a very large collection of training data on protein mutations. To assess the status quo of predicting the change in the free energy (DDG) upon the mutation such as the substitution of interfacial residues in binary protein complexes, using isothermal titration calorimetry, here we experimentally measured DDG of interfacial residue replacements in a collection of ten complexes having a biased interfacial amino acid composition (i.e., enriched in charged amino acids). A systematic comparison of the experimentally measured DDG with that of fifteen prediction algorithms showed that the linear correlation coefficient of the best-performing algorithm is only 0.26. This suggests a gap in the knowledge comprehending the contributions of free energy continues to exist, advocating for the development of experimental methods for large-scale measurements of the consequences of amino acid replacements in proteins.

ABS#256

Poster session, July 15

Structural and Thermodynamic Basis of Nearest-Neighbor Cooperativity in the Ring-shaped Oligomeric Regulatory Protein TRAP from ITC, native MS, and cryo-EM

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Cooperativity, which arises from allosteric communication between remote sites in macromolecules, is a fundamental process that affects nearly every biological process. However, the molecular basis and thermodynamic consequences of allostery at the microscopic level are typically difficult to discern, impeding the understanding and prediction of macroscopic properties. Here, we focus on allosteric interactions between ligand binding sites in the cyclic regulatory protein TRAP (trp RNA-binding Attenuation Protein), which assembles into a homo-12mer in Bacillus halodurans (Bha). Bha TRAP exhibits Trp-Trp cooperativity, whereby the binding of one Trp ligand affects the affinity of subsequent Trp ligands. We hypothesize that the binding state of adjacent Trp-binding pockets dominates Trp-Trp cooperativity. We parametrized a nearest-neighbor (NN) statistical thermodynamic model by fitting ligand-dependent bound populations with a polynomial that considers 2^12 (4096) possible Trp-TRAP configurations. Native MS titration data revealed strong positive homotropic cooperativity. Fitting with the NN model yielded an interaction coefficient $\alpha = 256$ and a weak intrinsic binding affinity of K0 $\sim$ 1 mM. To validate the NN model, we engineered linked protomers, allowing us to disable binding on alternating sites, eliminating the NN effect. The linked proteins lacking NN interactions exhibited weak non-allosteric bindings, supporting the dominance of the NN effect. Cryo-EM studies reveal ligand-induced rigidification of a β-sheet, indicating that the bound Trp stabilizes a conformation that energetically favors adjacent Trp binding. Overall, this study provides a quantitative and structural understanding of site-site allostery in TRAP,

with implications for other macromolecules that bind ligands on a cyclic lattice.

ABS#260

Poster session, July 13

Global protein conformational metastability response in isogenic animals to missense mutations and polyglutamine expansions in aging

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The conformational stability of the proteome has tremendous implications for the health of the cell and its capacity to determine longevity or susceptibility to age-associated degenerative diseases. For humans, this question of proteome conformational stability has the additional complexity from non-synonymous mutations in thousands of protein coding genes challenging the capacity of the proteostasis network to properly fold, transport, assemble and degrade proteins. Here, we quantify the proteome-wide capacity to such challenges using the isogenic organism Caenorhabditis elegans by examining the dynamics of global protein conformational stability in animals expressing different temperature-sensitive (ts) proteins or short polyglutamine (polyQ) expansions in the context of biological aging. Using limited proteolysis of native extracts together with tandem mass tag-based quantitative proteomics, we identify proteins that become metastable under these conditions and monitor the effects on proteome solubility and abundance. Expression of different mutant proteins in the same tissue identifies hundreds to a thousand proteins that become metastable affecting multiple compartments and processes in a cell autonomous and non-autonomous manner. Comparison of the network of metastable proteins, however, reveals only a small number of common proteins. The most dramatic effects on global proteome dynamics occur in aging with one-third of the proteome undergoing conformational changes in early adulthood. These age-dependent metastable proteins overlap substantially with ts proteins and polyQ; moreover, expression of polyQ accelerates the aging phenotype. Together, these results reveal that the proteome responds to misfolding one-at-a-time to generate a metastable subproteome network with features of a fingerprint for which aging is the dominant determinant of proteome metastability.

ABS#261

Poster session, July 13

Exploring the sequence determinants for activity and inhibition of classical protein tyrosine phosphatases

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Protein tyrosine phosphorylation is a prevalent post-translational modification that modulates enzyme activity, protein localization, protein stability, and protein-protein interactions. Protein tyrosine phosphatases (PTPs) are an important family of signaling enzymes that catalyze the dephosphorylation of tyrosine residues on proteins. Many diseases including cancers arise from the dysregulation of tyrosine phosphorylation, and tyrosine phosphatases have been proposed as therapeutic targets for the treatment of these diseases. The largest subfamily of tyrosine phosphatases, the classical PTPs, are characterized by a highly conserved catalytic cysteine and two additional motifs known as the WPD loop and the Q-loop, which facilitate a conserved catalytic mechanism. Despite these conserved features, the intrinsic catalytic rates of different PTPs, and their susceptibility to covalent inhibition, span multiple orders of magnitude. The molecular basis for these differences is still unknown. We characterized the intrinsic catalytic activities of an array of PTPs and tested an array of electrophiles and fragment scaffolds to investigate the required chemical parameters for the covalent inhibition of PTPs. We also assessed sequence divergence at key residues in PTPs to explain their differential activity and susceptibility to covalent inhibition. We are now exploring two parallel methods to analyze sequence-structure-function relationships in tyrosine phosphatases: (1) we are pursuing high-throughput scanning mutagenesis of the WPD loop in PTPs using a yeast selection assay and (2) we are using statistical coupling analysis to determine groups of covarying amino acids in PTPs, which will be used to design mutant PTPs for further characterization. We anticipate that our study will help elucidate the regulatory mechanisms of PTPs and guide the development of covalent probes and inhibitors for tyrosine phosphatases.

ABS#262

Poster session, July 14

Inactive to Active Conformational Changes of Human Insulin Receptor Ectodomain

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The human insulin receptor (hIR) is a receptor tyrosine kinase that plays a key role in glucose homeostasis and metabolic regulation. Although multiple structures of its extra-cellular part (ectodomain) have been resolved by X-ray and cryoEM, the pathway/mechanism of the ectodomain (ECD) conformational changes from the inactive apo state to the active insulin-bound state is still elusive. Before insulin binding, the structure of the ECD adopts a Λ-shape. After insulins bind, it changes to a T-shape. We performed molecular dynamics simulations using the explicit-solvent model. The conformational fluctuations of the wild-type dimer of hIR-ECD in its apo state show populations of conformations that are in between the insulin-free Λ shape and insulin-bound T shape. The simulation results of the structure-based model reveal the difference in thermal stability at different domain-domain interfaces. It has been hypothesized that prior to insulin binding, there exists a conformational state that is in between the apo L shape and insulin-bound T shape. Our simulations of a model system that mimics this conformational state support this hypothesis. Insulin might combine with this kind of conformation as opposed to the L shape observed in the X-ray.

ABS#264

Poster session, July 13

Activation, assembly, and recycling of dynein transport complexes by Lis1

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Cytoplasmic dynein-1 (dynein) is critical for the proper function of all eukaryotic cells, including cell division and long-range intracellular transport. Dynein exists largely in an autoinhibited 'Phi' conformation in cells and must undergo conformational changes to assemble an active complex with its coactivators: dynactin and an activating adaptor. Once active, ATP-hydrolysis drives dynein's movement toward the microtubule minus ends, from where dynein needs to be recycled back to the microtubule plus ends. Prior functional studies have shown that Lis1, an essential dynein regulator mutated in patients with the neurodevelopmental disease lissencephaly, is important for promoting the formation of active dynein complexes and colocalizes with dynein at the microtubule plus ends. How Lis1 aids in the activation and assembly of the dynein complex, and if Lis1 is important for dynein recycling remains unknown. I propose that Lis1 activates dynein by relieving its autoinhibited Phi conformation via reorienting dynein to prime it for assembly into active transport complexes. Once dynein reaches the microtubule minus ends, I hypothesize that Lis1's binding to dynein will lead to conformational changes and complex disassembly to allow for dynein's recycling back to the microtubule plus end. I will use cryogenic electron microscopy (cryo-EM) to determine the intermediate species of dynein-Lis1 complexes between the activation, assembly, and recycling pathways. I will generate structure-based functional hypotheses based on my cryo-EM data and test the functional implications of my discoveries via functional assays.

ABS#266

Poster session, July 15

Biological protein nano-springs as allosteric modulators and sensors of mechanical forces in the cell

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Tandem-repeat proteins (TRP) are characterised by their simplicity and modularity, structural malleability, and adaptability to new allosterically driven functions. There are several examples where a repeat scaffold appears to

drive allosteric mechanisms via its intrinsic dynamic flexibility; therefore, these proteins are a powerful model system to study allostery. At the same time, their exceptional amenability to rational design makes TRPs a powerful tool for protein engineering. Here we, first, report our findings on the allosteric function of PR65, the HEAT-repeat scaffold subunit of protein phosphatase PP2A, the inactivation of which is associated with cancer and neurodegeneration. We elucidate the structural dynamics of PR65 and function as an elastic connector upon binding of potentially therapeutic small molecule activators. For this, we use a battery of biophysical techniques, including HDX-MS and optical tweezers, combined with molecular dynamics simulations. Second, we present results on engineering TRPs as mechanosensitive sensors. Mechanical forces play a role in a wide variety of cellular processes, but the understanding of mechanical stimuli at the molecular level has been limited to date due to the lack of tools that can accurately measure low forces in vivo. Moreover, force sensors need to be customisable for specific uses and force regimes. Here we report a toolkit for measuring a range of different forces in the cell, made possible by the striking spring-like properties of the TRP class and their exceptional amenability to rational design. We design a panel of FRET-based tension sensors using consensus tetratricopeptide (CTPR) proteins as the mechanosensitive linkers. Optical tweezers experiments on the CTPR linkers show that the proteins respond to physiological forces in a highly distinctive manner. We dissect the mechanics of the proteins by mutationally creating and then characterising a series of variants with systematically modified properties to create a toolbox of customisable, calibrated force sensors.

ABS#269

Poster session, July 14

Determining the role of an extended loop above the active site of chlorogenic acid esterase from Lactobacillus helveticus

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Chlorogenic acid (CGA) is a phenolic antioxidant that is found in many foods in high concentrations. CGA esterases are biotechnologically relevant enzymes that hydrolyze CGA into caffeic and quinic acid. The structure of Lactobacillus helveticus CGA esterase is unusual compared to other CGA esterases since it features a large loop above its active site. Here, we investigate the functional importance of this loop. The loop contains a lysine residue that is hypothesized to form a hydrogen bond with the quinic acid moiety of CGA, increasing the binding affinity of the enzyme for CGA. Mutating the loop lysine to alanine (K187A mutation) led to a large increase in Km for CGA binding, supporting our hypothesis that the lysine residue is important for stabilizing CGA binding. Surprisingly, the kcat for CGA turnover for the K187A mutant increases slightly compared to the wild-type enzyme, suggesting that the rate of product release is limited by the breakage of the hydrogen bond between quinic acid and lysine 187. That said, the kcat/Km value was higher for the wild-type enzyme than the K187A mutant. The K187A mutation had no effect on either kcat or Km for the hydrolysis of the smaller substrates ethyl caffeic acid and ethyl ferulic acid, ruling out the possibility that lysine 187 is a direct participant in ester hydrolysis and further suggesting that the change in kcat for the K187A mutant is not due to the CGA esterase becoming a better catalyst, per se. Binding of ethyl caffeic acid and ethyl ferulic acid was weaker than CGA binding. We conclude that lysine 187 provides specificity to Lactobacillus helveticus CGA esterase as it enables the enzyme to selectively bind CGA over other potential substrates.

ABS#270

Capturing Protein Interactions (July 14, PM)

Investigation of the molecular determinants of picornavirus 3C protease and 3D RNA polymerase interactions with phosphoinositide-enriched replication membranes

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Cells infected with poliovirus (PV) undergo a remodeling of their intracellular membranes. These membranes serve as sites for replication and possibly genome packaging and may alter the conformation and function of viral proteins that directly bind to these phosphoinositide-containing membranes.

Previously, our lab demonstrated that the 3C protease interacts directly with phosphoinositide lipids (PIPs). However, those studies used short-chain, soluble PIPs. Here, we have conducted solution-state NMR studies with lipid nanodiscs to present these PIPs in a lipid bilayer context. More specifically, paramagnetic

relaxation enhancement (PRE) NMR experiments are used because they provide distance-dependent information due to increased relaxation of nuclei in proximity of paramagnetic ions. For these studies, we generated nanodiscs with phosphotidylcholine, PI4P and a paramagnetically labeled lipid (phosphorylethanolamine (PE)-diethylenetriaminepentaacetic acid (DTPA) (Gd)). The PRE experiments demonstrated that 3C interacts with the membrane through its positively charged N-terminal helix and the RNA binding region (residues 85-95). This binding mode was consistent with that proposed from previous molecular dynamics simulations.We have also confirmed PIP binding sites on the 3D RNA-dependent RNA polymerase using shortchain, soluble PIPs through chemical shift perturbations. Further experiments using lipid nanodiscs are needed to obtain more information about 3D-membrane interaction.

Our results help confirm PIP binding sites on these critical PV proteins, including providing the experimental data on the mode of membrane binding of PV 3C in a lipid bilayer context. Given that other positive-strand RNA viruses likewise remodel cellular membranes for RNA genome replication and/or virion assembly, the principles developed by studying the PV proteins will be applicable to similar viruses.

ABS#271

Poster session, July 14

A Photo-Crosslinking Strategy to Identify Tyrosine Phosphatase Substrates

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Protein tyrosine phosphorylation is critical for cellular function, and aberrant phosphorylation is tied to a wide range of human diseases. Identifying the substrates of specific tyrosine kinases and phosphatases has aided in our foundational understanding of human biology and disease origins. While there are many tools available to identify the substrates of tyrosine kinases, differences in active site chemistry have prevented these tools from being amenable to tyrosine phosphatases. Protein Tyrosine Phosphatase 1B (PTP1B), has been of particular interest due to the breadth of substrates it likely dephosphorylates, as indicated by its involvement in signaling processes that control angiogenesis, cell proliferation, and insulin mediated metabolic homeostasis. Identifying these substrates is critical to untangling the role of PTP1B in disease. Tyrosine phosphatases transiently interact with their substrates, making them difficult to identify by conventional methods such as immunoprecipitation. One option is to make substrate-trapping mutations, which modestly extend the lifetime of enzyme-substrate complexes, but typically require inactivating the phosphatase. In this study, we are using a photo-crosslinking strategy to covalently trap the enzyme-substrate complex. Using structural and evolutionary data, we have identified eight sites near the substrate binding pocket in classical tyrosine phosphatases for incorporating photo-crosslinking amino acids that do not compromise phosphatase activity. Photo-crosslinkers can be incorporated at these sites using Amber codon suppression in mammalian cells. Photo-crosslinking studies in mammalian proteomes are ongoing to asses mutant specific crosslinking efficiency. Our approach, which relies on the covalent capture of substrates with light, will elucidate the role of PTP1B in specific signaling pathways and cellular contexts. The protein substrates identified from this study will deepen our understanding of a wide range of cellular processes and potentially identify new therapeutic targets.

ABS#274

Poster session, July 13

Accuracy of Prediction of Weak Interactions in Proteins in the Deep-Learning Era

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Aromatic ring-mediated weak interactions such as cation–p (CP), CH–p (CHP), anion–quadrupole (AQ), etc. play very important roles in protein structure and function (e.g., conformational preference, stability, recognition, finely regulating function, etc.). In the deep-learning era, with unprecedented success in protein structure prediction, the availability of structures of natural and designed proteins is practically limitless. In this limitless milieu of protein structures, an assessment of the accuracy of identifying weak interactions in predicted structures is warranted for practical applications such as to facilitate the interpretation of perturbations of weak interactions in somatic, germline natural variants of proteins when experimental structures are unavailable, especially of the full-length protein. Our analysis shows that CP and AQ interactions in AlphaFold2 (AF2) predicted

structures can be identified with an accuracy of >70% with respect to the corresponding experimental structures (resolution < 3Å). The prediction accuracy however gradually falls below 50% as the resolution drops (resolution > 6Å). With the known background estimate of the prediction accuracy of weak interactions, an analysis of somatic disease, germline disease, and rare variants of human proteins with unavailable experimental structures was performed. The analysis showed that several weak interactions are perturbed in a diseased state and the perturbed weak interactions of diseased states can also include those involving disordered regions. These observations, in the CRISPR base editing era, will motivate in vivo experimentally probing of the site-specific perturbation of weak interactions in proteins.

ABS#276

Poster session, July 13

Exploring the Interaction between GST Rho-Class of Nile Tilapia (Oreochromis niloticus) and Microcystin-LR: An In Silico Molecular Docking Study

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Glutathione S-transferases (GSTs, EC 2.5.1.18) plays an important role in the elimination of toxins and xenobiotic compounds from the body of living organisms. Among the great variety of GSTs, the rho class GST is specific to teleosts and cephalochordates. In Nile tilapia (Oreochromis niloticus) this enzyme may be capable of detoxifying toxic compounds, such as microcystin-LR (MCLR), which is a toxin produced by cyanobacteria Mycrocistis aureoginosa. Microcystin is a toxin that can affect human and animal health and cause problems in aquatic ecosystems. In this work, we used an in silico approach to study the GST rho-class to give an insight on the interaction between the GST rho of Nile tilapia and the substrates MCLR, GSH, 1-Chloro-2,4-dinitrobenzene (CDNB) and the conjugates S-hexyl-glutathione (GSX), 1-(S-glutathionyl)-2,4-dinitrobenzene (GSDNB) and GS-MCLR. Molecular docking was performed in MOE 2022.10. The results revealed that the catalytic amino acid Ser13 of GST is essential for the interaction with the thiol group of GSH cysteine (Figure 1), as well as with the GSDNB, GSX and GS-MCLR conjugates. In addition, the tested compounds (CDNB and MCLR) were accommodated at the H-site, suggesting a favorable interaction with this hydrophobic region. Despite being a large molecule, MC-LR was well oriented in the H-site, indicating that the rho-class GST site is broad enough to bind with large molecules (Figure 2). In general, the GSH conjugates were accommodated in the G site, whereas the xenobiotic part was oriented toward the H site. The results suggest that the protein could be a potential bioremediation agent for aquatic environments contaminated with this toxin. However, it is important to note that purification and characterization of the GST rho from Tilapia is necessary to test its activity with this toxin experimentally.

ABS#280

Poster session, July 14

Selenoprotein S cellular roles and connection to SARS-CoV-2

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Selenoprotein S (selenos) takes part in the endoplasmic reticulum protein degradation pathway, NFkB signaling, and cytokines secretion, although, the function of selenos in these cellular processes is limited. We applied proteomics, in vivo and in vitro crosslinking, and pull-down to shed light on the selenos's cellular roles. Selenos is being recruited by many viral proteins and SARS-CoV-2 uses endoplasmic reticulum membranes and associated human proteins for its replication and to evade detection. Recently, selenos has been introduced to be associated with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) non-structural protein (nsp7). This protein is essential for viral RNA replication and transcription. It was unclear whether selenos and nsp7 interact directly and whether the interaction is possible when nsp7 forms a complex with the other components of the virus's replication machinery. Therefore, we used pull-down assays to show selenoprotein S binds nsp7, including when nsp7 is in complex with the coronavirus's RNA-dependent RNA polymerase. This places selenoprotein S at the heart

of the coronavirus's replication complex and marks it as the first human protein shown to directly interact with the viral replication complex. Cross-linking experiments were employed to map the interactions of selenoprotein S and nsp7 in the replication complex. We show that the hydrophobic segment of selenoprotein S is essential for binding nsp7. This arrangement leaves an extended helix and the intrinsically disordered region of selenoprotein S exposed and free to recruit additional proteins to the complex.

ABS#281

RNA-Protein Machines: Ancient Synergies (July 14, AM)

Dependence of P/E tRNA Hybrid Formation on Subunit Rotation in the Ribosome

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The ribosome is a massive two subunit nucleoprotein biomolecular assembly responsible for protein synthesis in all living cells. The ribosome and the tRNA molecules undergo several large-scale functional rearrangements during the protein elongation cycle. Here we explore two such rearrangements in the ribosome and tRNA molecules during the translocation step in bacteria, namely, P/E tRNA hybrid formation and inter-subunit rotation in the ribosome. We utilize multi-basin all-atom structure-based (SMOG) models (1,2) and the Ribosome Angle Decomposition (RAD) (3) method to study the dependence of P/E hybrid formation on rotation of the small subunit body with respect to the large subunit. Structure-based models provide a simplified energetic description, where experimental structures are defined to be the potential energy minima. We employ all-atom structure-based models to simulate spontaneous P/E hybrid formation (4) or transition of the P-tRNA from the P to E tRNA binding sites on the large subunit. By assessing the dynamics for different degrees of small subunit body rotation, we are identifying the precise physical relationship between these two large-scale collective processes. Mean first passage time (mfpt) for P/E hybrid formation as a function of subunit rotation revealed a non-monotonic dependence. The mfpt for P/E hybrid formation decreases as body rotation increases to a threshold value beyond which it increases. The all-atom SMOG model provides insights into the structural factors that govern the dynamics, including revealing individual proteins that introduce sterically-indued barriers that lead to non-monotonic behavior.

ABS#282

Poster session, July 14

Recombinant Expression and Refolding of the c-type and g-type Lysozymes from Totoaba (Totoaba macdonaldi)

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Lysozyme (EC 3.2.1.17) is known to catalyze the hydrolysis of the β-(1, 4) glycosidic bonds present in the peptidoglycan layer of bacterial cell walls causing the lysis of bacteria, but also plays a critical role in the innate immune response against bacterial pathogens. C-type and g-type lysozymes exhibiting unique features are found in teleost fish species, like totoaba (Totoaba macdonaldi), suggesting lysozyme´s critical role for its defense system. Previously, the c-type (TmLyzc) and g-type (TmLyzg) lysozymes genes from totoaba were characterized, coding for proteins of 143 and 193 amino acids, respectively. TmLyzc and TmLyzg were recombinantly expressed in BL21 cells at 25 °C, isolated from inclusion bodies and subjected to in vitro refolding experiments after purified under denaturing conditions. Refolding reactions where TmLyzg and TmLyzc were detected in soluble fractions were pooled and extensively dialyzed against 50 mM potassium phosphate buffer pH 8.2. The dialyzed protein solutions were clarified by centrifugation and the soluble fractions were concentrated and processed with precision protease to remove tags. Refolding of both lysozymes did not yield active enzymes until his-tag removal was achieved, possibly because their active site were somehow hindered by the presence of the his-tag. Lysozyme activity were analyzed with the solid-phase and turbidimetric assays. Further experiments will address the broad spectrum antibacterial activity of c-type and g-type lysozymes from totoaba. Being T. macdonaldi a critical endangered species, this knowledge will be crucial for establishing sustainable long-term cultures for its rearing, aimed to improve farming conditions, diagnosis and immunoprophylactic strategies.

ABS#285

Poster session, July 15

KCD: A Prediction Web Server of Circular Dichroism for Proteins

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We introduce a web server that predicts the far-UV circular dichroism (CD) spectra of proteins by making use of their three-dimensional (3D) structures from the Protein Data Bank (PDB). The algorithm is based on the DeVoe classical theory of optical activity through a knowledge-based model of circular dichroism (KCD) that also depends on a set of atomic complex polarizabilities, which are obtained from the analysis of a series of synchrotron radiation CD spectra together with their corresponding 3D structures from the PDB. The new training set of 115 proteins distinguishes the following secondary structures: α-helices, β-sheets, coils, and other structural elements. As a result, our model is in better agreement with the references of the training set, as well as with newly predicted spectra. At this point, it is worth mentioning the remarkable capabilities of the recent approaches based on artificial intelligence, which accurately predict the native structure of proteins. However, the structure of proteins is flexible and can be changed by a diversity of environmental factors such as interactions with other molecules, mechanical stresses, variations of temperature, pH, or ionic strength. Experimental CD spectra together with reliable CD predictions can be utilized to assess the secondary structural changes.

ABS#288

Proteins in Motion (July 15, AM)

Assessing how side chain conformational heterogeneity changes upon ligand binding

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While protein conformational heterogeneity plays an important role in many aspects of biological function, including ligand binding, its impact has been difficult to quantify. Macromolecular X-ray diffraction is commonly interpreted with a static structure, but it can provide information on the anharmonic and harmonic contributions to conformational heterogeneity. Here, through multiconformer modeling of time- and space-averaged electron density, we measure the conformational heterogeneity of 743 stringently matched pairs of crystallographic datasets that reflect unbound/apo and ligand-bound/holo states. When comparing the conformational heterogeneity of side chains, we observe that when binding site residues become more rigid upon ligand binding, distant residues tend to become more flexible, especially in non-solvent-exposed regions. We observe increased protein flexibility among ligand properties as the number of hydrogen bonds decreases and relative hydrophobicity increases. Across a series of 13 inhibitor-bound structures of CDK2, we find that conformational heterogeneity is correlated with inhibitor features and identify how conformational changes propagate differences in conformational heterogeneity away from the binding site. Collectively, our findings agree with models emerging from nuclear magnetic resonance studies suggesting that residual side-chain entropy can modulate affinity and potentially suggest a potential mechanism for the entropic cost of ligand binding.

ABS#289

Poster session, July 15

Interactions between Coarse-Grained Waters and Hydrophilic/Hydrophobic Atoms and Roles of Waters' Hydrogens

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Proteins function in waters. One critical ingredient of realistically simulating the functional process of a protein in a physiological condition is an accurate model of the interactions between the protein and water molecules. The physical properties of amino acids, specifically their hydrophilicity or hydrophobicity, are derived from the way in which they interact with water molecules. In this work, we set out to identify the underlying physical determinant of hydrophilicity or hydrophobicity. To this end, we develop a new method for coarse-graining the

potential energy surface of a system containing both proteins and water molecules by employing a technique used in coarse-grained normal mode analysis. Using the method, we coarse-grain each water molecule to a single-point solvent particle and decompose the coarse-grained potential energy surface into several components that describe the pairwise interactions between protein's atoms and solvent particles. We then examine the components to uncover how solvent particles interact with different atom types. Our results show that the hydrophilicity/hydrophobicity of different atom types can be determined from the components. Additionally, hydrophobic, hydrophilic, or amphipathic characteristics of amino acids can be well reproduced. Our investigation shows that the hydrogen atoms of waters play two significant roles in protein-solvent interactions: (i) forming different mean potential energy wells with different atom types through the preference of their poses near the atom types, (ii) assisting solvent particles escape from the energy well.

ABS#292

Membrane Proteins: From Natural to Designed (July 14, PM)

Solubility-Based Protein Design for Biomedical Applications

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Solubility and stability are key merits for proteins related to their functional performance. The manipulation of such properties represents important aspects of protein design for both mechanistic study and practical applications. On the one hand, increasing the solubility of membrane proteins can facilitate their large-scale synthesis, study and utilization that were hindered by molecules' hydrophobicity and tendency to aggregate in solution. Using the simple tool named QTY code, we designed soluble variants of multi-pass transmembrane proteins to be used as a novel type of bio-nanomaterials in several biomedical applications not previously attainable. Besides their potential as antibody-like decoy receptors for immunoregulation in vivo, we adopted these functional molecules as biospecific probes together with 2D-crystalline S-layer intermediate layer in affinity-based biosensors for specific detection of pathologically relevant biomarkers. A biomimetic top-down platform was established by combining protein design-based modular "dual-monolayer" biorecognition construct with graphene-based field-effect-transistor arrays, that can be further developed for point-of-care diagnostics and continuous health-monitoring. On the other hand, by introducing hydrophobic binding interfaces into fully soluble proteins, we were able to design protein species that may self-assemble into well-ordered nanoparticles for drug delivery with receptor-mediated uptake pathways, which showed enhanced efficiencies compared to vehicles prepared through traditional means. Our work clearly shown that be reversibly manipulating solubility on different types of proteins, it is possible to design novel species with superior functional performance for various biomedical applications.

ABS#294

Poster session, July 13

Mechanistic Basis for Neuropathological Distributions of Dynamin Related Protein 1, DRP1, in Mitochondrial Fission

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Dynamin-related protein 1 (DRP1) is the primary GTPase mechanoenzyme that mediates mitochondrial fission, and de novo heterozygous mutations cause neonatal lethality. Productive fission events are contingent on DRP1 self-assembly into a "collar" around the mitochondrion—visible as discrete puncta by immunofluorescence microscopy. However, several pathologic DRP1 variants are impaired in functional self-assembly yet remain capable of forming subcellular puncta, suggesting puncta formation is distinct from DRP1 self-assembly. To better understand this, DRP1-mediated changes to puncta formation and its relationship to mitochondrial fission were quantified for wild-type and pathological variants using MitoGraph [1] and other

quantitative image analysis tools. As expected, puncta reside on mitochondria consistent with the widely held view that puncta are pre-scission complexes of highly-assembled DRP1. By contrast, DRP1 puncta are also in the cytoplasm, and these puncta are more mobile by fluorescence recovery after photobleaching (FRAP) than would be predicted for highly-ordered pre-scission complexes. Moreover, DRP1 puncta reversibly respond to 1,6-hexanediol, a compound shown to disrupt weak interactions but not ordered assemblies. Interestingly, disease-causing DRP1 variants that are assembly-deficient also appear as puncta and display greater sensitivity to 1,6-hexanediol compared to wild-type DRP1. Recombinant DRP1 can also undergo liquid-liquid phase separation (LLPS) suggesting an unexpected basis for subcellular puncta. FRAP of these resulting phase-separated states, or droplets, suggests a highly mobile phase. Additionally, pathologic DRP1 variants have an enhanced propensity to phase separate compared to wild-type DRP1 that appears to be modulated by an intrinsically disordered domain. These variants are deficient in self-assembly indicating a link between DRP1 oligomeric state and LLPS. Thus, these data support a model in which DRP1 puncta may derive from LLPS that are requisite for its recruitment and activity in productive mitochondrial fission.

ABS#295

RNA-Protein Machines: Ancient Synergies (July 14, AM)

SUPREM: Super RNA EcoGII Methyltransferase Engineered by Ancestral Sequence Reconstruction

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N6-methyladenosine (m6A) is essential for physiological processes through the regulation of RNA stability and translation. However, due to the lack of synthetic biology tools, it is still a paramount challenge to understand their roles and functions in vivo. Since our knowledge of structural-functional relationships in RNA methyltransferases (RNMTs) is limited, the functional improvement of m6A RNMTs has been challenging for developing novel research tools. Herein by ancestral sequence reconstruction (ASR), we explore the sequence space of bacterial EcoGII methyltransferase (M.EcoGII), a promising candidate for engineering because of its lack of specificity for the binding motifs and its slight promiscuous activity towards RNA. We generated a variant SUPer Rna EcoGII Methyltransferase (SUPREM) with ∼6-fold higher expression level and 12-fold higher RNA methylation activity than the wild-type M.EcoGII. Nanopore direct RNA sequencing detected improvement of SUPREM retaining non-site-specific activity. By comparative mutational analysis of M.EcoGII variants, we identified an important residue for the RNA methylation activity of SUPREM. Further SUPREM is an optimal potential candidate for RNA methylation activity study in vivo.

ABS#296

Poster session, July 13

Indels: The Evolutionary Switches Bridging Protein Functions and Topologies

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The emergence of proteins from small peptides is a fundamental event in the origin of life. These early peptides likely underwent a gradual process of selection and evolution, where mutations led to changes in their amino acid sequences and consequently their structures and functions. We recently demonstrated how insertions and deletions (InDels) may have shaped the coenzyme binding pocket in Rossmann enzymes (Toledo-Patino S et al., 2022). We switched the binding of a redox coenzyme nicotinamide dinucleotide (NAD) towards a methylating one S-adenosylmethionine (SAM), Illustrating how early peptides may have bridged distinct chemistries with minor changes in their sequences.
Building upon this work, we have now used InDels to rationally engineer a switch in topological connections. We introduced a single InDel at the connecting loop of the β1α1 segment of a (βα)2x protein template. Upon duplication, the resulting (βα)4x unit adopts either a p-loo or a Rossmann topology in absence or presence of the InDel, as predicted by AlphaFold (Jumper J et al., 2021). The biophysical characterization of the resulting targets showed the proteins express solubly and are well folded. Their structural characterization is currently being validated with NMR.

Our preliminary results show that these seemingly unrelated topologies may have been connected in the origin of early peptides before reaching their contemporary globular states. These findings shed light on the mechanisms underlying fold evolution and the origins of complex protein structures. It demonstrates that insertions and deletions are a powerful phenomenon that that hides the mysteries of the emergence of proteins and the molecular processes that underpin it.

ABS#297

Poster session, July 13

Crystallographic Module in Amber 2023

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The process of crystallographic protein structure determination is driven primarily by X-ray diffraction data and secondarily by molecular-mechanics force field, which serves to regularize the protein's geometry. Seeking to enhance the modeling component, we have implemented a module for crystallographic calculations in the MD simulation platform Amber. Previously, we have shown that Amber hyphenated with the crystallography package Phenix can successfully refine protein models [1]. Such refinement procedure consists of a short MD run steered by crystallographic structure factors and performed on a crystal unit cell containing multiple protein molecules along with the (explicitly modeled) interstitial solvent. Here we report on the latest upgrade of crystallographic facilities in Amber. The new version contains functions to treat bulk solvent, scale calculated structure factors and evaluate the maximum-likelihood target function (ported from the cctbx library). The X-ray functionality of Amber no longer relies on external dependencies, so that the full advantage of GPU acceleration can be taken. This development results in ca. 25-fold speed-up for the core refinement procedure, reducing the computation time to just several minutes. In turn, this makes it possible to expand the refinement model from a single unit cell to a 2x2x2 supercell, leading to an appreciable improvement in Rfree (in some cases, by as much as 0.048). This work was supported by the joint NSFC-RSF grant to Yi Xue (award 32061133011) and Nikolai Skrynnikov (award 214400033). All authors make a call for peace.

ABS#298

Poster session, July 15

Design of specific protein inhibitors from natural effectors

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Various diseases such as cancer are driven by elevated activity of particular enzymes or aberrant protein-protein interactions. Protein-based inhibitors are attractive candidates for drug development as they possess the ability to bind to their targets with high affinity and high specificity. While many antibody-based therapeutics are being presently pursued, we engineer therapeutic proteins starting from natural protein effectors. Such proteins already bind to the correct epitope on their target and are non-toxic and non-immunogenic, presenting attractive candidates for drug development. Yet, natural proteins usually possess poor binding specificity as in their native environment they participate in many interactions and convey different signals. We developed methodology to convert such effectors into very specific protein inhibitors by supplying them with loop extensions that could reach non-conserved sites on their targets and recognize subtle amino acid differences. Our approach starts with computational design of loop extensions into natural effectors; these loops are subsequently optimized through experimental directed evolution. We applied this strategy to obtain inhibitors for two established drug targets in cancer: matrix metalloproteinases and Ras. In both cases, our engineered inhibitors demonstrated high binding affinity and superb specificity toward their targets in vitro and subsequent inhibition of pro-cancer pathways in cellular essays. Our strategy for natural effector redesign is universal and could be applied to target any disease-associated protein.

ABS#300

Poster session, July 14

Electrodetection of small molecules by conformation-mediated signal enhancement

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Amino acids are building blocks of biological molecules, and they play a crucial role in cellular metabolism and neurological mechanisms. Dysregulation of these metabolic processes is linked to chronic diseases, such as diabetes. Metabolic studies have therefore identified glycine and branched-chain amino acids (Leucine, Isoleucine, Valine) as potential biomarkers for the early detection of type-I-diabetes (T1D). We have developed an electrochemical biosensing platform that harnesses solute binding proteins, which undergo a conformational change upon target analyte binding, to generate a quantifiable signal proportional to analyte concentration. To test this sensing platform, we designed and characterized two biosensors, which detect glycine and leucine, respectively. These glycine and leucine biosensors were, in turn, validated through cyclic and differential pulse voltammetry using a redox label (ferricyanide), allowing for selective and sensitive detection of our targets over a wide dynamic range and with sensitivities as low as 1 nM and 100 nM respectively. Our work represents a new platform for electrochemical sensors, where redox probe access to screen-printed gold electrode surfaces can be altered by the conformational change of a solute binding protein. Altogether, these biosensors form an essential step towards the application of electrochemical sensing to the monitoring of diseases, leading to new miniaturized sensors with high sensitivity and low cost.

ABS#301

Poster session, July 15

What is the Best Way to Protonate Crystallographic Structures? Comparison of Protonation Tools Using 1HN-15N Residual Dipolar Couplings

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Nearly 88% of all protein structures in the Protein Data Bank were solved by X-ray crystallography. The vast majority of these structures lack proton coordinates. Here we employ NMR residual dipolar couplings (RDCs), which are exquisitely sensitive to proton coordinates, to evaluate a number of popular protonation tools, including Amber, CHARMM, H++, Chimera, Pymol, NMRServer, MolMol, Maestro, SCWRL4, Reduce, Phenix, and HAAD. The analysis involves 12 proteins, for which highly accurate 1HN-15N RDC data are available or have been measured in house. We found that the simple MD-based scheme involving energy minimization in implicit solvent produces the best results, with Amber having a slight edge over CHARMM. To gain further insight, we parameterized the orientation of NH vectors using two angles: in-plane angle γ and out-of-plane angle δ. For most protonation tools, these two angles are fully or partially constrained (e.g. δ is set to zero). In contrast, protein structures protonated by Amber show an appreciable spread in γ and especially in δ (standard deviations of 1.4° and 4.6°, respectively). This is consistent with the well-known variability of the dihedral angle ω, characterizing the deviation of peptide bond from planarity. Of interest, residues in α-helices are mostly associated with positive δ values (mean of 1.6°), whereas β-sheet and coil conformations are biased toward negative δ values (means of -0.5° and -1.0°, respectively). In addition, we examined a number of neutron structures and ultra-high-resolution X-ray structures, where hydrogen atoms are often visible in the electron density maps. Strikingly, most of these structures display degenerate γ, δ distributions, indicating that their hydrogen atoms were built using relatively crude algorithms. In summary, we present an MD-based protonation protocol that can robustly and accurately add missing hydrogens to protein structures. We acknowledge the joint NSFC-RSF grant to YX (award 32061133011) and NRS (award 21-44-00033).

ABS#303

Poster session, July 14

The Reshaping of Protein Structure by Crystal Packing

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As the mainstay technology for structure determination of biomacromolecules, X-ray crystallography has produced the majority of the high-resolution protein structures in the Protein Data Bank. These structures not only help to elucidate protein function, but also serve as a principal training dataset for AI-based structure prediction algorithms. One has to bear in mind, however, that

crystallographic coordinates of protein molecules are to some degree sensitive to crystal packing. To investigate this subtle effect, we chose two well-studied model proteins: human ubiquitin (Ub) and hen egg white lysozyme (HEWL). A total of 16 Ub structures representing four different crystal forms, as well as 343 HEWL structures representing six different crystal forms, have been analyzed. For both proteins, structures that belong to the same crystal form show markedly lower pairwise RMSD compared to structures that belong to different crystal forms (e.g. 0.27 Å versus 0.63 Å for Ca atoms in HEWL), suggesting that the crystal packing is responsible for certain minor differences in protein conformation. Using MD simulations of a crystal supercell, we tested this hypothesis by observing the time-evolution of a protein structure in its native crystal lattice or otherwise in a different (non-native) crystal lattice. Remarkably, we observed that the average conformation of a protein, when embedded in a non-native lattice, gradually transitions towards the new conformation associated with this non-native lattice. Our results provide the computational evidence for slight but noticeable reshaping of protein structure caused by crystal packing, underscoring the need to consider crystal form as an integral part of X-ray structural information. We acknowledge the joint NSFC-RSF grant to YX (award 32061133011) and NRS (award 21-44-00033).

ABS#305

Poster session, July 14

Study of Disease-Associated Variants of Human Ornithine Transcarbamylase

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Every year 14,000-77,000 individuals are diagnosed with ornithine transcarbamylase deficiency, OTCD (1). OTCD occurs when mutations in the ornithine transcarbamylase (OTC) gene lead to biochemically inactive or absent OTC resulting in the accumulation of ammonia since it cannot be converted to urea. Some phenotypes of OTCD are very severe and result in clinical symptoms of vomiting, lethargy, coma, cerebral edema and ultimately death. The severity of the symptoms is determined by the specific mutation which entails the onset of the disease. Early onset OTCD which presents during the neonatal period, is often fatal. Late onset OTCD tends to have a milder phenotype; in most cases patients do not know they have this deficiency for years or decades. Currently, more than 420 mutations in the gene have been identified and 270 are point mutations, yet very little biochemical characterization has been done on these variant proteins. In order to expand our knowledge and understanding of the disease we analyzed all 270-point mutations computationally. Each variant was analyzed using Partial Order Optimum Likelihood (POOL), which was developed in our laboratory (2). Protein variants that showed the least and greatest variation in computed chemical properties were selected to be experimentally characterized. The disease variants with the computed properties that differed the most from wild-type were the most kinetically hindered. Ultimately, the insight gained from characterizing these mutants could aid in developing personalized therapies in the future.
Acknowledgement: NSF CHE-1905214, MCB-2147498

ABS#307

Poster session, July 14

MiD49/51 function as a long-chain fatty acyl-coenzyme A sensors on the mitochondrion to activate Drp1

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Mitochondrial fission occurs in many cellular processes, but the factors inducing fission are poorly understood. Here, we show that long-chain acyl coenzyme A (LCACA) is an activator of two related mitochondrial fission proteins, MiD49 and MiD51, which are receptors for the dynamin GTPase Drp1. LCACAs like palmitoyl-, oleoyl-, and stearoyl-CoA induce oligomerization of MiD49 and MiD51, activating their ability to stimulate Drp1 GTPase activity. The effectiveness in inducing oligomerization decreases with acyl chain length, becoming negligible for octanoyl-CoA. Palmitic acid or coenzyme A alone, lyso-phosphatidic acid, or palmitoyl-carnitine do not induce MiD oligomerization, suggesting specificity for LCACAs. The stoichiometry of LCACA:MiD in the oligomer is approximately 1:1, suggesting binding to the previously identified nucleotide-binding pocket. ADP has been suggested as an MiD51 ligand, but LCACA binds

MiD51 with significantly higher affinity. A point mutation in the nucleotide binding pocket reduces LCACA binding and the oligomerization effect of LCACA for both MiD51 (R342A) and MiD49 (Y195A). In cells, LCACA binding mutants do not assemble into puncta on mitochondria (suggesting an oligomerization defect) or induce the effects on mitochondria characteristic of these proteins. These results suggest that LCACA is an endogenous ligand for both MiDs, inducing Drp1 recruitment and mitochondrial fission.

ABS#308

Poster session, July 13

Distinct Phosphosites Regulate Pdr1 Activity and Azole Resistance in Candida Glabrata

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Candida glabrata is the second most common cause of systemic Candida infections, and these infections are increasingly resistant to frontline therapeutics. Resistance to azole-class antifungals in C. glabrata is mediated by Pdr1, a Zn(II)2Cys6 transcription factor that initiates the transcription of drug efflux pump genes. The literature cites several clinically observed strains of C. glabrata with gain of function mutations that make Pdr1 hyperactive and cause high levels of drug resistance. Previous work in our lab has indicated that mutating specific Pdr1 phosphosites to alanine or glutamate restores azole sensitivity in C. glabrata. In this study, these drug sensitive Pdr1 phosphosite mutations were combined with gain of function mutations using site-directed mutagenesis to investigate how this affects C. glabrata azole resistance. Using agar gradient diffusion assays, we found that one of phosphosite mutations increased fluconazole sensitivity when combined with all gain of function mutations, one phosphosite mutation did not restore sensitivity when combined with gain of function mutations, and 2 phosphosite mutations cause azole sensitivity only with specific gain of function mutations. We are currently using RT-qPCR to measure transcription of drug efflux pump genes in the sensitive strains. Overall, our results suggests that the different phosphosite mutations have different mechanisms that affect azole sensitivity, and this could potentially be applied to the development of new therapeutics.

ABS#309

Modern Anti-viral Strategies (July 13, AM)

Targeting Spike Glycans to Inhibit Viral Entry

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SARS-CoV-2 Spike harbors glycans which function as ligands for lectins. Therefore, it should be possible to exploit lectins to target SARS-CoV-2 and inhibit cellular entry by binding glycans on the Spike protein. Burkholderia oklahomensis agglutinin (BOA) is an antiviral lectin that interacts with viral glycoproteins via N-linked high mannose glycans. Here, we show that BOA binds to the Spike protein and is a potent inhibitor of SARS-CoV-2 viral entry at nanomolar concentrations. Using a variety of biophysical tools, we demonstrate that the interaction is avidity driven, that BOA crosslinks the Spike protein into soluble aggregates, and that multivalency is essential for BOAs ability to inhibit SARS-CoV-2. Using virus neutralization assays, we demonstrate that BOA effectively inhibits all tested variants of concern as well as SARS-CoV 2003, establishing that glycan-targeting molecules have the potential to be pan-coronavirus inhibitors.

ABS#310

Poster session, July 13

Recruitment of double-stranded DNA break repair proteins through the RNF8-HERC2 phosphothreonine-dependent interaction

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Double-stranded DNA breaks (DSBs) occur when the phosphodiester-sugar backbone of both DNA strands is severed. Typically, a cell senses and initiates an immediate response to properly repair the damage, but if the

DSB is incorrectly repaired or ignored, the cell will enter a state of genomic instability. Genomic instability contributes to tumorigenesis and cancer cell proliferation, so it is essential to improve our understanding of the mechanisms involved in the DSB response. Ring finger protein 8 (RNF8) is an E3 ubiquitin ligase known to be involved in the recruitment of DSB response proteins through phosphothreonine-dependent interactions with its forkhead-associated (FHA) domain. The RNF8 FHA domain is thought to interact with the flexible C-terminal tail of HECT and RLD-containing E3 ubiquitin ligase 2 (HERC2) but their direct interaction and role in the DSB response remains unclear. This study aims to better understand the recruitment of DSB response proteins by confirming and characterizing the HERC2-RNF8 interaction via pulldown assays, isothermal titration calorimetry (ITC), and multidimensional nuclear magnetic resonance (NMR) spectroscopy. Using various HERC2 constructs, a direct interaction between the RNF8 FHA domain and the phosphothreonine HERC2 tail peptides was confirmed and thermodynamically characterized using ITC. This interaction was found to be dependent on the phosphorylation of HERC2 residue T4827. Our NMR chemical shift perturbation experiments also identify specific RNF8 FHA domain residues that contribute to this phosphothreonine-dependent interaction. These cumulative findings demonstrate that the formation of RNF8-HERC2 complex contributes to maintaining genome stability by facilitating the recruitment of other DNA damage response proteins during the DSB response.

ABS#311

Poster session, July 14

The role of conformational entropy in antibody binding

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Mainstream antibody engineering strategies mostly focus on antigen-binding interfaces, also known as complementarity-determining regions (CDRs). However, the affinity required for therapeutic applications is difficult to acquire solely by changing the interface residues after key residues have already been optimized for antigen specificity. Numerous molecular dynamics (MD) simulation studies have shown that affinity maturation and reduction of antibody plasticity and flexibility are closely related. However, the dynamic contribution of antibody-antigen interactions remains challenging to quantify experimentally. Recent work from this lab has demonstrated that protein-ligand interactions often involve significant changes in conformational entropy, which directly contribute to the free energy of binding. To investigate the conformational entropy contribution to antibody binding, we employed a well-characterized picomolar-affinity anti-fluorescein antibody,4-4-20, as a model system. The single-chain variable fragment (scFv) of 4-4-20, the smallest recombinant antibody fragment that retains full binding activity, was isotopically labeled with Nuclear Magnetic Resonance (NMR) -active nuclei (1H, 15N, and 13C). The dynamic disorder of the amino acid side chains of scFv 4-4-20 in its free and ligand-bound states to fluorescein was determined using advanced NMR relaxation experiments. The goal is to determine the contributions of conformational entropy to the thermodynamics of antigen binding. Backbone and sidechain NMR spectra of the anti-fluorescein scFv 4-4-20 in its free and bound forms have been obtained. The chemical shift difference between the free and bound spectra suggested that scFv 4-4-20 undergoes structural changes upon binding to fluorescein. Analysis of changes in fast methyl-bearing side chain motion will be presented. Ultimately, our goal is to develop a general strategy to engineer antibodies to enhance the free energy of binding to its cognate antigen based on conformational entropy. This work was supported by Texas A&M University and Mathers Foundation.

ABS#312

Poster session, July 14

Hereditary Spastic Paraplegia Linked Mutations in Rac1 Cause Structural Changes that Disrupt ROS Accumulation

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Ras-related C3 Botulinum Toxin (Rac1) is a small GTPase of the Rho family that is involved in cytoskeletal organization, transcription, gene expression, and cell proliferation. Studies have shown that Rac1 may play an important role in reactive oxygen species (ROS)

accumulation. Maintaining appropriate cellular levels of ROS is critical during neurodevelopment as an excess of ROS can damage various macromolecules, and consequently cause cell death due to oxidative stress. Seven point mutations in the RAC1 gene have been identified in patients with hereditary spastic paraplegia (HSP) that have been suggested to disrupt ROS accumulation regulation, but the molecular basis for Rac1 deregulation remains unclear. To address this, various Rac1 HSP substituted proteins were measured using circular dichroism to assess for differences in the global secondary structure and fold of wild-type Rac1 and the HSP disease-linked substitutions. Isothermal titration calorimetry (ITC) was conducted to assess the ability of Rac1 HSP substituted proteins to bind to GTP/GDP and thereby properly regulate ROS accumulation. Pulldowns assays were also performed between the Rac1 HSP variants and the E3 ubiquitin ligase HACE1 to determine whether the substituted residues could affect Rac1 degradation. Our study demonstrates that some of the Rac1 HSP proteins are unable to interact with HACE1 suggesting that the disruption of Rac1-HACE1 interactions likely contributes to the HSP phenotype.

ABS#313

Poster session, July 14

Discovery of novel potential PET hydrolases from polar marine environments

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Plastic contamination is a human-made, global environmental problem with high repercussions not only on our flora and fauna but also with undetermined consequences to human health [1]. Among plastics, polyethylene terephthalate (PET) stands out for its representation of 10% of the annual production of plastics worldwide, accumulating as waste at rates parallel to its production levels and comprising 90% of marine waste [2,3]. Fortunately, the discovery in the last decades of PET hydrolases (PETases) that efficiently degrade this plastic at temperatures between 60-80ºC has paved the way for the potential biological recycling of PET [4].

Reasoning that similar cold-active enzymes must also be present in highly polluted environments, and driven by recent discoveries of PETases that can degrade this polymer at 25ºC and the circulation of plastic pollutants to polar regions through the ocean currents, we performed a bioinformatic search for novel PETases in three marine metagenomic reference catalogs that include samples from Arctic and Antarctic regions. For this work, we used hidden Markov models (HMM) built from amino acid sequences of experimentally characterized PETases.

Our bioinformatic analysis led to the identification of 710 novel potential PETases, distributed from the marine surface up to 3500m in depth, clustered in four clades separately from the evolutionarily related diene-lactone hydrolase family and with a single clade that groups together with all the known PETases. Enzymatic and structural characterization of a subset of 10 potential novel PETases from this clade using the aliphatic polyester polycaprolactone as model substrate and X-ray crystallography confirmed that 7 of these enzymes have polyesterase activity, spanning different optimal temperatures ranging from 14ºC-50ºC and that their structure corresponds to the canonical α/β hydrolase fold. These novel PETases provide further evidence of the global presence of plastic-degrading enzymes in all natural environment.

ABS#314

Poster session, July 14

Exploring the Regulation of the HECT Ubiquitin E3 Ligase UBE3C by Calmodulin

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Ubiquitin-protein ligase E3 C (UBE3C) is a poorly understood member of the Homologous to E6AP Carboxyl Terminus (HECT) E3 ubiquitin ligase family that contains a putative N-terminal IQ motif. UBE3C has been found to be linked to numerous diseases, including upregulation in multiple types of cancer, neurological disorders, and asthma, though its exact mechanisms are unclear. IQ motifs have been shown to be a potent binding partner for the calcium binding protein Calmodulin (CaM), which serves as a calcium regulatory protein through differential binding to substrates based on conformational changes in response to environmental calcium concentration. Typically, the CaM/IQ motif interaction is Ca2+-independent, where it preferentially associates with CaM in the absence of calcium. The presence of an IQ motif in

UBE3C suggests that CaM may play a regulatory role in UBE3C's function. To examine this complex, a UBE3C N-terminal truncation containing the IQ motif was constructed, overexpressed in E. coli, and purified to study the interaction between this CaM/IQ motif under differential calcium concentrations using biophysical techniques including pull down assays, circular dichroism, and isothermal titration calorimetry. Our studies aim to better understand how this interaction between UBE3C's IQ Motif and CaM affects UBE3C ubiquitylation activity and its role in disease.

ABS#318

Poster session, July 15

Biophysical and structural characterization of transcription factor-DNA binding in Drosophila melanogaster

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Homeotic genes are responsible for determining the identity of numerous anatomical structures in eukaryotes during early embryonic development. These genes encode for homeodomain transcription factors (TF-HDs) that regulate transcription by binding to specific regulatory DNA sequences. Mutations to these TF-HDs can lead to serious implications in embryonic development and body-segmentation abnormalities. These TF-HDs, which are highly conserved across all eukaryotic species, consist of helix-turn-helix DNA binding domain where the third helix (aka "the recognition helix") preferentially binds to the major groove of DNA with a 5'-TAAT-3' core. However, the affinity and the importance of flanking DNA sequences that these HDs bind and recognize remains poorly understood. To address this, we have systematically examined the binding affinity and specificity of various Drosophila melanogaster (fruit fly) TF-HDs with double-stranded DNA using Isothermal Titration Calorimetry (ITC). D. melanogaster was chosen as a model organism due to its shorter lifespan and similar genome homogeneity to humans. Chimeras TF-HDs were also constructed to investigate the importance of the sequence in the recognition helix when binding to the DNA major groove. Structural determination of the TF-HDs was also performed using multidimensional nuclear magnetic resonance (NMR) spectroscopy. The major findings of this collaborative interdisciplinary study will help to improve our understanding of TF-HD sequence specificity for various DNA sequences and further clarify the important roles of TF-HDs during embryo development in eukaryotes.

ABS#320

Poster session, July 14

Synthesis, Characterization, and Cellular Imaging of a Cyan Emitting Fluorescent α-Amino Acid for Live-cell Protein Imaging

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With the advancement in chemical probes and microscopy, protein labeling has widely attracted the interests of chemical biologists to monitor protein interactions with other biomolecules, as well as protein dynamics and signaling, conformation changes like folding/unfolding in live-cells. Among other labeling approaches, small-molecule based approaches like incorporation of fluorescent unnatural α-amino acids (FAA) into proteins for live-cell imaging is gaining wide research interests as they pose no or minimal disturbances in structure and functionality of target proteins. Here, we report the synthesis of a novel fluorescent α-amino acid 4-dibenzothiophen-4-yl-L-phenylalanine (DBT-FAA) capable of emitting cyan light in the visible region using an optimized Suzuki cross coupling reaction methodology in a good yield. It emits fluorescence emission signals with a major peak at ~440 nm. It has a high quantum yield (0.74) and resistant to photobeaching in aqueous solution. The DBT-FAA readily enters Hela cells, giving strong emission signals in cytosol upon 405 nm laser excitation. The cells remain viable and normal morphology at 50 μM DBT-FAA concentration, suggesting no or very minimal toxicity. Further research on site-specific incorporation of DDT-FAA into peptides and proteins to unravel the biological processes for broader applications in therapeutics and biochemical research is underway in our lab. We thank Dr. Y. Wei

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ABS#321

Poster session, July 15

Understanding Alzheimer's Disease Using Cryo Electron Microscopy

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Dementia is an umbrella term for a collection of symptoms that are caused by disorders affecting the brain. It affects more than 50 million people worldwide. The most common is Alzheimer's disease (AD), which affects 50-60% of people with dementia. Alzheimer's is a progressive disease and it involves parts of the brain that control thought, memory, and language. In 2020, as many as 5.8 million Americans were living with AD. Scientists do not yet fully understand what causes AD, but they believe that is caused by changes that accumulate with age, as genetic mutations and protein folding disorders. Finding treatment for these neurodegenerative diseases required characterization of mechanisms that drive neurodegeneration and key proteins and protein complexes. However, these key biological players are tough to study due to multiple states or conformations. These large and/or dynamic systems present a challenge to traditional methods of 3D structural determination as X-Ray crystallography or NMR. Fortunately, cryo-electron microscopy (Cryo-EM), has emerged as a well-suited approach for the determination of native protein function and the dynamics of complex biological systems. In Cryo-EM, specimens are rapidly frozen (vitrified) so that their biologically relevant native states are preserved and is then possible to obtain structural details of the specimen at near-atomic resolution. This technique is leading to new insight into numerous biological processes, as AD. Cryo-EM structures of tau amyloid filaments from human brain will be presented. These structures reveal that distinct tau folds characterize many different diseases. We demonstrate that post-translational modifications of tau modulate filament assembly, and that previously observed additional densities in AD and CTE filaments may arise from the presence of inorganic salts, like phosphates and sodium chloride. In-vitro assembly of tau into disease-relevant filaments will facilitate studies to determine their roles in different diseases, as well as the development of compounds that specifically bind to these structures.

ABS#323

Poster session, July 14

Unraveling the Half and Full Site Sequence Specificity of the Saccharomyces cerevisiae Pdr1p and Pdr3p Transcription Factors

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The Pleiotropic Drug Resistance (PDR) network is an important regulatory system in yeast involved in the transport of various toxins and drugs out of the cell. The transcription factors Pdr1p and Pdr3p, key regulators of this network, bind to specific sequences called PDR responsive elements (PDREs) in the promoter region of PDR genes to control expression. However, the exact mechanisms underlying the differences in the regulons of these proteins are not well understood. A combination of genomic occupancy profiling (CUT&RUN) and in vitro binding assays were employed to characterize the differences in DNA sequence specificity between the two transcription factors. The results indicate that interaction between the transcription factors and PDRE sites is not solely determined by the core sequence, but also influenced by the surrounding regions. Pdr1p preferentially binds type A and B PDREs, in contrast to Pdr3p's preference for type C and B PDREs. Flanking sequences have a substantial impact on overall affinity, with each protein possessing a different specificity for flanking sequences that reflects the relative genomic occupancy of the PDRE. Moreover, although Pdr1p and Pdr3p are known to dimerize on PDRE sites, they also have the capacity to bind half sites with high affinity. These results highlight how sequence variation in PDREs impacts the DNA binding affinity of Pdr1p and Pdr3p leading to differential regulation within the PDR network by the two transcription factors.

ABS#325

Undergraduate Research Session

Elucidating the Active Site Environment and Mechanism of a Cancer-Associated Variant of DNA Polymerase Theta

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DNA is constantly being damaged from a variety of factors, one of which being UV light. DNA polymerases are enzymes that play an important role in DNA replication and repair. Human DNA Polymerase Theta (Pol theta) is involved in DNA repair and helps to protect cells from damaging environmental factors, like UV, despite being an error prone, low fidelity enzyme. Understanding the dynamic mechanism in which Pol theta incorporates correct versus incorrect nucleotide during repair is pivotal to understanding its low fidelity status. Moreover, elucidating the mechanism of nucleotide incorporation of a variant of Pol theta associated with melanoma may provide clues into the development and progression of the disease. Many high-fidelity DNA polymerases experience a global conformational change during correct nucleotide incorporation but lack this movement with incorrect incorporation. To observe this conformational change and subsequent fidelity we can utilize 2-aminopurine fluorescence on a templating DNA strand to monitor fluorescence changes at the DNA polymerase active site. These changes in fluorescence are due to pi stacking when the DNA polymerase is shifting to integrate the correct nucleotide. Our objective to is to compare incorporation rates and active site microenvironments during phosphodiester bond formation of wild-type Pol theta with cancer-associated variants to better understand how Pol theta may protect against DNA damage. Our preliminary results suggest that WT experiences a decrease in fluorescence signal as expected with correct nucleotide incorporation. Interestingly, we observed little to no fluorescence change with the cancer associated variant, L2538R, under the same conditions suggesting that the variant has a different active site environment and potential nucleotide incorporation mechanism compared to WT. These findings provide insight into the global mechanism of cancer and the potential role of Pol theta in melanoma.

ABS#327

Poster session, July 14

Structural and Functional Characterization of a Secreted Toxoplasma Complex Required for Host Cell Invasion

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Toxoplasma gondii, the apicomplexan parasite that causes toxoplasmosis, is an obligate unicellular parasite that actively invades and resides within host cells to grow and facilitate its parasitic life cycle. In addition to toxoplasmosis, apicomplexans are the causative agents of many leading diseases, including malaria and the diarrheal disease cryptosporidiosis, caused by Plasmodium and Cryptosporidium spp., respectively. While, individual apicomplexan species have evolved invasion factors specific to their hosts, they all rely on several core invasion proteins conserved amongst apicomplexans. One such complex, called the "CLAMPlex", is secreted to the surface of parasites during invasion and is essential for active invasion in all tested apicomplexans. We have established that the CLAMPlex consists of three interacting proteins: CLAMP, CLIP and SPATR. However, the specific functional domains and the activities of this complex are poorly understood, as better-characterized model organisms lack recognizable homologs.

To address this gap, we optimized the immunoprecipitation of the native complex directly from the parasite and, in parallel, performed recombinant expression of CLAMP, CLIP and SPATR. The purified proteins were used to generate a nanobody libraries, and subsequently to select CLAMPlex-binding nanobodies from the libraries using phage display. We identified nanobody hits against the interacting proteins in the complex that will be recombinantly expressed and used to target the CLAMPlex and block invasion. Together with in vivo experiments in future, this will allow us to functionally define the essential regions of the CLAMPlex and establish this conserved complex as a prime vaccination candidate against apicomplexan parasites.

ABS#328

Poster session, July 15

Cotranslational formation of disulfides guides folding of the SARS CoV-2 receptor binding domain

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Many secreted proteins, including viral proteins, contain multiple disulfide bonds. How disulfide formation is coupled to protein folding in the cell remains poorly understood at the molecular level. Here, we combine experiment and simulation to address this question as it pertains to the SARS-CoV-2 receptor binding domain (RBD). We show that the RBD can only refold reversibly

if its native disulfides are present prior to folding. But in their absence, the RBD spontaneously misfolds a nonnative, molten-globule like state that is structurally incompatible with complete disulfide formation, and which is highly aggregation prone. Thus, the RBD native structure represents a metastable state on the protein's energy landscape with reduced disulfides, indicating that non-equilibrium mechanisms are needed to ensure native disulfides form prior to folding. Our atomistic simulations suggest that this may be achieved via co-translational folding during RBD secretion into the endoplasmic reticulum. Namely at intermediate translation lengths, native disulfide pairs are predicted to come together with high probability, and thus under suitable kinetic conditions, this process may lock the protein into its native state and circumvent highly-aggregation prone nonnative intermediates. This detailed molecular picture of the RBD folding landscape may shed light on SARS-CoV-2 pathology and molecular constraints governing SARS-CoV-2 evolution.

ABS#329

Poster session, July 14

Biochemical and Cellular Characterization of Mutational Effects in the Oncogenic Phosphatase SHP2

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The protein tyrosine phosphatase Src homology region 2 domain-containing phosphatase-2 (SHP2), is an important hub in many crucial cellular signaling pathways. Mutations in PTPN11, the gene encoding SHP2, underlie several leukemias and congenital disorders. SHP2 consists of a phosphatase domain and two phospho-tyrosine recognition domains (N-SH2 and C-SH2) which control phospho-dependent localization and allosterically regulate the phosphatase domain. Many mutations in PTPN11 disrupt the interactions between N-SH2 domain and the phosphatase domain, which keeps SHP2 in its auto-inhibited conformation. Indeed, these mutations cause an increase in SHP2 phosphatase activity even in the absence of stimuli. However, several disease-associated PTPN11 mutations do not conform to this model, suggesting alternative mechanisms through which PTPN11 mutations lead to SHP2 pathogenicity. The broad goal of this project is to elucidate different mechanisms of pathogenicity across disease-relevant SHP2 variants, through biochemical, biophysical, and cell-biological approaches. First, using proximity-labeling proteomics, we are identifying SHP2 interactors in both wild-type and mutant states. Our data so far suggests altered localization for two disease-associated SHP2 mutants. Second, we discovered that the T42A mutation in the N-SH2 binding pocket causes a change in the binding affinity and specificity of the N-SH2 domain. As a result, SHP2 activation can be more easily attained for some ligands, but not others, which provides a potential mechanism of rewired signaling. Taken together, our work will provide insights into the diverse forces driving SHP2 pathogenicity, which will aid our ability to combat SHP2-related disorders.

ABS#330

Poster session, July 14

Insights into Antibody-Antigen Interactions for a Glycoprotein Binding Antibody through Comprehensive Complementarity-Determining Region Mutagenesis

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The immune system depends on antibodies to detect and eliminate foreign pathogens. Recent advances in sequencing and structural biology have allowed for comprehensive profiling of complementarity-determining regions (CDRs) within antibodies, providing new insights into antibody evolution and function. However, understanding the energetics of antigen recognition sites within CDRs is limited. We used mutational scanning and structure determination to identify essential binding sites, enhancing our comprehension of interactions between antibody CDRs and antigens. In this study, an anti-tumor single-chain variable fragment, 3E8 scFv, that binds to TAG-72, a mucinous glycoprotein with cancer-specific glycans sTn and Tn, was employed to identify potential binding regions and sites by substituting the original CDR sequences with the most commonly

occurring amino acids in the CDR regions of the database. Only the heavy chain CDR (HCDR) loop mutants showed a decrease in binding affinities, with HCDR3 having the most significant reduction. The application of alanine single mutants to CDRs resulted in reduced binding affinity, but only around 50% showed impairment compared to the wild-type and 10% had no detectable binding signal. Most single mutants in the CDR loops showed moderate decreases in melting temperature, while some mutants exhibited significant loss of stability, indicating a role in structure stabilization. Finally, the antigen binding fragment of 3E8 was crystallized, revealing that the "hot spots" responsible for binding are clustered together, creating a potential binding pocket for the antigen that is a subset of the full pocket defined by the CDRs. The findings offer valuable information on the thermodynamic impact of CDR residues and the interactions between antibody CDRs and antigens. Our work underscores the importance of defining the crucial sites in CDR loops in antibody engineering and further studies on CDRs are needed to develop more effective antibody-based therapeutics.

ABS#332

Poster session, July 14

Global vs. Local Specialization: An Examination of the Divergence in Overall vs. Clustered Amino Acid Enrichments in Extremophilic DNA Polymerase I Homologs

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Bioinformatic analyses of extremophilic proteins often seek to define global amino acid enrichments that may underlie extreme temperature stability. While some amino acids are consistently enriched in thermophilic or psychrophilic proteomes, many trends differ significantly with different data sets and approaches. Here, we take a localized approach to understanding protein extremophilicity through bioinformatics. We have examined approximately 1000 homologs of DNA Polymerase I (Pol I) from a phylogenetically diverse set of bacteria and generated structural models of their ligand-bound DNA polymerase domains. Using these models, we have calculated enrichments of amino acids in close proximity to the bound DNA. In addition, we have calculated the frequency of three-dimensional clustering of particular amino acids. Our results help differentiate enrichments that confer global properties from those that are critical to local functions. For example, we find that thermophilic Pol I homologs are enriched in prolines overall, but their active sites show no such enrichment. In contrast, glycine shows almost no overall enrichment in extremophilic Pol I homologs, but significant enrichment in the active sites of pyschrophilic homologs. In addition, many psychrophilic homologs possess three-dimensional glycine clusters throughout their structures, while their thermophilic counterparts have almost none, and instead show an increase in the number of attractive charge-charge pairings. Our analyses found several other amino acids with surprising patterns of opposing global vs. local enrichment. This method highlights trends that may be masked by global bioinformatic analyses and provides a strategy for selecting protein regions for targeted engineering efforts.

ABS#334

Poster session, July 14

Effects of Pdr1 Phosphorylation on Fluconazole Resistance in Candida glabrata

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Candida glabrata is a pathogenic fungus that has gained notoriety due to its prevalence as a hospital-acquired infection and increasing resistance to frontline azole class antifungal treatments. In 2015, the Candida family was ranked the fourth most common cause of bloodstream infections, and for C. glabrata the mortality rate is estimated to be as high as 50%. The Zn(II)2Cys6 class transcription factor Pdr1 plays a role in the development of azole resistance by initiating the transcription of drug efflux pumps, but the molecular mechanisms regulating Pdr1 activity are still under investigation. Based on data from other Zn(II)2Cys6 transcription factors, we hypothesized that phosphorylation plays a role in regulating C. glabrata Pdr1. To test this hypothesis, we used the NetPhosYeast server to predict phosphosites in C. glabrata Pdr1, and we selected high-probability sites based on comparison with known phosphosites in the homolog S. cerevisiae Pdr1. Site directed mutagenesis was used to create a library of alanine and glutamic acid Pdr1 variants to mimic “always on” or “always off” phosphorylation states, respectively. A C. glabrata pdr1Δ strain was

complemented with these variants, and fluconazole resistance was assessed through spot dilution assays and agar gradient diffusion assays. We identified 7 phosphosite mutations that resulted in strains with single-digit μg/mL fluconazole minimum inhibitory concentrations, similar to the pdr1Δ strain. Current studies are using RT-qPCR to measure mRNA levels for the Cdr1, Pdh1, and Snq2 drug efflux pumps in the fluconazole sensitive strains. Since phosphorylation has been shown to affect fluconazole sensitivity, this suggests that kinase inhibitors could be potential therapeutics to sensitize C. glabrata cells to azoles.

ABS#335

Poster session, July 14

Hydrogen-Deuterium Exchange (HDX) and X-ray Crystallography Reveal Novel Mechanisms of Allosteric Modulation in PTP1B

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Protein Tyrosine Phosphatase 1B (PTP1B) is a well-known therapeutic target for several diseases, including diabetes and cancer. However, the highly conserved active sites of PTPs make developing specific and bioavailable small-molecule modulators challenging. Allosteric sites on proteins have long been considered potentially useful in developing specific targeted therapeutics, but the mechanisms that convey a signal through a protein's structure remain poorly understood. Based on crystal structures of PTP1B bound to small-molecule fragments from an initial crystallographic fragment screen, 140 small molecules were designed to target the allosteric binding pocket at loop 16, a recently identified allosteric site in PTP1B [1,2] that is not highly conserved across PTP structures. We then used biophysical experiments to characterize these compounds' structural and functional effects on PTP1B and explore the allosteric potential of the L16 site. Each compound was assessed using a binding and activity assays. Among the designed ligands, we identified binders that potently inhibited PTP1B and ones that had no discernable effect on enzyme kinetics. The structural and dynamical changes these compounds brought about in PTP1B were further analyzed using hydrogen-deuterium exchange mass spectrometry (HDX-MS) and X-ray crystallography and compared to those changes induced by previously known allosteric and active site inhibitors. Despite being designed to target the same L16 binding pocket, the novel ligands induce distinct structural and dynamic responses in PTP1B consistent with the engagement of multiple PTP1B allosteric pockets to exert their action on phosphatase structure and activity. Additionally, several ligands were found to inhibit the closely related T-Cell Protein Tyrosine Phosphatase (TCPTP), another highly validated target for insulin resistance and cancer, albeit to different extents than for PTP1B. This study uncovers promising new allosteric footholds for long-studied therapeutic targets PTP1B and TCPTP, providing new structural and dynamical insights into the mechanisms of the allosteric network in phosphatases.

ABS#337

Poster session, July 15

Small Orphan Proteins with Global Impact: Evolution of the Nitrogenase G-subunit

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Catalytic innovations related to geobiological cycles have played a crucial role in the history of our planet's atmosphere, surface, and biosphere. Thus, understanding their evolution can provide powerful insights about past geochemical environments and Earth-life integration. Nitrogenases are the only enzymes that convert atmospheric molecular nitrogen into bio-available ammonia. Despite their singular importance, their structure and mechanisms have experienced relatively few major changes since their emergence ∼3 billion years ago [1]. Among the few innovations within this system, we find alternative vanadium and iron nitrogenases. These enzymes differ from the more abundant molybdenum nitrogenase in their metal cofactors and multimeric composition [2]. In this work [3], we explore the evolution of alternative nitrogenases from a so far underexplored perspective in protein evolution: the emergence and adoption of small subunits by larger complexes. The G-subunit is a functionally essential part of the vanadium and iron nitrogenase complexes. Through phylogenetic inference, we reconstructed the history of the interactions between the G subunit and the overall nitrogenase complex. These interactions were characterized by sequence- and structure-based computational techniques. We find that these interactions allowed this enzyme to diversify into alternative nitrogenases, enabling organisms to expand into ecological niches with different metal availabilities.

ABS#338

Poster session, July 13

Mini-Protein Guided Endosomal Escape: Scope and Mechanism

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Biologics can be exploited to produce highly specific therapeutics. However, most biologics experience obstacles when being delivered—degradation and poor cytosolic delivery. Since most biologics are too large and hydrophilic to directly penetrate the plasma membrane, they enter the cell through endocytosis. Here, it will need to escape the endocytic pathway or be directed to lysosomal degradation. (1) The Schepartz lab discovered a mini-protein called ZF5.3 that escapes from late endolysosomes by exploiting a previously unrecognized portal for endosomal escape.(2,3) I aimed to explore the ability of ZF5.3 to deliver anti-Ras monobodies and simultaneously apply photo-catalysis methods to map its journey from the cell surface into the cytosol of a mammalian cell. Monobodies are evolved small binding proteins that offer great promise as inhibitors of protein-protein interactions.(4) Their limitation is the inability to access the cytosol and desired targets. I proposed to prepare and analyze the properties of a fusion between ZF5.3 and the anti-Ras monobody NS1. The genetic fusion NS1-ZF5.3 reaches the cytosol at therapeutically relevant concentrations (>200 nM) (Figure 1A). Future experiments aim to test its activity in-cellula. To maximize the potential of ZF5.3 as a delivery vehicle, we need to investigate its mechanism. Previous studies revealed that endosomal escape of ZF5.3 demands the activity of the homotypic fusion and protein sorting (HOPS) complex.(3) Yet, questions remain regarding which proteins or lipids guide ZF5.3 to this location when endosomal escape occurs. I proposed to identify these direct partnerships using a novel Iridium photocatalyst-promoted proximity labeling reaction and quantitative proteomics (Figure 1B).

ABS#340

Undergraduate Research Session

Incorporation of Noncanonical Amino Acids in Candida Glabrata

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Candida glabrata is a nosocomial pathogenic fungus that causes candidiasis infections, which have high mortality rates in immunosuppressed and immunocompromised populations due to acquired drug resistance. Previous work to characterize protein-protein interactions regulating drug resistance in C. glabrata have used co-immunoprecipitations. However, these experiments do not give information about the interacting surfaces and often miss capturing weak or transient interactions. Genetic code expansion incorporates noncanonical amino acids site-specifically in proteins and enables using bioorthogonal reactions to covalently capture and map protein-protein interactions in their native environment, but this technique has never been applied in C. glabrata. We have developed and are currently optimizing genetic code expansion tools to incorporate the photocrosslinking amino acid p-benzoylphenylalanine (pBpa) into proteins in C. glabrata. Proof of concept was demonstrated by incorporating pBpa into superfolder GFP at position Y151 (Y151pBpa sfGFP) using an orthogonal translation system consisting of an E. coli tyrosyl-tRNA synthetase (EcTyrRS) and tRNA-CUA pair. Proper functioning of the bioorthogonal EcTyrRS/tRNA-CUA pair was evaluated using western blots to quantify the expression of full length Y151pBpa sfGFP when pBpa is added to the culture medium. Additionally, we are evaluating the presence of photocrosslinked sfGFP dimers after irradiating live cells with 365 nm light. Successful development of this genetic code expansion method will expand the tools available to investigate C. glabrata's proteome, and our lab will apply these tools to study protein-protein interactions regulating drug resistance.

ABS#341

Protein Evolution: Lessons from the Past (July 13, PM)

Rescuing from the DEAD: Understanding Substrate-Mediated ATP Hydrolysis through Deep Mutagenesis of a DNA Polymerase Clamp Loader

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Clamp loaders are AAA+ ATPases that facilitate processive DNA replication by loading a sliding clamp on primed DNA. This process is reliant on ATP hydrolysis triggered by DNA substrate binding to the clamp loader. The underlying principles that govern substrate-mediated catalysis in AAA+ are still not well understood. Like many other ATPases, T4 bacteriophage clamp loaders contain a highly conserved DExD motif within each active site of the pentameric ATPase. In the distantly related ATPase family, DEAD-box RNA helicases, the final aspartic acid residue of the DEAD motif mediates structural rearrangements necessary to couple RNA binding to ATP hydrolysis. We take inspiration from this mechanism and study a partial loss-of-function mutation to the final aspartic acid of the DExD motif (D110C) in T4 bacteriophage clamp loaders through deep mutagenesis [1] and biochemical assessment. Through a variety of biochemical assays, we find that the mutated (D110C) clamp loader is unable to bind primed DNA and has compromised ATP hydrolysis activity. We then use the D110C clamp loader as the basis for a saturation-mutagenesis experiment that identifies regions in which mutations can rescue phage propagation. The nature of these mutations suggests that intramolecular interactions present in the wild-type protein need to be broken to rescue the activity of the D110C clamp loader. Our data suggest that the DNA-free state of the clamp loader represents a low energy conformation that is converted to the active state by DNA binding. Our results show how deep-mutagenesis experiments can provide valuable insight concerning the mechanisms of complex molecular machines.

ABS#342

Poster session, July 13

Mechanistic Studies and Engineering of LOV Photoreceptors in Fungal Systems

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Via photosensory modules such as Light-Oxygen-Voltage ("LOV") domains, blue light can be converted from a physical stimulus into a control for biological processes. This process entails light-driven conformation changes between the photosensor and attached effector domains, creating a "photoswitch" with specific biochemical and cellular outputs. Metagenomic and genomic surveys have shown a tremendous diversity of these effector domains across thousands of LOV-containing proteins, including a Helix-Turn-Helix (HTH) DNA binding domain in the bacterial transcription factor EL222 to the Regulator of G-protein Signaling (RGS) domain in the fungal membrane associating protein BcLOV4. Here I will present a variety of cellular studies of these proteins expressed in heterologous settings, letting us both examine structure-function relationships for these LOV photoreceptors and examine their suitability for controlling biological processes. I will present data on the LOV-HTH EL222 and RGS-LOV BcLOV4 proteins, where I found model driven site-directed mutagenesis revealed structural residues important to functionality that has driven a more accurate modeling of their dynamic states. This work has led to novel cycling mutations making them ideal targets for precision tool development. Harnessing this information, I am currently engineering novel light-activated "optogenetic" tools for controlling cellular biology.

ABS#343

Poster session, July 14

Adaptable, Turn-On Monobody (ATOM) Fluorescent Biosensors for Multiplexed Detection in Cells

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Fluorescent reporters of intracellular molecules are greatly desired for imaging applications. A challenge in biosensor design is to develop a single molecule, fluorescent protein (FP)-based platform that can be easily adapted to recognize any target of choice. We addressed this challenge by fusing antibody-like binding domains, known as monobodies, to an FP in such a way that target binding activates fluorescence. The resulting adaptable

turn-on monobody (ATOM) class of sensors are engineered by the insertion of a circularly permuted monobody into surface-exposed loops of fluorescent proteins. We demonstrate modularity by designing biosensors for three different proteins using previously designed monobodies. The generalizability of the design is shown by engineering FPs from two distinct lineages, allowing multi-color imaging. Lastly, we illustrate the utility of these biosensors for imaging proteins in the mitochondria and endoplasmic reticulum. Fluorescence activation involves ligand-dependent chromophore maturation with fluorescence turn-on ratios of >20-fold in cells and up to 100-fold in vitro. The reported class of biosensors enable fast design to detect a ligand of choice and study a variety of cellular processes.

ABS#345

Poster session, July 14

Investigating the biophysical basis for allosteric regulation of tyrosine phosphatases

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Tyrosine phosphatases are signaling enzymes that help regulate the phosphorylation states of proteins. Their function is critical for cell communication, proliferation, and migration. Changes in enzymatic activity due to variations in phosphatase conformational states can lead to disease. The phosphatases PTP1B and SHP2 have been identified as drug targets due to their roles in metabolic diseases and cancer, however, we do not fully understand the underlying allosteric mechanisms for their tunability. Elucidating the molecular basis for the regulatory mechanisms that fine-tune phosphatase function will improve our understanding of their protein-protein interactions in cells. Moreover, investigating whether nature has evolved allosteric communication networks that are conserved across family members will reveal insights into less well-studied phosphatases. With this motivation in mind, we first studied PTP1B in the context of its interaction with the adaptor protein Grb2 using NMR and HDX-MS. In addition, we examined the requirements for PTP1B-Grb2 binding and the potential impact of Grb2 binding on PTP1B function. Next, we studied SHP2 which, unlike PTP1B, has two regulatory SH2 domains in addition to its phosphatase domain. Mutations in these phosphotyrosine-recognition domains have been shown to increase SHP2 basal activity without impacting binding to other tyrosine phosphorylated peptides/proteins. We are using biophysical techniques to reveal how ligand interactions impact the conformation of the SH2 domains that regulate SHP2 activity. In this way, we will better understand the allosteric contributions of the SH2 domains on SHP2 function. Together, these biophysical studies will characterize evolutionarily-conserved mechanisms for allosteric communication across tyrosine phosphatase family members.

ABS#347

Poster session, July 14

Small Changes, Big Improvements: High-Quality "Prepared" Models of All X-Ray Structures in The Protein Data Bank for Broad Use

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The vast majority of biomedical and drug discovery projects begin by downloading a structural model from the Protein Data Bank (PDB). However, the qualities of these structures (which date back to 1976) vary greatly, and can adversely impact project duration and cost. We hypothesized that low model qualities can be improved to leverage the "quality in, quality out" paradigm, saving time and money on projects utilizing these input proteins. First, we used 6 industry-standard quality metrics to develop an aggregate "Quality Score" from 0% (worst) to 100% (best). We then assessed the ability of Rosetta's Relax preparation algorithm to improve the Quality Scores of 487 unique X-ray protein structures (representing a diverse cross-section of the PDB) by Relaxing and validating each model, and then calculating its RMSD and change in Quality Score upon Relaxation. Crystal contacts, electron density data, and water molecules were also considered to assess the effect of mimicked crystallographic conditions on Quality Score. We tested and analyzed over 40 unique variations of the Relax protocol in an automated pipeline across 3 cluster architectures. A Pareto-optimal Relax variant was found to increase all

model Quality Scores to an average of 99.4% ± 0.8% with sub-Angstrom all-heavy-atom RMSDs from their starting structures, plus a reasonable runtime and near-identical reproducibility across architectures (manuscript in preparation). This Relax algorithm was then followed by the PDB-REDO pipeline for re-phasing, which ultimately resulted in the most optimal models. We are now Relaxing all crystal structures from the PDB in partnership with Cyrus Biotechnology and PDB-REDO, and publicly releasing the method and ongoing results for broad use. Our pipeline can be used to prepare X-ray structural models as high-quality inputs for protein engineering projects, and the prepared database can serve as a high-quality dataset for machine learning, AI, and other big data applications.

ABS#349

Poster session, July 15

Friend or foe? The potential role of the proteasome in amyloid beta nucleation

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Alzheimer's disease (AD) is an uncured, fatal, neurodegenerative disease that impacts ~10% of persons over the age of 65. Both the development and progression of AD are associated with the de novo assembly, or nucleation, of amyloid beta 42 (Aβ42) fibrils. This conformational ordering of Aβ42 monomers into a highly ordered amyloid fibrils is both kinetically and thermodynamically limited, making it an extremely rare event. As the occurrence of AD cannot be explained via randomness, it is plausible that the kinetic/thermodynamic nucleation barriers to Aβ42 can be overcome by disease-specific factors. One extrinsic factor thought to facilitate the formation of Aβ42 fibrils is the loss of proteasomal function with age. It is hypothesized that age-related declines in proteasomal function, and general proteostasis, facilitate the conformational transition of Aβ42 by increasing the local concentration of both monomers and oligomers. Indeed, Aβ42 oligomers are known to bind and inhibit the proteasome, potentially exacerbating this transition. However, as Aβ42 nucleation is also kinetically limited, increased concentration alone is not sufficient to initiate a seeding event. Therefore, our central hypothesis is that the proteasome creates a direct interface required for the formation of Aβ42 nuclei. Using distributed amphifluoric FRET (DAmFRET) in HEK293T cells, we show that Aβ42 self-assembly is reduced in the presence of the proteasomal inhibitor MG132 (10μM; 24h). This effect was not observed in the presence of the autophagy inhibitor chloroquine (10μM; 24h), suggesting that Aβ42 nucleation is in part dependent on proteasomal function. Additionally, we observed that subcellular localization of Aβ42 impacts its ability to self-assemble. Using DAmFRET, we show that nuclear localization of Aβ42 lowers the kinetic nucleation barrier, whereas cytoplasmic localization enhances this barrier. Taken together, these data suggest that both the proteasome and subcellular localization are principal factors governing the conformational transition of Aβ42 monomers into highly ordered fibrils.

ABS#352

Poster session, July 13

Investigations into the Phase Separation and Nuclear Role of Tau

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Tau is a microtubule-associated protein and pathological hallmark of Alzheimer's disease (AD), most infamous for becoming hyperphosphorylated and fibrilizing into neurofibrillary tangles (NFTs). Beyond this role, mounting evidence suggests that tau localizes into the nucleus and plays unknown roles in DNA protection and heterochromatin regulation. Intriguingly, frontotemporal dementia mutants (P301L) of tau show loss of genetically silent heterochromatin clusters. This has been associated with aberrant expression of heterochromatic genes and in other studies, of transposable element activation in AD patient brain tissue. Similar effects of heterochromatin relaxation and gene dysregulation have been observed in tau knockouts, suggesting that loss of tau is pathologically relevant. Recent literature showed that tau undergoes liquid-liquid phase separation (LLPS). Studies have not fully described the functional role of nuclear tau in gene regulation, and whether this involves LLPS. Our work demonstrates that tau has an intrinsic ability to phase separate with, and oligomerize chromatin, likely through its DNA-binding domain, and this is regulated by phosphorylation. In addition, tau phase separates with heterochromatin protein 1α (HP1α), an essential heterochromatin constituent, which may promote its partitioning into heterochromatin despite its lack of DNA-

sequence specificity. These data imply a potential role for tau LLPS in the maintenance of chromatin compaction with aging.

ABS#353

Poster session, July 13

Multimodal Machine Learning for Protein Variant Effect Prediction and De Novo Design

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Proteins are governed by biophysical principles, which recasts their variant effect prediction and (re)design as a principle-driven optimization problem. Meanwhile, growing protein data exists in a multitude of modalities including texts, images, graphs, and geometries, which drives the emerging formulation of data-driven multimodal machine learning. The data-driven formulation, with principles incorporated properly, can enable broad exploration of the protein universe and overcome the limited quantity, quality, and transferability of the protein phenotype data. I will introduce our multimodal machine learning methods including (1) protein structure-informed language models and (2) deep generative models conditioned on desired properties such as structural folds and specific functions. Importantly, I will demonstrate their use in predicting protein variant effects in protein thermostability & activity, cell fitness, and disease phenotypes, anticipating SARS-CoV-2 variants of concerns, and designing functional proteins.

ABS#354

Structures of Mega-Complexes (July 13, PM)

Molecular Determinants of AP-3 Membrane Engagement and Cargo Selection

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AP-3 belongs to the Adaptor Protein (AP) complex family of heterotetramers which are responsible for regulating vesicular transport throughout the cell (1). AP-3 coordinates trafficking between the trans-Golgi network (TGN) and the early endosome/lysosome system (1). It accomplishes this by engaging an organelle membrane, a transmembrane-spanning proteinaceous cargo, the small GTPase Arf1, and vesicular coats (e.g. clathrin). Historically AP-3 has been characterized via immunofluorescence and genetic knockdowns, but biochemically it has never been successfully reconstituted in vitro (2). AP-3 exhibits a high degree of plasticity not otherwise observed among complexes within the family (1). Curiously, it has been documented that certain transmembrane-spanning cargos are selectively recognized by certain AP complexes, while other cargoes are promiscuously trafficked (2). The molecular basis for this discrimination or promiscuity remains unknown, especially pertaining to AP-3 dependent cargoes. Unlike any of the other AP complexes, AP-3 is known to have clathrin-dependent and independent functionality, a duality that makes AP-3 unique (2). From a biochemical perspective though, much remains unknown about this dynamic and exceptional AP complex. In order to investigate the molecular underpinnings of AP-3's functionality, we have successfully reconstituted the human isoform of AP-3, and determined its structure via single-particle cryogenic electron microscopy (cryo-EM) alone and in the context of a membrane. To do this, we resorted to an unconventional method of grid preparation. Unexpectedly, our findings indicate that AP-3 is regulated completely differently than other AP complexes. Our structures and accompanying biochemical characterization provide insight into the basis for how AP-3 engages cargo, the membrane, and post-translationally modified Arf1. Our structures are currently the only three-dimensional reconstructions of a human specific AP complex, providing important insight for diseases resulting from genetic defects in AP-3 subunits.

ABS#355

Poster session, July 13

Calmodulin Interacts with the IQ Motif of the HECT E3 Ubiquitin Ligase UBE3B Through a Calcium-Independent Mechanism

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Kaufman oculocerebrofacial syndrome (KOS) is an ultra-rare autosomal recessive disease that presents with developmental setbacks along with a distinct set of ocular and

facial deformations. The cause of this disease has been credited to mutations in the UBE3B gene that encodes for the homologous to E6AP C-terminus (HECT) E3 ubiquitin ligase UBE3B. UBE3B has an N-terminal IQ motif composed of mostly positively charged amino acids that was previously shown to bind to the calcium-binding protein Calmodulin (CaM) which decreases UBE3B-dependent ubiquitylation activity. The molecular mechanism and structural basis for CaM-UBE3B complex formation are currently unclear. To address this, we conducted a series of pulldown assays and isothermal titration calorimetry using a UBE3B IQ motif truncated construct in the presence of various CaM constructs. Our results show more prominent interactions between the IQ motif and UBE3B happened with CaM constructs containing its C-terminal lobe and in the absence of calcium. Our studies aim to uncover the CaM-dependent regulatory mechanism of UBE3B and how UBE3B malfunction contributes to the KOS phenotype.

ABS#356

Poster session, July 15

Predicting multiple conformational states via sequence clustering and AlphaFold2

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AlphaFold2 (AF2) has revolutionized structural biology by accurately predicting single structures of proteins and protein-protein complexes. However, biological function is rooted in a protein's ability to sample different conformational substates, and disease-causing point mutations are often due to population changes of these substates. This has sparked immense interest in expanding AF2's capability to predict conformational substates. We demonstrate that clustering an input multiple sequence alignment (MSA) by sequence similarity enables AF2 to sample alternate states of known metamorphic proteins, including the circadian rhythm protein KaiB, the transcription factor RfaH, and the spindle checkpoint protein Mad2, and score these states with high confidence. We used our clustering method, AF-cluster, to screen for alternate states in protein families without known fold-switching, and identified a putative alternate state for the oxidoreductase DsbE. Similarly to KaiB, DsbE is predicted to switch between a thioredoxin-like fold and a novel fold. This prediction is the subject of future experimental testing. Further development of such bioinformatic methods in tandem with experiments will likely have profound impact on predicting protein energy landscapes, essential for shedding light into biological function.

ABS#357

Poster session, July 15

Experimental Validation of Multiple Conformational States Predicted via AlphaFold2 and Sequence Clustering

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Improving abilities to understand and predict multiple conformational substates of proteins holds great promise in furthering our understanding of protein function and disease mechanisms. For instance, metamorphic proteins, or proteins which change their folded structure as part of their biological function, perform diverse functions, yet their evolution and their functions are poorly understood. The accuracy of AlphaFold2 (AF2) at predicting single structure has garnered interest in its ability to predict multiple conformations of metamorphic proteins, yet in its default settings, it is unable to predict the different states of these proteins. Here we use the AF-cluster method, which can predict multiple states of metamorphic proteins, to investigate the evolutionary distribution of predicted structures for the model fold-switching protein KaiB and validate our predictions using NMR spectroscopy. We find that predictions of both ground state and fold-switched states of KaiB were distributed in clusters across the KaiB family. One of KaiB variants from the cyanobacterium family was unexpectedly predicted with high accuracy to be stabilized in the fold-switch state and we show this prediction is accurate by NMR spectroscopy. Furthermore, we used our method to find a minimal set of mutations that were able switch

the equilibrium between the two metamorphic states of KaiB from Rhodobacter Sphaeroides and confirm by NMR spectroscopy that these mutations indeed switch the equilibrium populations of the two states. This study highlights the great potential in integrating bioinformatic tools like the AF-cluster method with structural biology techniques such as NMR spectroscopy to identify new metamorphic proteins and may open ways of targeting their multiple states for therapeutic discovery.

ABS#358

Poster session, July 13

Understanding SHP2 activity and regulation using deep mutational scanning

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SHP2(Src homology region 2 domain-containing phosphatase-2) is an important signaling protein. It's involved in multiple signaling pathways like the Ras/-Raf/MEK/ERK pathway, the PI3K pathway and the Jak/STAT pathway. Mutations in SHP2 have been shown to be the cause of various cancers and developmental diseases. However, for many of these mutations it remains unclear if, and how, they alter SHP2 structure and conformational dynamics. In addition, not all known pathogenic mutations that can potentially alter SHP2 structure and activity have been studied. We are using deep mutational scanning to study the mutation-activity relations of SHP2. We first established and validated a yeast selection assay which faithfully reports SHP2 function by coupling cell viability to phosphatase activity. Using this assay, we characterized a saturation mutagenesis library of SHP2 we generated in-house, which included all possible point mutations in SHP2. Then, we used deep sequencing to examine the library composition before and after selection, to yield an activity profile of all the SHP2 variants in the library. By analyzing the activity differences, we were able to infer how key interactions of certain residues with their surrounding environment can regulate intra- or inter-domain structural features in wild-type SHP2. Moving forward, we are working on doing deep mutational scanning under different selection stress, and in presence of SHP2 inhibitors or activators, to further study how SHP2 activity is regulated in different contexts. These insights can further our understanding of the diversity of SHP2 pathologies, and ways to counteract them.

ABS#359

Capturing Protein Interactions (July 14, PM)

Thermal Stability of Monomeric, Dyad and Triad NanoLuc Constructs and Their Assisted Refolding Via Addition of E. coli Chaperones (DnaK, DnaJ, GrpE)

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Chaperone proteins are an essential component in protein homeostasis, with one of their vital tasks concerning protein (re)folding. With not too many robust model client proteins found in literature to examine chaperone assisted refolding, we aim to introduce a new highly bioluminescent protein, NanoLuc (NLuc), an alternative to the traditionally used bioluminescent Firefly Luciferase (FLuc). With a high thermal denaturation temperature (58°C), small molecular weight of 19 kDa (FLuc is 62 kDa), we developed constructs with tandem repeats of NLuc (dyad and triad NLuc). Using thermal denaturation refolding studies and Circular Dichroism (CD) experiments we find striking differences among the monomeric NLuc, dyad and triad NLuc. Interestingly, at 58°C monomeric NLuc demonstrates thermal stability while both dyad and triad NLuc lose their thermal stability with a bioluminescence drop of up to 80% after 20 min (monomeric NLuc has a 20% drop at same timepoint). The addition of DnaK, DnaJ, and GrpE resulted to more than 70% of refolding of both dyad and triad NLuc, a great contrast from the spontaneous refolding results, which show no recovery for both protein constructs suggesting that both constructs are useful models for studying chaperone mechanisms. CD experiments of these constructs also demonstrate a different behavior with the monomeric NLuc losing its β-sheet conformation at elevated temperatures, a behavior not as predominant in the dyad NLuc. Additionally, our Coarse-Grained Molecular Dynamic (CG-MD) simulations provide great insight into the refolding of thermally unfolded monomeric dyad and triad NLuc constructs. Consistent with the experiment, we found that monomeric NLuc is able to successfully refold, while the dyad and triad had a 78% and 63% of

misfolding, respectively. Lastly, we used MD simulations to examine the probability of domain swapping and found a non-negligible contribution of it in NLuc misfolding.

ABS#360

Poster session, July 13

In Silico Prediction of Linear Antibody-Epitopes via Alphafold 2

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Characterizing how antibodies bind to their target protein is important for understanding how they perform their functions, which could be neutralizing a pathogen during an immune response, live cell imaging, protein-tagged purification, or other immunoassays. However, determining the binding epitope of an antibody can be a time and labor-intensive endeavor with significant cost. To overcome this problem, we developed an AlphaFold2 [1] based pipeline to predict linear epitopes for antibodies. This pipeline takes a single chain variable fragment (scFv) sequence and a target antigen sequence, then splits the antigen sequence into sliding window peptides. The potential complex between the scFv sequence and each of these peptides is predicted via AlphaFold2. While generating structures, AlphaFold2 also assesses prediction confidence via the pLDDT metric [2]. Using pLDDT, we can assess how well AlphaFold2 thinks each antigen-derived peptide sequence binds to the antibody scFv sequence. Notably, we find that the frequency of correctly identifying experimentally validated epitope sequences within the antigen can be increased by splicing the CDR loops of interest from the antibody onto favorable (soluble and stable) scFv scaffolds like 15F11 (pdb 5B3N) and 2E2 [3]. The resulting pipeline correctly predicted linear epitopes for well characterized antibody:epitope pairs such as HA and Myc, as well as novel ones like mBG17 [4], an antibody that binds tightly to the SARS-CoV-2 nucleocapsid protein. The predicted orientation of the epitope in mBG17 was verified with competition ELISA assays, demonstrating that our AlphaFold2 pipeline can accurately predict linear epitope sequences de novo. Overall, this work demonstrates that our AlphaFold2 pipeline can accurately identify epitopes for linear antibodies using only the antibody and antigen sequences, significantly accelerating linear epitope identification.

ABS#361

Poster session, July 15

Reciprocal Amino Acid substitutions within TMD2 of Streptococcus mutans YidC1 and YidC2 Exchange Paralog Functions due to Altered Phospholipid Occupancy

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Unlike Gram-negative bacteria which contain a single YidC, Gram-positive bacteria, mitochondria, and chloroplasts generally harbor two paralogs of the YidC/Oxa/Alb family of membrane-localized chaperone insertases. The oral pathogen Streptococcus mutans often serves as a model organism for other Gram-positive species. Elimination of S. mutans YidC2 has far more apparent and severe consequences than elimination of YidC1, yet the underlying mechanism mediating paralog-specific functions of YidC2 compared to YidC1 is unknown. A previously observed W138R gain of YidC2-like function mutation within transmembrane domain 2 (TMD2) of S. mutans YidC1 led us to mutate the analogous position, S152, within YidC2. Furthermore, deletion of the sole gene encoding S. mutans cardiolipin synthase resulted in sensitivity to multiple environmental stressors similar to the phenotype observed following deletion of yidC2. This led us to speculate that specific interactions of YidC1 and YidC2 with phospholipids present in the S. mutans cytoplasmic membrane contribute to their respective functional activities. We used Coarse Grain Molecular Dynamics (CGMD) simulations with the Martini2.2 Forcefield to evaluate interactions of YidC1, YidC2, and substitution derivatives, with phospholipids prevalent within the S. mutans membrane. Lipid occupancy profiles, particularly for cardiolipin, were altered in YidC1 and YidC2 substitution constructs to mimic characteristics of the other wild-type paralog. CGMD also identified a preference for YidC1 and YidC2 dimer formation compared to monomers, and revealed that YidC2 dimer function likely depends on increased flexibility relative to YidC1 dimers. While, YidC1W138R/A strains acquired the ability to support S. mutans growth under conditions of acid and osmotic stress, YidC2S152W/A strains lost these

typical YidC2-associated properties and acquired YidC1-like behavior. Collectively, our results demonstrate that differences in phospholipid occupancy at a pivotal residue within TMD2 of YidC1 and YidC2, respectively, governs their ability to confer paralog-specific attributes.

ABS#362

Undergraduate Research Session

Specificity studies of small molecule inhibitors targeting the ankyrin-repeat oncoprotein gankyrin

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The oncoprotein gankyrin contains seven ankyrin repeats and is overexpressed numerous cancers. Gankyrin interacts with the proteasomal subunit S6ATPase to form the 19S regulatory cap of the 26S proteasome that is responsible for protein turnover in the cell. It contains a featureless, concave surface to facilitate a diverse range of essential protein-protein interaction, some recently being identified in the onset of cancer development. Of these, gankyrin acts as a chaperone for the RING E3 ubiquitin ligase MDM2 to enhance ubiquitylation of the tumor suppressor protein p53, however, its overexpression increases p53 degradation that leads to enhanced cancer cell migration and tumorigenesis. Inhibitors of gankyrin protein-protein interactions are hypothesized to obstruct tumor formation and growth, but the precise mechanism for how these small molecules inhibit gankyrin is currently unknown. We describe our biochemical and biophysical analyses of various small molecules using ITC, CD, and NMR to pinpoint where they bind to gankyrin and how they affect the 3D structure of gankyrin. TO determine if these small molecules are specific for gankyrin, we also assessed their effect on other ankyrin repeat containing proteins such as RFXANK, Ankra2, Trabid, and DARPin using similar biochemical approaches. This interneciary collaborative project between the Spratt Lab at Clark University and the Muth Lab at St John's University aims to produce and characterize potent small molecule inhibitors of gankyrin that will induce apoptosis in cancer cells.

ABS#363

Poster session, July 13

Expanded Co-Crystals as Scaffolds for DNA-binding Protein Guests

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X-ray crystallography is a useful technique for determining the structures of biomolecules which can provide insight into their function. However, growing biomolecular crystals that are suitable for x-ray diffraction(XRD) is a challenging hit-and-miss process that typically requires significant efforts to optimize high sample purity and search for suitable growth conditions. Porous scaffold crystals provide a possible solution to these traditional crystallography challenges. Our designed co-crystals consist of a DNA-binding protein and cognate DNA blocks that are designed to expand the crystal structure at the DNA junctions, while preserving the overall lattice topology. Moreover, the target crystals are highly modular with respect to the inserted DNA nucleotide sequences. In turn, this modularity allows us to grow crystals that display the binding sequence for several different classes of DNA-binding guest molecules, including guest proteins. Experimentally, we have been able to monitor porous co-crystal uptake of fluorescently labeled guest proteins using confocal microscopy. Attempts to use XRD to determine the structure of guest proteins inside host co-crystals are ongoing.

ABS#364

Poster session, July 14

Progress Towards the Development of a Kinetic FRET-Based Assay for the Study of HECT E3 Ligase Inhibition by Small Molecules

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Homologous to E6AP Carboxyl Terminus (HECT) E3 ubiquitin ligases are key players in the ubiquitin-signaling pathway that manages protein localization, viral immune response, and targeted protein degradation, among many other cellular activities. HECT, C2, and WW domain containing E3 ubiquitin protein ligase 2 (HECW2) is a poorly studied member of the HECT E3 ubiquitin ligase family that has been linked to the ubiquitylation of lamin proteins in the nuclear envelope. The malfunction of nuclear envelope proteins is believed to play an important role in the group of diseases caused by lamin mutations, collectively known as laminopathies. A prime example includes Hutchinson-Gilford Progeria Syndrome (HGPS), which is characterized by rapid uncontrollable aging of children. Many researchers currently study the ubiquitylation activity of HECT E3 ubiquitin ligases using gel-based activity assays, however, these are generally unreproducible and lack the ability to acquire quantitative kinetic data. To address this, we have been working towards the development of a sensitive FRET-based kinetic assay to more precisely investigate the transfer of ubiquitin to and from HECW2. This assay is being prepared to analyze potential small molecules identified using a proprietary machine learning algorithm that could inhibit HECW2 ubiquitylation activity. Our novel assay approach can also be adapted to examine the kinetic parameters of other members of the HECT E3 ubiquitin ligases and help in the future screening of other molecules for HECT-dependent diseases including laminopathies.

ABS#365

Peptide Modalities: Size Doesn't Matter (July 14, AM)

Implementing a Novel Proteome-Wide Biochemical Screen to Define Sequence Motifs That Support Binding to the Core Autophagy Protein LC3B

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LC3B is one of six paralogous proteins critical in the regulation and execution of autophagy, a conserved proteostasis pathway that delivers proteins, protein complexes, and organelles to the lysosome via a double-membraned vesicle. LC3B is a ubiquitin-like protein that is reversibly conjugated to phosphatidylethanolamine (PE) on both the inner and outer autophagosomal membranes and engages in an extensive protein-protein interaction network that includes cargo destined for degradation as well as proteins involved in autophagosomal transport and lysosomal fusion. LC3B is thought to interact with many partners via a degenerate four-residue short linear motif (SLiM) known as the LC3-interacting region (LIR) often found in disordered regions of the binding protein. Notably, this LIR motif is of low information content, and core motif instances alone are not sufficient to explain binding. To discover additional sequence elements that modulate the binding of LIR motifs and to identify novel candidate LC3B interaction partners, we developed and deployed a human proteome-wide screen using 36-amino acid peptides. We identify over 10,000 binding peptides with many bearing the core LIR motif. We further observed strong enrichment for acidic residues up to 15 positions N-terminal to the core LIR motif, suggesting additional sequence specificity not encoded in the core LIR motif. We also found novel noncanonical motifs that bind to LC3B with micromolar (uM) affinity. Our discoveries broaden the definition of the LIR motif, expand the network of candidate LC3B interaction partners, and provide a valuable starting point for the design of LC3B binders and therapeutics to modulate autophagy.

ABS#368

Poster session, July 14

Training a Message Passing Undirected Graph Neural Network for Protein Sequence Design Using Structural Ensembles and Multiple Sequence Alignments

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De novo protein design requires sequence design methods that predict the sequence of a desired protein from backbone coordinates. State-of-the-art sequence design methods use message passing graph neural networks that treat the protein backbone as a graph, with atoms as nodes and edges defined by proximity in 3D space, to learn structure-to-sequence relationships. We improve on these methods by reframing the sequence design task to incorporate protein flexibility and by updating the neural network architecture to better represent interactions between atoms. Instead of treating

sequence design as a one-to-one structure-to-sequence problem, we treat sequence design as a many-to-many problem of mapping a structural ensemble to a multiple sequence alignment (MSA). We generate structural ensembles by applying noise to the backbone coordinates in torsional space (i.e., by updating phi and psi dihedral angles). This ensures the noisy structures remain physically realistic because bond lengths and angles are unchanged. Our architecture operates over an undirected graph instead of a directed graph. This architecture better represents interactions in a protein structure, as two atoms share a single interaction energy. We evaluate our model in three ways. First, we calculate the native sequence recovery (NSR) and MSA recovery (MSAR). We define the latter as the ability to predict the likelihood of observing each possible pair of residues in the MSA. We match the benchmarks' performance on NSR and outperform the benchmarks on MSAR. Second, we show that, compared to benchmarks, our model better predicts binding and folding energies. Third, we use AlphaFold to predict the structure of sequences designed by our model and by baselines and find improved alignment between the native structures and the AlphaFold predictions for our model. These results demonstrate the value of building protein flexibility into sequence design models and of using a model architecture that best represents protein structures.

ABS#369

Poster session, July 14

Mass Spectrometry and Molecular Dynamics Simulations Untangles Structure and Function of Protein Condensates

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RNA-binding proteins can undergo liquid-liquid phase separation (LLPS) serving as a major regulator of the cellular stress response. RNA-binding proteins are present in phase separated compartments, such as stress granules. These contain RNA recognition motifs and low-complexity domains characteristic of disordered proteins, and are important for LLPS but also toxic aggregation. The low stability and heterogeneous interactions of disordered proteins makes it challenging to obtain structural information on the droplet states of proteins. Here, we use native mass spectrometry (MS), and molecular dynamics (MD) simulations to explain the self-assembly mechanisms of three RNA-binding proteins that can undergo LLPS and aggregation. Through fusion to an LLPS-compatible spider silk domain, we produced natively soluble forms of the proteins FUS, TDP-43, and hCPEB3 that are implicated in neurodegeneration, cancer, and memory storage. We then followed pH-controlled self-assembly using ion mobility mass spectrometry (IM-MS), which enables us to monitor conformational changes in a wide range of conditions, from denaturing to native. We find that FUS monomers undergo a pronounced unfolded-to-globular transition, whereas TDP-43 oligomerizes into partially disordered dimers and trimers during LLPS. hCPEB3 remains disordered though forms fibrillar aggregates rather than undergoing LLPS. Based on the IM-MS data, we can perform MD simulations, obtaining atomistic models of FUS and TDP-43 assembly intermediates. In these models, FUS has the lowest b-sheet content, hCPEB3 has the most, and the b-sheet content of TDP-43 is intermediate between that of the other two proteins. Our results show that despite their superficial similarity, all three proteins adopt distinct configurations in their assembled states. We speculate that these specific assembly architectures serve to regulate RNA processing and translation in specific ways depending on biological context.

ABS#370

Poster session, July 13

The search for the hypothetical RNA joinosome: trans-splicing complex in the mitochondria of Diplonema papillatum

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Freshly transcribed RNA undergoes numerous transformations to become a mature mRNA ready for translation. We are studying an unprecedented mode of RNA processing in the marine microeukaryote Diplonema papillatum, where mitochondrial genes are fragmented into 40-550 bp pieces called modules. Each is encoded on one

of the 81 chromosomes of the genome, and is transcribed individually [1]. Then, all the module transcripts of a gene are assembled in the correct order to form a mature contiguous mRNA by a mode of trans-splicing that has yet to be uncovered, as it is clearly distinct from all splicing mechanisms described thus far. Since the modules bear 3′PO4 and 5′OH ends (instead of the usual 3′OH and 5′PO4) [2], we posit that an RtcB-type RNA ligase, capable of ligating these types of ends, is the catalytic component of the hypothetical protein complex called the joinosome that assembles the RNA modules. Of the three RtcB genes found in D. papillatum's nuclear genome [3], RtcB1 is the top candidate of being part of the joinosome as it bears a mitochondrial import signal. The hypothetical joinosome must also include RNA-recognition factors proteins, nucleic acids, or otherwise that act as matchmakers of the module transcripts. Our main goal is to isolate the postulated joinosome by co-immunoprecipitation of D. papillatum RTCB1 bearing a C-terminal ProteinA tag and identify its components by tandem mass spectrometry and nucleic acid sequencing. The analyses promise to reveal a novel molecular mechanism of RNA trans-splicing, which could lead to the development of a molecular toolbox for synthetic biology, genetic engineering, and potentially even gene therapy.

ABS#371

Poster session, July 14

Antibiotic Fragments have Activity and Bind on Their Target Protease

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Multidrug-resistant tuberculosis is considered a global health crisis. Successfully treating drug-resistant tuberculosis infections may require exploiting novel molecular targets in teh causative pathogen, Mycobacterium tuberculosis. ATP-dependent Clp proteases, which contribute to protein homeostasis and pathway regulation, are one emerging class of novel antibacterial targets in mycobacteria. Theres essential enzymes consist of an unfoldase (ClpX or ClpC1) and a barrel-shaped ClpP1P2 peptidase that assembles from separate ClpP1 and ClpP2 peptidase rings. Acyldepsipeptides (ADEP) are complex peptide antibiotics that inhibit Clp proteases by binding to the ClpP2 ring of ClpP1P2 and blocking unfoldase binding. ADEPs can kill mycobacteria in culutre and in models of infection, but their potency is relatively low. Our objective here was to test if simplified ADEP fragments, lacking the complex peptide macrocycle, retain their binding characteristics to ClpP1P2. We solved multiple co-crystal structures of ADEP fragments in complex with M. tuberculosis ClpP1P2. These structures confirm that the core ADEP binding component restains its ability to bind ClpP1P2 in the context of ADEP fragments. Surprisingly, we also found that ADEP fragments are able to bind to peptidase active sites within the barrel, and mimic pepetide substrate in modulating ClpP1P2 assembly. Our studies provide a path for future development of ADEP derivatives to achieve improved potency.

ABS#373

Poster session, July 15

Knowledge-Based Energy Scale for Inserting Amino Acids into the Membrane

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The distribution of amino acids on membrane proteins reflects their positioning and orientation in the lipid bilayer. Here, using an expansive dataset of known structures of membrane proteins, we calculated the propensities of occurrence for each amino acid at different z-depths along the membrane normal axis. From the protein structures, we identified whether an amino acid side chain is buried within the protein or exposed to the environment, and calculated the propensities correspondingly. The propensity differences between “exposed” and “buried” positions for some amino acids quantitatively show their preferences for both the z-depth in the membrane and the position on the protein. From the differences in propensities at different z-depths, we calculated the pseudo-energies of moving an amino acid from the aqueous environment into the membrane at each z-depth. This energy scale reflects the physiological process of inserting peptides into the membrane, and correlates with experimental data on peptide partitioning and transmembrane segment insertion. Our knowledge-based amino acid propensity and energy scales provide a reliable reference for positioning of membrane proteins in

the membrane, and can be used for membrane protein structural characterization, prediction, and design.

ABS#374

Poster session, July 13

Effects of Drying and Additives on Protein Structure at the Residue Level

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Overcoming the inherent instability of protein-based drugs and industrial enzymes via refrigeration increases the cost associated with their transportation and storage (1). Drying can eliminate the need for refrigeration but often leads to protein unfolding, aggregation and inactivation (2). To combat these problems, proteins are often dried with protective additives known as excipients (3). However, excipients are typically employed empirically, and their mechanism of protection is poorly understood (4). To increase our knowledge of excipients and dry proteins, we employ liquid-observed vapor exchange NMR, a technique that reveals how drying and addition of excipients affect protein structure at the residue level (5). We conclude that vacuum-drying gives more protection than freeze-drying. Moreover, adding desiccation tolerance proteins from tardigrades (CAHS D) improves protection, and protection by CAHS D is concentration dependent; lower concentrations lead to better protection.

ABS#376

Poster session, July 15

How enzymes impart catalytic power to amino acids in the active site

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Amino acid side chains, such as the carboxylic acid groups of aspartate and glutamate, or the primary amine of lysine, are weak Brønsted acids and bases for the free amino acids in solution. How do enzyme active sites transform them into strong acids, bases, or nucleophiles? Evidence is presented that enzymes impart catalytic potency to amino acids in the active site through couplings that 1) Alter the strength of acidity/basicity and, simultaneously; 2) Expand the buffer range. To achieve an expanded buffer range, a residue must be strongly coupled either: 1) to a like-charged residues with an intrinsic pKa difference less than 1 pH unit; or 2) to an oppositely-charged residue where the intrinsic pKa of the anionic residue is higher than that of the cationic residue, with an optimal difference that is dependent on the coupling energy. Strength of acidity or basicity, as represented by shifts in pKa, are controlled by coupling to other charged residues; the expanded buffer ranges enable the correct protonation states to be populated at the operational pH. Examples are reported, showing how nearby aspartates and glutamates play significant supporting roles to catalytic aspartates and glutamates. Similarly, catalytic lysines are supported by neighboring tyrosines or cysteines, where the Tyr or Cys has the higher intrinsic pKa, or by other lysines, where the intrinsic pKa difference is less than 1 pH unit. Supported by NSF CHE-1905214, MCB-2147498 and a Fulbright Faculty Research Award.

ABS#377

Poster session, July 15

Mitochondrial-derived Peptides: Are They Actually Important?

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Mitochondrial-derived peptides are encoded by mitochondrial DNA but have biological activity outside mitochondria. Eight of these are encoded by sequences within the mitochondrial 12S and 16S ribosomal genes. These are humanin, MOTS-c, and the six SHLP peptides, SHLP1-SHLP6. These peptides have been shown to have various effects in cell culture and animal models, including neuroprotection, enhancement of insulin sensitivity, and inhibition or promotion of apoptosis. To gauge their importance in vertebrate evolution, an analysis of synonymous codon bias in the peptide coding sequences is presented. Because they lie in RNA genes, such bias should only occur if the peptides have undergone purifying selection, that is, their amino acids have been conserved to maintain biological function. Humanin shows the strongest bias for synonymous codons, followed by SHLP6 and possibly SHLP4. In contrast, SHLP1, SHLP3, and SHLP5 show no significant bias, while the situations

for MOTS-c and SHLP2 are more complex. MOTS-c also shows no significant bias, though the analysis cannot rule out that a highly conserved pentapeptide within MOTS-c, MGYIF, might have undergone natural selection in mammals. For SHLP2, the results are unclear for primates, though vertebrates in general show no significant bias. Curiously, a cluster of codons with significant bias was observed preceding and in the same reading frame as SHLP2. Named SHLP2b, this cluster has the standard start codon, ATG, in most primates, whereas humans and most other vertebrates have the alternate start codon, GTG. The results suggest synonymous codon bias analysis could be used to discover additional mitochondrial-derived peptides.

ABS#382

Poster session, July 13

Smart Polymers, Smart Sensors: Exploring the Promise of Elastin-Like Polypeptides

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Proteins with compelling functions (e.g., molecular recognition, stimuli-responsive "smart" polymer behavior) are not efficiently translated into industrially viable sensors. This is in part because elements of the sensor fabrication process, such as labeling proteins with probes or immobilizing them on a surface, are often incompatible with stable protein function. If it were easier to design proteins to perform within the context of a given application or independent of context, developing protein-based sensors would be dramatically simplified. Elastin-like polypeptides (ELPs) are genetically-engineered "smart" polymers that have been studied for over 30 years, often with an eye toward biosensing applications, but their potential in this domain has yet to be realized. We hypothesize that a substantial barrier to predictable ELP function in the context of devices and nanomaterials is the knowledge gap between how proteins behave in solution, where they are most frequently studied for their reversible, tunable coacervation, versus how they behave immobilized on surfaces; and how modular protein components function separately versus how each function is influenced when components are physically linked together. The overall objective of this work is to fill in these knowledge gaps with engineering principles toward predictable design of ELP fusion-based analyte-responsive polymers. Toward this end, we present quantitative insights obtained through light scattering and zeta potential measurements into how ELP protein-protein and protein-interface interactions are influenced by ions and the environment, with implications for the rational design of ELP-based surfaces and materials with enhanced performance.

ABS#383

Poster session, July 13

Computational investigations of interdomain allostery in FXR

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Nuclear receptors are ligand-regulated transcription factors responsible for modulating gene expression. The farnesoid X receptor (FXR), like every other nuclear receptor, has a multidomain structure that contains a highly dynamic hinge region connecting the DNA binding (DBD) and ligand binding (LBD) domains. By understanding interdomain interactions, we can gain insight into how ligand binding influences DNA binding specificity. Here, we use molecular dynamics simulations of the full-length receptor to investigate the role of the flexible hinge region in communicating signals between the two domains. We investigate FXR in various ligand-bound and oligomeric states and extract information about allosteric signaling pathways from MD trajectories. These studies will provide insight enabling the design of ligands that selectively regulate interdomain allostery in FXR.

ABS#384

Poster session, July 13

A maestro in the making: beta-sheets conduct differential binding patterns in orchestrating selective ligand affinity between LXR-alpha and beta

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Nuclear receptors are transcription factors that can regulate gene expression upon activation by binding of ligands (1). Liver X receptors alpha and beta (LXRa/b) are cell-specific nuclear receptors that are differentially involved in cholesterol and lipid metabolism (3). Their high structural similarity, sharing approximately 77% identity in their ligand binding domain, has posed a difficulty in developing therapeutics targeting and regulating the activities of these paralogous proteins with high selectivity. In our study, we observe that the beta sheet region in each of these receptors can differentially modulate the binding of endogenous ligands (oxysterols), exhibiting a receptor-specific selective binding. We will show how the beta sheet regulates the dynamics of alpha-helix 5 (H5) and alpha helix 8 (H8) and therefore is able to facilitate high specificity and selective discrimination among various endogenous ligands. Our study will enable us to rationally develop targeted therapeutics against dysregulated gene expressions in various diseased states.

ABS#386

Poster session, July 13

Determining Where CowN and Nitrogenase Interact Using Protein Crosslinking

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Nitrogenase catalyzes the reduction of atmospheric dinitrogen into ammonia. Nitrogenase is inhibited by the environmental gas carbon monoxide (CO). Nitrogen-fixing bacteria rely on the protein CowN for protection from CO. NHS-diazirine crosslinking previously demonstrated that CowN and nitrogenase interact. However, this experiment did not determine which amino acid residues were important in establishing the interaction. Here, we explore the CowN-nitrogenase interaction using the crosslinkers EDC and sulfo-SIAB. EDC covalently crosslinks carboxylic acids and amines whereas sulfo-SIAB crosslinks amines and sulfhydryl groups. EDC crosslinking results were inconclusive. In contrast, the CowN-nitrogenase complex was detected with sulfo-SIAB, meaning the interaction site contains a sulfhydryl group from a cysteine and an amine from a lysine residue. A separate sulfo-SIAB crosslinking experiment was conducted with C90A CowN, which does not have any cysteines. This experiment also yielded a crosslinked complex, indicating that the cysteine involved in binding was located on nitrogenase and lysine was located on CowN. These crosslinking results are consistent with activity assay data that indicate that the C90A CowN variant is able to fully protect nitrogenase from CO inhibition. Work is ongoing to determine which cysteine on nitrogenase mediates the interaction with CowN.

ABS#387

Poster session, July 13

Aspergillus niger Prolyl Endoprotease from Nutritional Supplement Capsules for Use in Hydrogen-Deuterium Exchange Mass Spectrometry

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A prolyl endoprotease from Aspergillus niger (ANPEP) has been shown to be useful in the protein digestion step of peptide-level Hydrogen Deuterium Exchange (HDX) Mass Spectrometry (MS) experiments because it is active in HDX MS conditions and can cleave after proline (1). We sourced ANPEP from widely available gluten-degrading nutritional supplement capsules to integrate into traditional online digestion HDX MS workflows. We investigated the identity and activity of ANPEP in the capsules by dissolving the pill contents in traditional quench buffer and centrifuging, taking the supernatant for subsequent testing with peptide mapping and enzyme activity assays. The major protein component (22% by mass) of the capsules was found to be, in fact, active ANPEP, in relatively pure form. This ANPEP was easily isolated from the capsule fillers and immobilized on POROS-20AL resin. Its specificity was compared to both porcine pepsin and nepenthesin II. Comparative digestion of alcohol dehydrogenase showed that ANPEP performed similarly to pepsin and nepenthesin II, covering 98.7% of the sequence with a redundancy of 16.79. In digestions of a mixture of six proteins, we found that ANPEP preferred to cleave after alanine and proline (43% and 39% cleavage, respectively) with other amino acids ranging from 10-25% cleavage. In comparable studies, porcine pepsin preferred leucine and phenylalanine (33%

and 32% cleavage, respectively) with other amino acids ranging from 5-20% cleavage. Nepenthesin preferred leucine and lysine (54% and 48% cleavage, respectively), with other amino acids ranging from 7-38% cleavage. For the amino acid on the N-terminal side of the cleavage point, ANPEP had some preference for histidine, serine, or valine. Overall, ANPEP's comparable functionality to both pepsin and nepenthesin II, as well as its biased specificity, make it a valuable enzyme to consider when conducting HDX MS studies, especially given that it is easily obtainable from nutritional supplement capsules.

ABS#389

Poster session, July 15

Structure and Activity of Metabolic Proteins in Living Cells

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Cellular metabolism is a complex network of enzymes and chemical reactions, finely tuned to meet the cell's energy needs. Recent research suggests that the cell regulates its metabolism not only through control of the activity of the enzymes involved but also through exquisite spatial control of the components of metabolism via liquid-liquid phase separation (LLPS). Understanding how and why this spatial organization occurs is critical to understanding how metabolism adapts to stress and disease. Optical photothermal infrared microscopy (OPTIR) is a promising structurally specific tool for investigating how proteins and metabolites organize themselves without bulky fluorescent labels. Here, we investigate phosphofructokinase (PFK), the enzyme responsible for the committing step in glycolysis, the preferred pathway for energy production in cancer cells. Under certain conditions, PFK forms filaments in vitro and LLPS in cells but we do not know the structure of PFK within the LLPS condensates. It is also unclear which conditions promote filamentation inside cells and how they alter global and local glycolytic activity. To characterize the structural states of PFK inside cells, we measure changes in the amide I and azide modes of azidophenylalanine (azF)-labeled PFK in response to perturbations in the environment. We observe unique spectral features for stressed and unstressed PFK-transfected cells, suggesting that conformational constraints imposed by LLPS promote filament assembly and regulate glycolytic activity.

ABS#394

Poster session, July 14

How catalytic lysines in enzymes gain their catalytic properties

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The lysine side chain is a weak base and is fully protonated at neutral pH in the free amino acid. How does it become a strong base or nucleophile in enzyme active sites? Computational analysis using Partial Order Optimum Likelihood (POOL) was performed on 75 enzymes that represent all six major enzyme classifications and a variety of different folds. All contain one or more lysine residues that have been found to be catalytically active in previously reported experiments. The catalytic lysine residues reported here have strong coupling interactions to other residues that obey one or both inequality expressions reported by Coulther, Ko and Ondrechen in 2021. Specifically, the catalytic lysines are strongly coupled to at least one other lysine residue with intrinsic pKa difference within 1 pH unit, or else are strongly coupled with tyrosine or cysteine residues wherein these anion-forming residues have an intrinsic pKa higher than that of the lysine. The interactions help us in identifying roles of these supporting residues and also identify arrays of interacting residues that are characteristic of specific biochemical functions, thus supplying functional information that can be used to annotate protein structures of unknown function. This analysis provides insight into how catalytic lysines achieve their catalytic power. Acknowledgement: NSF MCB-2147498 & USPakistan Knowledge Corridor; MM and HE supported by NSF DBI-2031778.

ABS#395

Poster session, July 13

Identification and characterization of alternative sites and molecular probes for SARS-CoV-2 target proteins

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Due to the SARS-CoV-2 pandemic which began in early 2020, there is still a pressing need for effective antivirals to improve outcomes for infected patients. Using computational methods, we predicted the binding sites of several viral protein targets and screened over one million potential compounds. YASARA was used to visually inspect and clean up the target structures prior to active site prediction. Our group's own machine learning (ML) algorithm, Partial-Order Optimum Likelihood (POOL), was applied to predict probable, biochemically active binding sites using computed electrostatic and chemical properties and surface topology data. While POOL correctly predicted the catalytic sites, it also predicted secondary sites that are likely active recognition sites for the main protease and methyl transferase. Simulated docking of compound libraries was performed using Schrodinger Glide in Standard Precision (SP). Compounds with docking scores ≲ -7.00 advanced to Extra Precision (XP). Targets include non-structural proteins 1, 3, 15, and 16, as well as the human ACE2 receptor (in complex with spike protein). Docking scores were promising between -9 and -14. Compound libraries used included Enamine, ZINC, and CAS. Examples of promising hits include Ceftolozane (-12.287), 5-methyltetrahydrofolate (-12.108) and Folotyn (-11.201). Good hits warrant further development including in vitro and in vivo studies to determine suitability as antivirals. Supported by NSF grants CHE-1905214 and CHE-2030180. IB, AH and JS supported by NSF DBI-2031778.

ABS#396

Poster session, July 14

SiteTack: A Deep Learning Model for PTM Site Prediction that Improves Accuracy by Tacking on PTM Information

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Protein post-translational modifications (PTMs) occur when a chemical functional group is added to a protein in the cell,most commonly being phosphorylation. PTMs increase proteome diversity, regulate cellular processes, modulate proteinactivity, and are important in disease. Hence identification of PTMs are vital to understanding biology and medicine.Recently, deep learning has been used to predict PTM location with significant success, yet there are still limitations inthese methods as they are only available for common PTMs and have limited accuracy for some PTMs likephosphorylation. To improve PTM prediction accuracy, we developed a sequence based deep learning algorithm whichalso took as input known PTM sites in the prediction. Recent experimental work has validated this approach forphosphorylation where Johnson et al. found that 129 of the 303 kinases they tested were specifically selected for aphosphorylated Thr (with similar assumption for Ser) or Tyr in at least one spot in the motif [1]. MusiteDeep [2-4] andothers are examples of sequence-based models which we have improved upon by including other PTM sites in the trainingsets. Specifically, our sequence-based model takes in amino acid sequences with labeled PTM sites, embeds them into avector, processes them through a 2D convolutional layer, max pooling layer, and a series of dense layers to predict theprobability of a modification at a given site. Without labeling, our model is on par with previous models like MuSiteDeep [2], TransPhos [5], and PhosIDN [6]. However, with labeling, we are able to improve upon our model with anunprecedented AUC of 0.932 and an accuracy of 85.9% for phosphorylation. Ultimately, this project builds uponprevious models and highlights the importance of PTMs in addition to the canonical amino acids in protein substraterecognition.

ABS#401

Poster session, July 13

Structural Studies of Small Molecule ARNT PAS-B Binders as Novel Modulators of Transcriptional Response

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Hypoxia Inducible Factors (HIFs) are heterodimeric oxygen-regulatable transcription factors, the dysregulation of which are implicated in several forms of cancer. These complexes are comprised of an oxygen regulated α subunit (HIF-1α, 2α, 3α) and a β subunit, Aryl

Hydrocarbon Receptor Nuclear Translocator (ARNT). These heterodimers recruit several ancillary proteins in their gene regulatory functions, including coiled-coil coactivators (CCCs) which bind via the ARNT PAS-B domain. While a HIF-2α specific small molecule therapeutic, belzutifan, has been developed using a strategy previously established by our group, a compound interfering with ARNT/CCC interactions would be a pan-HIF (HIF-1α, 2α, 3α) inhibitor useful for research and therapeutic applications. To this end, we have used NMR and X-ray crystallography to identify the binding modes of KG-548 and KG-655, two small molecule fragments that we showed to disrupt ARNT PAS-B/CCC interactions in vitro. Notably, KG-548 binds exclusively to an exterior binding site on the β-sheet surface, whereas KG-655 occupies both this surface binding site and a hydrated internal cavity. Combining our experimental data and computational approaches, we report structural models of both binding modes and explore the binding potencies of small molecule derivatives of the two well-characterized leads. Investigations of the binding and inhibitory properties of KG-548 and KG-655 in the larger HIF2α-ARNT complex, suggest that results derived from the simpler ARNT PAS-B system translate well into the larger functional protein complex, an essential aspect of developing novel tool compounds.

ABS#402

Poster session, July 14

In vivo characterization of Haloacid Dehalogenase-like Proteins in Trypanosoma brucei

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Human African Trypanosomiasis (HAT) and Chagas Disease are parasitic diseases caused by trypanosomes, ancient single-celled eukaryotes. Therapeutics to these parasitic infections are often lacking, encounter drug resistance, and/or are toxic to the host. There is a need for new therapeutics. In this vein, we present the characterization of three Trypanosoma brucei haloacid dehalogenase (HAD)-like proteins. All three T. brucei HADs were acquired from bacteria through horizontal gene transfer (1). HAD32, HAD35, and HAD42, named for the molecular weight of the protein, are important to the life cycle of the parasite. We seek to ask the in vivo function of the HAD proteins and if they are related. RNA interference (RNAi) is a tool that halts production of a protein in the cell and can be used to deduce protein function. We have generated RNAi trypanosome cells lines targeting individual and combinations of HADs and assessed their growth and phenotypes. Identifying HAD-interacting proteins will also lend functional clues for these enzymes. We've carried out interaction studies between HADs and have begun exploring non-HAD interacting proteins. Together, these data will begin to bring into focus the function of Trypanosoma HAD-like proteins.

ABS#404

Poster session, July 13

What Are Ancient Eukaryotic Trypanosomes Doing with Bacterial Haloacid Dehalogenases (HADs)

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Transmission of the unicellular eukaryotic parasite, Trypanosoma brucei, is responsible for human African trypanosomiasis (HAT, also known as sleeping sickness) commonly found in sub-Saharan Africa. Transmission of T. cruzi can lead to Chagas disease. Considering the need for effective and affordable therapeutics for these global health threats, one avenue to combat this challenge is to identify and characterize the activity and physiological role of proteins that are unique to the parasite and absent or different from the host. To pursue this goal, we are characterizing a repertoire of T. brucei haloacid dehalogenase-like (HAD) enzymes acquired by the parasites from bacteria via horizontal gene transfer (1) and now have become an integral component of normal T. brucei growth (2). Sequence analysis revealed three putative HADs in T. brucei: TbHAD32 (32kDa), TbHAD35 (35kDa), and TbHAD42 (42kDa). The putative His-tagged HADs were expressed in bacteria and purified to approximate homogeneity. Each HAD has a phosphatase motif. Accordingly, we screened each protein for activity with a variety of potential phosphate-containing substrates and kinetically characterized those with activity. We also explored how the presence of one HAD impacts the activity of another in vitro. The findings of our in vitro characterization will guide our exploration of the in vivo activity of Trypanosoma HADs.

ABS#407

Poster session, July 15

Ergothioneiine in an Enzyme: Creation of a Mutant of Thioredoxin Reductase Containing 2-thiohistidine

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The objective of our study was to insert the novel amino acid 2-thiohistidine, an analogue of ergothioneine, into an enzyme to confer a gain of catalytic function. The enzyme that we chose was P. falciparum thioredoxin reductase (PfTrxR), which contains a C-terminal di-cysteine active site. We hypothesized that since ergothioneine is an excellent scavenger of singlet oxygen, we should be able to create a mutant containing 2-thiohistidine such that catalysis of singlet oxygen could be achieved. The reaction of 2-thiohistidine or ergothioneine with singlet oxygen results in a 5-oxo-derivative that can be reduced back to the original form by attack of a thiol onto the 4-position of the 2-thio-imidazole ring. PfTrxR is a reductase that reduces electrophilic substrates of this type such as dehydroascorbic acid. We hoped that by incorporating 2-thiohistidine as part of the polypeptide chain, catalysis could be accelerated due to the resulting loss in entropy. We used protein semisynthesis to create the mutant. Following enzyme purification, we used standard enzyme assay methods to measure the activity of the mutant and wild type enzyme against various substrates including singlet oxygen. The results of our spectrophotometric assay of the consumption of NADPH show that our mutant was able to catalyze the reduction of singlet oxygen, but the wild-type enzyme was not. This is an example of protein engineering to confer a gain of function that is not possible with the 20 standard proteinogenic amino acids. We are currently testing our mutant for other gains of function. Our results open the door to engineer proteins and peptides containing this novel amino acid with resulting gain in catalytic function.

ABS#409

Poster session, July 15

Unexpected control of protein self-association by translation initiation

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Living proteomes are necessarily far from equilibrium. It is paradoxical, then, that reducing protein influx -- which should promote equilibration -- instead, prolongs life in various model systems. Phase transition to thermodynamically favored but rare self-assemblies like amyloids often disrupts normal protein function and proteostasis. We therefore reasoned that, kinetic barrier to energetic equilibration via self-assembly of the native state to probabilistic amyloid-like states must exist, and that it decreases with protein influx.

To measure kinetic barriers to phase transition of proteins, we used Distributed Amphifluoric FRET (DAmFRET) that quantitatively reads out protein self-assembly at single cell resolution, over a wide range of concentration of the query protein, in living cells. In an inducible manner, we expressed structurally diverse self-assembling proteins from a high-copy plasmid in yeast. We controlled translation initiation specifically using characterized uORF or Kozak mutations, while maintaining the same level of query protein expression by titrating plasmid copy number. Remarkably, all reductions in translation initiation hampered seed formation irrespective of the query protein structure and expression level. Various manipulations to enhance polypeptide interaction and residence time on the polysome enhanced aggregation of the query proteins, pointing to co-translational polypeptide interaction being crucial to such protein self-assemblies. Indeed, a boiling stable protein that forms a dodecamer, assembled co-translationally, thereby extending the implications of translation initiation beyond nucleation-limited rare self-assemblies to protein self-association, broadly.

Finally, our observations are true in both yeast and mammalian cells alike, thereby underscoring the widely conserved nature of this phenomenon. Thus, translation initiation strength potentially dictates the behavior of a wide range of protein classes, across eukaryotes, in a strikingly profound manner. We are exploring both - the underlying physical mechanism, and physiological implications of this phenomenon, via proteomics, mathematical modeling, computational mining of RiboSeq datasets.

ABS#410

Aggregates, Amyloids, or Condensates? (July 16, AM)

Structural determination of neurodegenerative disease-associated proteins inside cells

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The misfolded proteins associated with neurodegenerative disease can adopt a variety of different conformations, some of which are toxic. Because these proteins have identical amino acid sequences, the cellular environment clearly influences the final state, yet most structural studies do not include the cellular context and, perhaps because we are not studying the correct conformation, not a single therapeutic strategy for these diseases addresses the underlying protein misfolding pathology. Using new sensitivity-enhancement technology for solid state NMR spectroscopy, Dynamic Nuclear Polarization, we study protein structure in native environments - inside living cells - to reveal how both healthy and disease-relevant cellular environments influence protein structure. Because NMR reports quantitatively, with atomic level precision, on all sampled conformation, it can not only report on structural polymorphs but also provide experimental restraints on regions of intrinsic disorder, complementing insights from cryo-electron microscopy and tomography. Using this approach, we recently demonstrated that an amyloid fibril with a solved cryo-EM structure was polymorphic and found that when those fibrils were used to seed amyloid propagation in mammalian cells, the minority polymorph in the purified setting became the majority polymorph inside cells. Selective amplification of one of two polymorphs informs on the mechanism of protein-based inheritance of amyloid aggregates which will advance our ability to rationally intervene in protein-misfolding diseases.

ABS#411

Poster session, July 13

Further Elucidating the Structure-Function of a Natural Antimicrobial Domain by Rational Design

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As virulent plant pathogens continue to threaten global agriculture production, the need to develop more effective antimicrobial agents becomes urgent. Natural plant defense systems include the plant-specific insert (PSI), a lipid-interacting, antimicrobial domain of typical plant aspartic proteases and member of the saposin-like protein family. The PSI of different plants sharing similar primary structure can exhibit highly-specific vesicle leakage and fusogenic activity, the relationship of which remains largely unknown. A greater understanding of PSI structure-function will help uncover its antimicrobial mechanism and bolster strategies to combat plant pathogens. Using a rational design strategy, the effect of altering single residues of the wild-type common potato PSI (StPSI) was examined using computational and fluorescence analyses to further elucidate PSI-membrane interactions. Multiple sequence alignment was used to identify two conserved tryptophan (W) residues in StPSI as candidate sites for mutation into alanine (A) or phenylalanine (F). The resulting variants were compared to StPSI in terms of protonation state prediction by continuous constant pH molecular dynamics (MD), and interaction with a model lipid bilayer of equimolar phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in 0.15 M NaCl assuming a pH 3 environment by all-atom MD. Fusion efficiency for model liposomes was determined using the fluorescence resonance energy transfer (FRET) assay. The introduced mutations were predicted by MD analyses to alter the net charge, internal salt bridge network, and surface properties of StPSI and impair its membrane anchoring depending on the location and type of the substituting amino acid. W-to-A and W-to-F mutations resulted in decreased and increased fusion efficiency, respectively, as determined by FRET experiments. These results underscore the importance of distinct residue types at critical locations in steering interactions at the protein-membrane interface, insights of which can be applied to the engineering of membrane-active proteins with enhanced or tailored activity.

ABS#412

Protein Folding and Function in Context (July 13, AM)

Protein Mechanical Stability as a Determinant for Type Three Secretion

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The Type Three Secretion System (TTSS) mediates translocation of virulence effector proteins directly from the bacterial cytosol into host cells[1]. Effector proteins are folded in the bacterium and must be unfolded for secretion through the narrow needle of the TTSS[2]. However, the TTSS unfoldase is weak, and fusion of effectors with thermodynamically stable proteins such as GFP, ubiquitin or dihydrofolate reductase (DHFR) stall the unfoldase thus preventing secretion[3, 4]. This suggested a model where effectors have evolved to be thermodynamically labile[3-5]. Here, we first show that the unfolding free energy (ΔG°unfold) of the Salmonella effectors SptP and SopE2, as well as the E. coli effector NleC and its non-effector homolog Protealysin, are between 5 and 10 kcal/mol, typical for globular proteins and similar to published ΔG°unfold for GFP, ubiquitin, and DHFR. Next, we utilize single molecule atomic force microscopy to show that effector proteins all unfolded at low force (Funfold ≤ 17 pN @ 100 nm/s). Moreover, they are mechanically compliant, as measured by the distance to the transition state (Δx‡ between 1.5 and 2.7 nm), making them among the most mechanically labile proteins studied to date by AFM. In contrast, the non-effector homolog of NleC, Protealysin, is more mechanically robust (Funfold > 38 pN) and brittle (Δx‡ < 0.6 nm), approaching the published mechanical characteristics of GFP, ubiquitin, and DHFR that stall in the TTSS. These results support a novel paradigm: that effector protein unfolding and secretion by TTSS is a mechanical process and that effectors have evolved to be mechanically labile, so they can be unfolded by the weak TTSS unfoldase, but thermodynamically stable, to refold and remain active in the host cell[6]. This would represent a unique evolutionary pressure that provides a rationale for the extreme sequence divergence observed between TTSS effector proteins and their non-effector homologs.

ABS#415

Poster session, July 15

Heat Shock Protein Induction in Drosophila melanogaster as a Model System of Glial Tauopathy

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Tauopathies are a subclass of neurodegenerative diseases characterized by the aggregation of hyperphosphorylated tau protein in the central nervous system. Meanwhile, heat shock proteins (HSPs) play an essential role in sustaining proteostasis by supporting protein folding and preventing harmful aggregation.(1) Perplexingly, HSPs are unable to manage tau proteotoxicity. As a result, we seek to uncover how glial tau aggregation and age impact the chaperone response in a Drosophila melanogaster model by performing a holistic screening of HSP induction when faced with these stressors.
Through the use of the GAL4/UAS system, the human tau isoform 0N4R was overexpressed in D. melanogaster glia. Sixteen experimental groups were created to test the effects of glial tau, age, and induced heat shock on HSP induction. D. melanogaster mRNA was extracted from the CNS via TRIzol homogenization and isopropanol precipitation, all samples were subjected to qRT-PCR. Each HSP exhibited a unique heat shock response suggesting the importance of each protein's unique contribution to the proteostasis machinery. Notably, Hsp70 and Hsp27 demonstrated an increased expression in the presence of tau that disappeared with age in females while only exhibiting an increase in response to stress and tau in aged males. Overall minimal changes were observed in basal expression except for Hsp27 expression in young flies, suggesting an intrinsic role for Hsp27 in the early stages of tau expression. Interestingly, HSP responses exhibit sexual dimorphism to stress and aging, which may be an important mechanism contributing to the sexual dimorphism observed in the onset, progression, and severity of tauopathies.

ABS#417

RNA-Protein Machines: Ancient Synergies (July 14, AM)

Insights into HIV-1 Gag-RRE Interactions: Exploring Rev and Gag Binding & Competition on RRE Stem 1

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The Human Immunodeficiency Virus (HIV), specifically HIV-1, requires nuclear export of the unspliced viral RNA genome, but host cell surveillance systems prevent such export. HIV-1 bypasses these restrictions by coding for the nuclear-cytoplasmic shuttle protein, Rev, which binds to a structured genomic RNA element, called the Rev Response Element (RRE). Two high-affinity Rev binding sites have been identified, termed Stem 1A (S1A) and Stem 2B (S2B) [1]. Gag, a viral cytoplasmic protein that mediates genome packaging, has been shown to bind to the RRE near or overlapping with the S1A site, giving rise to the possibility that Gag: RRE interactions destabilize the Rev: RRE complex, thereby enabling Rev recycling [2]. This project seeks to stoichiometrically, thermodynamically, and structurally characterize these events, including the possible competition between Rev and Gag for the RRE S1A binding site. We employ a series of truncated Stem 1 RNA constructs, a Rev ARM peptide, and the nucleocapsid domain of Gag, NC, in EMSA, ITC, SEC, and NMR experiments. We find that NC may preferentially bind S1A over Rev Peptide, and the purine-rich bulge is crucial for Gag: RRE binding. Understanding this interaction could establish a physiologically relevant basis for RRE-Gag binding and provide insight when designing therapeutics that target HIV proteins or RNAs.

ABS#418

Poster session, July 14

Optimization of Expression and Purification of the Antiviral Protein HERC5 for Structural Determination

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Interferon Stimulated Gene 15 (ISG15) is a tandem ubiquitin-like protein that works with E1-E2-E3 cascading enzymes to interfere with viral replication. HECT domain and RCC1-like domain-containing protein 5 (HERC5) is a unique HECT E3 ubiquitin ligase responsible for the specific attachment of ISG15 onto viral proteins as part of the host's innate immune response. HERC5 has demonstrated ISG15 regulation in various viral infections such as human immunodeficiency virus (HIV), influenza, and SARS-CoV-2. The antiviral activity displayed by the interaction between HERC5 and ISG15 makes them promising drug targets to develop antiviral treatments that can inhibit the replication of viral proteins. Despite its critical role in preventing viral proliferation, the 3D structure of HERC5 remains unknown. In addition to the C-terminal HECT domain responsible for attaching ISG15 onto substrates for viral protein degradation, HERC5 protein has an N-terminal domain consisting of five regulator chromatin condensation (RCC1) motifs that form a □-propeller RLD domain. The RLD domain plays a direct role in the recognition and coordination of viral substrates, however, the first 155 amino acids of the domain were found to cause chronic solubility issues during protein purification. Removing these amino acids allowed for the purification of HERC5. Using a □155 HERC5 construct generously donated to us by Dr. Art Haas (University of Louisiana), we have been working towards optimizing the expression and purification of a soluble and pure HERC5 protein for structural determination. These studies will be important to decipher the specific mechanism that the antiviral HERC5 protein uses to covalently attach ISG15 onto its viral protein targets.

ABS#420

Membrane Proteins: From Natural to Designed (July 14, PM)

The Simple QTY Code for Protein Design

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The simple QTY code is based on two key molecular structural facts: 1) all 20 amino acids are found in naturally occurring alpha-helices regardless of their distinct chemical properties: (a) hydrophilic, (b) hydrophobic and (c) amphiphilic; 2) several amino acids share striking structural similarities despite their different chemical properties; for example, glutamine (Q) vs Leucine(L); Threonine (T) vs Valine (V) and Isoleucine (I); and Tyrosine (Y) vs Phenylalanine (F). Using the simple QTY code, we replace 40%-60% amino acids L, I, V, F in transmembrane a-helices with amino acids Q, T, Y, the water-soluble QTY variants still maintain the stable structures and ligand-binding activities in the chemokine receptors. The AphlaFold2 predictions proved the QTY code validity. The simple QTY code is a likely useful tool and has big impact for designs of water-soluble variants of

previously water-insoluble GPCRs, glucose transporters, solute carrier transporters, ABC transporters, potassium ion channels, and perhaps aggregated proteins. https://en.wikipedia.org/wiki/QTY_Code

ABS#421

Capturing Protein Interactions (July 14, PM)

Tepsin Binds LC3B To Promote ATG9A Export And Delivery At The Cell Periphery

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Tepsin is an established accessory protein found in Adaptor Protein 4 (AP-4) coated vesicles, but the biological role of tepsin remains unknown. AP-4 vesicles originate at the trans-Golgi network (TGN) and target the delivery of ATG9A, a scramblase required for autophagosome biogenesis, to the cell periphery. Using in silico methods, we identified a putative LC3-Interacting Region (LIR) motif in tepsin. Biochemical experiments using purified recombinant proteins indicate tepsin directly binds LC3B, but not other members, of the mammalian ATG8 family. Calorimetry and structural modeling data indicate this interaction occurs with micromolar affinity using the established LC3B LIR docking site. Loss of tepsin in cultured cells dysregulates ATG9A export from the TGN as well as ATG9A distribution at the cell periphery. Tepsin depletion in mRFP-GFP-LC3B HeLa cells using siRNA knockdown increases autophagosome volume and number, but does not appear to affect flux through the autophagic pathway. Re-introduction of wild-type tepsin rescues ATG9A cargo trafficking defects. In contrast, re-introducing tepsin with a mutated LIR motif does not fully rescue altered ATG9A subcellular distribution. Together, these data suggest roles for tepsin in cargo export from the TGN, as well as delivery of ATG9A-positive vesicles near the cell periphery and overall maintenance of autophagosome structure.

ABS#423

Poster session, July 14

Exploring Desiccation Tolerance: is p26 the Key?

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Exploring Desiccation Tolerance: is p26 the Key?
Preserving biomedically essential materials in the frozen state is a well-established method for some biologics. Still, current challenges include the cytotoxic effects of cryoprotective agents on cells and the financial burdens associated with maintaining extremely cold temperatures below -80 °C. Dry preservation of biologics mitigates the challenges associated with maintaining cold-chain logistics. Lyophilization protocols that preserve the activity of biologics after rehydration allow sample storage at room temperature, safer handling of specimens, and extend the shelf-life. While dry-state storage has been implemented for some relatively simple biological molecules, preserving complex materials, such as mammalian cells, has yet to be achieved. We study the small heat shock protein (sHSP) p26 from the brine shrimp Artemia franciscana, an animal that can survive in an entirely desiccated state. A. franciscana upregulates the expression of proteins associated with repair pathways, including p26. Unfortunately, previous studies to evaluate the structure and function of p26 have been limited due to low protein yields. We improved p26 production by optimizing the gene sequence for expression in E. coli and discovered that p26 is highly salt sensitive. The protein precipitates at NaCl levels above 50mM but adding 100mM trehalose or sucrose lessens the salt sensitivity. Bioinformatic analysis of the amino acid sequence of p26 indicated that the protein likely undergoes liquid-liquid phase separation (LLPS) during water stress, which may be part of the mechanism by which it protects brine shrimp embryos during desiccation. Current optimizations to the purification methods include salting-out as the first purification step, followed by ion exchange chromatography. Establishing an optimal purification method for this protein will allow for further exploration of the mechanism(s) by which p26 protects biological materials in the dry state and has the potential to offer novel avenues for protecting water-stress-sensitive biologics via lyophilization.

ABS#424

Poster session, July 14

Structure-Function Studies of Melanoma-Antigen Gene B2

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The dysregulation of the melanoma-antigen gene (MAGE) family of proteins is a hallmark of cancer cell proliferation and metastasis. In particular, overexpression MAGEB2 in non cancerous cells increases cell proliferation. Additionally depletion of MAGEB2 in cancer cells results in lack of cell viability. Next generation RNA-Seq experiments identified differentially regulated genes in HEK293 cells overexpressing MAGEB2. Interestingly, we observed increased expression of a number of proto-oncogenic genes such as PRL, TRIM11-1, and NUSAP1 compared to HEK293 controls. Additionally, we also observed decreased expression of tumor suppressor factors such as PCDHA13, UBE2F-SCLY, ZMYM4-AS1, TRIM43B, and CXCL10. To characterize the molecular mechanism of action of MAGEB2, we analyzed the protein structure calculated by alpha-fold compared it to predictions made by I-Tasser and RoseTTa fold. The structure of MAGEB2 comprises a MAGE homology domain which is largely conserved across the MAGE family and a largely unstructured N-terminus. To further characterize the molecular pathway of MAGEB2 and identify direct protein-protein interactions, immunoprecipitation experiments were conducted using cells overexpressing myc-tagged MAGEB2. Mass spectrometric analysis identified a total of 19 proteins co-eluting from anti-myc agarose beads. Notably, these 19 proteins are involved in various regulatory functions related to cancer such as DNA repair, cell cycle regulation, and protein synthesis. In total, these data suggest MAGEB2 contributes to cancer by influencing the regulation of specific molecular hallmarks and suggest MAGEB2 may be a viable target for the development of inhibitors that can be used for therapeutic interventions.

ABS#425

Poster session, July 13

Targeting the Oncoprotein Gankyrin with Small Molecules to Alter its 3D Global Fold

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Gankyrin (aka PSMD10) is a seven ankyrin repeat containing protein that helps to form the 19S regulatory cap of the 26S proteasome. Gankyrin plays an important role in regulating numerous oncogenic and inflammatory pathways through its ability to form protein−protein interactions (PPIs) with other intracellular proteins. Gankyrin has been reported to be overexpressed in many different cancer types making it an interesting drug target, however, the exact mechanism(s) used by gankyrin and its involvement in cancer development is still unclear. To address this, we conducted circular dichroism (CD), nuclear magnetic resonance (NMR) spectroscopy, and isothermal titration calorimetry (ITC) to examine various small molecules and their effect on gankyrin's 3D structure. This collaborative project between the Spratt Lab at Clark University and the Muth Lab at St. John's University is working towards discovering new cancer treatments by targeting ankyrin-repeat containing proteins. Our results show small-molecule gankyrin inhibition may emerge as a promising therapeutic strategy for breast and lung cancer. By targeting the ankyrin-repeats in gankyrin, it may be possible to develop novel strategies to create more effective cancer therapies.

ABS#426

Poster session, July 15

Topological Analysis of the Type 3 Secretion System Translocon Pore Protein PopD in Native Membranes

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The type III secretion system (T3SS) is employed by several pathogens to promote infection of host cells. The T3SS is a syringe-like apparatus that spans across the double membrane of bacteria, protruding 40-80 nm into the extracellular space and connecting with target cell membrane. In Pseudomonas aeruginosa, two secreted proteins PopB and PopD are found associated with the target eukaryotic cell membrane. These two proteins are proposed to form a transmembrane protein complex

(or translocon) that is required for endotoxin translocation into the target cell cytosol. The structure and topological arrangement adopted by PopD and PopB in functional translocons is not known.

To determine the topology of PopD in functional translocons we employed an approach that combines i) the use of a probe that sense the exposure of a protein segment to the host cytosol and ii) the analysis of single cysteines accessibility to a membrane-impermeant probe. For this purpose, several PopD variants containing the GSK-tag peptide were employed to determine what segments of PopD are exposed into the target cell cytosol. To identify what regions are exposed to the exterior of the target cell, single cysteines were introduced at different regions of PopD and their accessibility was determined using PEG5K-maleimide, a cysteine specific membrane impermeable reagent. Combining the PEG assay and the GSK-tag phosphorylation assay we found regions of membrane-inserted PopD exposed inside the target cell and regions exposed to the extracellular space. These results suggest that the P. aeruginosa translocon adopts a topology different from that proposed in current models.

ABS#427

Poster session, July 13

MSFP REU: Undergraduate "Collaborate from Home" Research in Macromolecular Structure and Function

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When the COVID-19 crisis shut down most undergraduate research opportunities, we developed an online, remote, computer-based Research Experiences for Undergraduates on the topic of Macromolecular Structure and Function that was funded by the USA National Science Foundation. The REU provided a mentored research experience and training in professional skills to assist the participants in pursuing a degree and future career in STEM. This fully virtual project involved faculty at four geographically-distributed institutions specializing in diverse but complementary approaches to study macromolecular structure and function. Importantly, its online "collaborate-from-home" format made it accessible to students during the pandemic to participate fully in the research, professional development, and other activities of the program.

ABS#429

Poster session, July 15

Protein supersaturation powers innate immunity and programmed cell death

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The response of cells to existential threats such as virus invasion often involves semi-crystalline polymerization of certain signaling proteins, but the highly ordered nature of the polymers has no known function. We hypothesized that the undiscovered function is kinetic in nature, emerging from the nucleation barrier to the underlying phase transition, rather than the material polymers themselves. We explored this idea using fluorescence microscopy and Distributed Amphifluoric FRET (DAmFRET) to characterize the phase behavior of all 116 members of the death fold domain (DFD) superfamily, the largest group of putatively polymerizing modules in human immune signaling. We found that a subset of the DFDs polymerized in a nucleation-limited manner that caused a switch-like transition from one cell state to another. The nucleation barriers were retained in the corresponding full-length signalosome adaptor proteins. We then conducted a comprehensive screen to identify nucleating interactions between DFDs. Nucleating interactions occurred primarily between members of the same DFD subfamily and within known signaling pathways, revealing extreme specificity of nucleation-mediated signaling through the network. We nevertheless uncovered multiple heterotypic interconnections between the different cell death subroutines of pyroptosis and extrinsic apoptosis, and went on to validate cross-talk between these pathways in vivo. In the process, we discovered that the inflammasome adaptor protein ASC is endogenously constitutively supersaturated, indicating that innate immune cells are thermodynamically fated to undergo inflammatory cell death and that the nucleation barrier delays this inevitability. Finally, we showed that anastasis, the recovery of cells from apoptotic signaling, results from the absence of nucleation barriers in apoptosome DFDs. Our results collectively imply that innate immunity comes at the cost of occasional spontaneous cell death and inflammation, which may contribute to the progressive nature of age-associated inflammation.

ABS#430

Protein Evolution: Lessons from the Past (July 13, PM)

Leveraging catalytically promiscuous enzymes to understand the basis for enzyme specificity and epistasis among homologs

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Catalytic promiscuity (the ability to catalyze non-biological reactions in the same active site as a native biological reaction) frequently contributes to the evolution of new enzyme activities. [1] Thus, studying catalytically promiscuous enzymes offers insight into both evolutionary mechanisms and the biophysical basis of enzyme specificity. As a model system, we investigate evolution of N-succinylamino acid racemase activity (NSAR) within the NSAR/o-succinylbenzoate synthase (OSBS) subfamily. This unique subfamily will enable us to generate a complete account of all specificity determinants, because global effects, which affect both activities, can be disentangled from effects on specificity, which differentially affect the two activities. Using ancestral sequence reconstruction, we identified two specificity-determining residues. One mutation enlarged the active site, facilitating binding to larger substrates. [2] The other residue, R266 in Amycolatopsis sp. T-1-60 NSAR/OSBS, enabled the nearby K263 to act as a general acid/base catalyst, which was required for NSAR but not the ancestral OSBS activity. [3] To confirm that R266 was co-opted to enable this new catalytic mechanism, we mutated R266 in four other NSAR/OSBS enzymes. However, effects of the mutation on reactivity of K263 were only observed in one enzyme. In the other three enzymes, mutating R266 reduced thermostability and compromised both NSAR and OSBS activities. Thus, epistasis due to differential effects on global structure among homologs might have masked the effect of the mutation on reactivity of K263. Furthermore, mutations at both specificity-determining positions reduce, but do not eliminate NSAR activity, suggesting that additional positions also help determine specificity. Together, identifying residues that impact specificity in different ways (substrate binding and catalysis) is paving the way toward fully characterizing the ensemble of specificity determinants and effect of epistasis on NSAR/OSBS enzymes. Strategies and concepts derived from our studies will guide investigations about the basis and engineering of specificity in other proteins.

ABS#431

Poster session, July 15

Delineating The Cytotoxic Potential of Different Natural Cytotoxicity Receptors in NK Cells

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The engagement of Natural Cytotoxicity Receptors (NCRs) on Natural Killer cells can activate potent innate immune responses, but the mechanism by which different NCRs activate NK cells is not clear. In particular, we are interested in recruiting NKs to the tumor microenvironment by engaging these activating receptors and determining the cytotoxic potential of different NCRs. To achieve this, we used phage display selections to identify antibody binders to NKp30, NKp46, and CD16, and developed bispecific NK engagers (BiNKs), fusing these binders to another binder targeting a tumor-associated antigen in a bispecific scFv-Fc format. These BiNKs displayed nanomolar affinity to their respective antigens in vitro via BLI and showed high affinity to antigen-expressing target cell lines via flow cytometry. In immune cell killing assays, all BiNKs demonstrated varying degrees of cytotoxicity beyond the canonical Fc-mediated antibody-dependent cell-mediated cytotoxicity. Armed with these novel BiNKs, we are poised to investigate the behavior of different NK activating receptors, as understanding NK engagement is crucial for the development of improved NK cell therapeutics.

ABS#434

Poster session, July 15

Molecular Pathomechanisms in Lipoamide Dehydrogenase Deficiency

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(Dihydro)lipoamide dehydrogenase (LADH) is a homodimeric flavin-disulfide oxidoreductase that catalyzes the oxidation of the multienzyme complex cofactor (dihydro) lipoamide, using NAD+ as a co-substrate, serving as a common third subunit (E3) to the mitochondrial alpha-keto acid dehydrogenase complexes for pyruvate, alpha-ketoglutarate, alpha-ketoadipate and branched-chain alpha-keto acids. Clinically relevant, disease-causing mutations in hLADH may result in compromised protein stability, folding and catalytic activity, enhanced reactive oxygen species (ROS) generation, and/or dissociation from the above multienzyme complexes that affect several central metabolic pathways simultaneously and lead to the often prematurely lethal hLADH deficiency. We determined the high-resolution crystal structures of the wild type hLADH and nine of its disease-causing variants at resolutions ranging from 1.44 to 2.89 Å to analyze the structural bases of hLADH deficiency. The investigated substitutions reside in either the active site (P453L), the cofactor-binding region (G194C, I318T, I358T), or the dimer interface (G426E, D444V, I445M, R447G, R460G). The P453L substitution triggered a significant remodeling of the active site that correlates well with the almost complete lack of residual activity and the severe clinical outcomes. The G426E and G194C substitutions altered the charge distribution and dynamics near the nicotinamide-binding site, but did not induce significant structural changes, which is in accord with the respective residual LADH activities and relatively milder clinical manifestations. The D444V, I445M, R447G and R460G substitutions all reside farther away from the active site on the homodimer interface of hLADH, however, they are still associated with significant losses in catalytic activity. Our structural data revealed considerable perturbations in the so-called H+/H2O channel, leading to the active site, for the above four variants. In I318T-hLADH, a crucial interaction of the catalytic base His452 (with Glu457) was slightly modulated. Finally, the I358T substitution primarily perturbed an FAD binding interaction (with His329). Results are discussed in context with clinical/biochemical data.

ABS#435

Poster session, July 15

Molecular Insight into Amyloid-induced Membrane Damage

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Amphipathic peptides can induce leakage in biological membranes, either through a detergent-like mechanism where they dissolve the lipids, or by creating pores on the surface of the membrane. This study presents all-atom simulations showing that membrane-bound amphipathic peptides self-assemble into beta-sheets that can form either stable pores or extract lipids from the membrane surface. Our simulations reveal that the non-polar side chains of peptides deposited on the membrane strongly interact with the acyl tail of the lipids, enabling lipids to be pulled out of the bilayer by oligomeric structures, causing detergent-like damage. These strong interactions also distort the orientation of lipid tails near the peptides, although this distortion is minimized around pore structures. Furthermore, beta-sheets bound to the membrane become twisted, with one end partially penetrating the lipid bilayer, enabling the interaction of peptides on opposite leaflets and leading to the formation of a long transmembrane beta-sheet that initiates poration. From our simulations, we suggested that the twisted beta-sheets can assist peptides in penetrating the membrane and forming pores, especially when the peptides are present on a single leaflet. In contrast, fibril-like structures cause little damage to lipid membranes because the non-polar side chains in these structures are not available to interact with the acyl tail of lipids. Understanding the mechanisms of membrane damage caused by amphipathic peptides is crucial for developing new therapeutic strategies to treat diseases associated with peptide-induced membrane damage.

ABS#436

Poster session, July 14

Understanding the Molecular Basis by which the Disordered Kir6 C-terminal Tail Controls KATP Channel Trafficking and Gating

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ATP sensitive potassium (KATP) channels link the cell metabolism to membrane electrical activity by sensing cytoplasmic ATP/ADP concentrations. KATP channels are comprised of four pore-forming Kir6 subunits surrounded by four copies of the regulatory sulfonylurea receptor (SUR). Kir6.x contains two transmembrane helices with N- and C-terminal domains extending into the cytoplasm. The C-terminal region of Kir6.x is comprised of structured ATP-binding core and a disordered

C-terminal Tail spanning the terminal 50 residues, not observed in published cryo-EM structures. However, proper trafficking of the KATP channel requires the interaction of the SUR and Kir6 Tail by unknown mechanisms. Furthermore, the Kir6 Tail contains several sites for phosphorylation and disease-causing mutations leading to diseases such as diabetes mellitus and hyperinsulinism. The proposed study aims to reveal the molecular mechanism by which the disordered Kir6 Tail regulates its structure and activity through interactions with other KATP channel regions. These interactions will be studied with the effects of phosphorylation and disease-causing mutations. To understand the structure-function relationship, the Kir6.2 tail was overexpressed in E. coli and labelled with 13C and/or 15N isotopes. NMR experiments were performed to obtain the 1H, 13C and 15N resonances and to calculate secondary structure propensity showing that the Kir6.2 Tail possesses alpha helical propensity within the disordered region and beta strand propensity near the phosphorylation site. NMR studies demonstrates interactions between Kir6.2 Tail and the first nucleotide binding domain (NBD1) of SUR, which is increased with phosphorylation. To understand the functional consequence of this interaction, the affinity of SUR NBD1 for ATP in the presence the Kir6.2 Tail was measured using fluorescence spectroscopy and demonstrated no differences, suggesting an alternative mechanism of action. This study shows that the disordered Kir6.2 Tail possess residual secondary structure and makes interaction with SUR NBD1 which may be indicative of a trafficking state.

ABS#437

Poster session, July 14

Targeting MEIG1 and PACRG Protein Interaction for Male Contraception by Molecular Modeling

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Interactions between the meiosis expressed gene 1 (MEIG1) and Parkin co-regulated gene (PACRG) protein are critical for mature sperm cell formation, and targeting either protein could be a viable contraceptive strategy. To gain insights into the underlying mechanism and dynamics of MEIG1-PACRG binding, we applied Gaussian accelerated molecular dynamics (GaMD) simulations and post-GaMD analysis. Our results suggest that MEIG1 residues W50 and Y68 play a crucial role in stabilizing MEIG1-PACRG binding by forming a complex network of interactions with PACRG. Moreover, our work demonstrates similar dynamic properties between human and mouse models, implying that the findings from mouse proteins can be applied to the proteins in developing human sperm. Using the Fpocket binding pocket detection algorithm, we identified a primary ligand binding pocket on the MEIG1 and PACRG interaction surface near W50 and Y68. We then performed virtual screening and molecular docking to test nearly one million drug-like compounds for their ability to bind the targeted pocket. This work provides a critical step forward in the development of male-based birth control by deepening our understanding of the MEIG1 and PACRG interaction and narrowing the list of potential drug candidates for future studies.

ABS#438

Poster session, July 14

Stabilization of the Protein-Protein Interface in Protein-DNA Co-Crystals

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Obtaining well-ordered crystals is a bottleneck challenge for biomolecular X-ray crystallography. The process of optimizing growth conditions can be long and tedious. This project relies on porous protein-DNA co-crystals as a modular scaffold with the potential to enable scaffold-assisted structure determination for DNA-binding proteins, largely circumventing the need for trial-and-error crystal growth. Previous work has succeeded in creating co-crystals with enlarged pores by insertion of a short expansion DNA duplex strut. However, the expanded crystals are more fragile. Therefore, the current project aims to stabilize these crystals via rational design of the adventitious protein-protein crystallographic interfaces. The highest priority stabilization strategy aims to engineer disulfide bonds across z-axis protein-protein interfaces within the crystal by mutating select residues to cysteine. These residues are hypothesized to then be able to spontaneously form disulfide bonds across the protein interface under oxidizing conditions. Two such designed mutant proteins are undergoing expression in E. coli. Following purification, the solvent-accessibility will be confirmed via the

Ellman's reagent assay. Crystals will be grown under reducing conditions using these mutant proteins as building blocks. X-ray diffraction and Ellman's reagent will be used to confirm the formation of disulfide bonds.

ABS#439

Poster session, July 15

Specificity of Picornavirus 3C Protein Interactions with RNA control elements

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Viral proteins are often responsible for multiple functions in part to maximize the genomic information content. The picornavirus 3C protein is a great model system for this. The 3C(D) protein is the major protease responsible for most of the polyprotein cleavage events and is also important for regulating the host cell's response through the cleavage of critical host cell proteins. Poliovirus (PV) is best studied from the picornavirus family. PV-3C protein interacts with cis-acting replication elements also (CREs; oriL, oriI, and oriR) within the viral genome to regulate replication and translation events. The mechanism and preference of various RNAs by PV-3C protease are unknown, especially given that these RNA replication elements lack an obvious consensus sequence or secondary/tertiary structure.

We have studied the interactions of PV-3C protease with RNAs having no secondary structures and another set of RNAs having different secondary structures. The RNAs were designed from oriL/oriR replication elements and selected random nucleotide sequences to understand the specificity of PV-3C towards RNAs. We have utilized Nuclear Magnetic Resonance spectroscopy (NMR) and Isothermal Titration Calorimetry (ITC) for our study. NMR chemical shift perturbations showed the interaction of PV-3C with unstructured and secondary structured RNAs. Unstructured RNAs have more global effects on the delta chemical shift of all amino acid residues of PV-3C compared to structured RNAs interaction. Some of the residues from 6-20, the C-terminal part (Blue colour surface in the schematic below), and the RNA binding region (residue 82-89, purple colour surface below) are having a similar effect with all the different RNAs, while it shows dynamic (fast on and off rates) interaction overall. ITC experiments reveal the interaction is exothermic. Our study opens a new avenue for understanding novel biomolecular interactions important in regulating the multifunctional roles of 3C in hacking the host genome and viral replication process.

ABS#441

Poster session, July 14

Predicting mutation-driven changes in protein conformational landscapes

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While protein structure determination of a single, static, lowest energy conformational state has improved rapidly over the last decade, we lack high throughput methods for understanding and predicting conformational dynamics that drive protein function in vivo. To address this gap, we developed a high throughput hydrogen-deuterium exchange (HDX) pipeline to report on changes to secondary structure stability across a mutagenized protein library. We are building our protein library using state-of-the-art protein design approaches1 to generate hundreds of designed proteins for 100 conserved protein folds, with >80% sequence diversity among library members. The HDX pipeline uses matrix-assisted laser desorption/ionization with mass spectrometry (MALDI/MS) to rapidly observe global conformational differences across the entire protein library, enabling identification of specific mutants with altered secondary structure rigidities. These mutants are iteratively analyzed by a batched HDX liquid chromatography with mass spectrometry (HDX-LC/MS) method for further characterization of conformational changes in conserved protein folds resulting from mutations. By iteratively combining the speed of MALDI/MS with the higher resolution of HDX-LC/MS, we were able to effectively probe a far greater number of proteins than previously possible. We augmented this increased throughput by batching and later deconvoluting proteins in single HDX-LC/MS injection and found that signal loss was minimal. Using a glycosyl hydrolase fold as our model, we identified new patterns of relative secondary structure stabilities that can inform further modeling of the conformational landscape of this fold and other protein folds. Building on recent improvements in protein structure determination and prediction, our method illuminates secondary structure stabilities and interactions in the otherwise static models of soluble proteins, in increasingly higher throughput. This pipeline could be paired with modeling and design methods to understand and engineer conformational changes in natural and artificial proteins.

ABS#442

Poster session, July 14

Structural and Functional Characterization of KCTD5-associated CRL3 for Gβγ Ubiquitination

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The ubiquitin-proteasome system controls a wide range of normal and pathological processes. In this system, the covalent attachment of ubiquitin to a target substrate protein is mediated by a cascade of three enzymes including a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3). In most cases, E3 ligase complexes determine substrate specificity. The multi-subunit Cullin-RING ligases (CRLs) comprise the largest family of E3s in which a Cullin protein serves as a central assembling scaffold to bridge substrates to E2 enzymes for ubiquitin transfer. In CRL3 complexes, the Cul3 C-terminal domain associates with the RING-finger protein (Rbx1) to recruit the E2, while the N-terminal domain interacts with BTB domain-containing substrate receptor proteins. KCTD5 is a homo-pentameric BTB domain protein that acts as a substrate receptor in Cul3 E3 ligase complexes. Gβγ subunits have recently been identified to be associated with KCTD5. Here, we present the characterization of CRL3KCTD5 ubiquitin ligase complex required for Gβγ ubiquitination. We report the cryo-electron microscopy structure of a 5:5:5 assembly composed of KCTD5, Cul3 and Gβγ. We use structure-guided mutagenesis to probe the workings of the complex and measure Gβ and Gγ ubiquitination. Our findings show how a CRL3KCTD5 complex targets Gβγ proteins for ubiquitination and advances our understanding of how Gβγ complexes are regulated by this post-translational modification.

ABS#444

Poster session, July 14

Dimer Dissociation is the Rate-limiting Step of KaiB Fold Switching

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Cyanobacteria harbor the simplest biological clock, composed of three proteins (KaiA, KaiB, KaiC), which constitute a phosphorylation oscillator whose periodicity is regulated by the metamorphic protein KaiB. These proteins are characterized by adopting dissimilar yet thermodynamically stable structures, each with its own function. In the case of KaiB, a structural interconversion occurs between a tetramer of asymmetric dimers, or ground-state (gsKaiB), and a thioredoxin-like monomer, or fold-switch state (fsKaiB).

Although both structures and their functions are well described, how the structural interconversion occurs and what is its rate-limiting step are unknown. Here, we employ molecular dynamics simulations using structure-based models (SBM) with dual-basin Gaussian contact potentials to explore the fold-switch of KaiB. From these simulations, dimer dissociation is identified as the rate-limiting step of KaiB fold-switch, following a three-state refolding pathway with accumulation of a monomeric intermediate (gs2 ⇄ 2gs ⇄ 2fs). Lastly, the importance of oligomer stability for the fold-switch of KaiB is demonstrated by experimental analysis of two mutants with altered periodicity, for which a shift towards population of the dimer and monomer species and local structural features compatible with fsKaiB are observed by size exclusion chromatography and hydrogen-deuterium exchange mass spectrometry, respectively.

Altogether, these results suggest that biophysical analysis of the transition state for the structural interconversion between gsKaiB and fsKaiB can be exploited for rational design of the periodicity of the cyanobacterial circadian clock.

ABS#446

Poster session, July 14

Glycosylation Rectifies the Entropic Component of Skp1's Disordered C-terminus on Homodimerization in a Pathogenic Protist

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Skp1/F-box protein (FBP)/Cul1 (SCF) E3 ubiquitin ligases are responsible for polyubiquitin-dependent degradation of intracellular proteins within all eukaryotes. Protein targets are specifically recognized by a variety of different FBPs which bind to Skp1, indirectly linking them to the E2 ubiquitin donor. Within the parasite Toxoplasma gondii, Skp1 undergoes glycosylation of a hydroxyproline residue in its disordered C-terminus, which has been shown to alter its FBP binding repertoire. Skp1 also homodimerizes in a manner which competes with FBP binding, which may be relevant in vivo given its estimated <50 nM Kd based on analytical ultracentrifugation. The role of the 34 C-terminal amino acids, which include the glycosylation site and are disordered in free Skp1 but contribute to the interface with FBPs, was examined by truncation. The Kd rose to over 400 nM suggesting a substantial contribution to dimerization. Surprisingly, replacement with a scrambled sequence nearly restored the stronger affinity. Interestingly, glycosylation of native Skp1 mimics loss of this region. Prior NMR evidence indicates that glycosylation increases order within this region, including increased α-helix formation as seen in complexes with FBPs. We therefore propose that the intrinsically disordered C-terminal tail promotes dimerization by an entropic force that is countered by increased order imposed by glycosylation. This in turn increases the amount of monomeric Skp1 available for FBP binding. Since oxygen is required to form the hydroxyproline glycan anchor, this process may underlie, via ubiquitin-mediated degradation, the ability of the parasite to navigate natural variations in oxygen over the course of infection.

ABS#447

Proteins in Motion (July 15, AM)

Modulating Enzyme Function via Dynamic Allostery and structure changes within Biliverdin Reductase B family

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Biliverdin reductase B (BLVRB) families catalyze the NADPH-dependent reduction of multiple flavin substrates, which are critical for maintaining cellular redox regulation. Here, we've begun identifying the role of dynamics and allosteric communication in BLVRB function by studying the changes to both structure and dynamics during the catalytic cycle. Through methods including NMR and kinetics studies, we've discovered that the oxidation state of the coenzyme (i.e., a single hydride) induces global changes to both protein/coenzyme structure and dynamics within multiple members of the BLVRB family. Importantly, the same allosteric networks affected by the oxidation state of the coenzyme also modulate catalytic function. Considering that BLVRB adopts one of the most common protein folds found within some 46,000 members of the SDR family, our studies have wider implications for how such enzymes exploit dynamics and allostery to modulate function.

Studies presented here the structural/dynamic changes of human BLVRB and its coenzyme through the catalytic cycle and then focus on a key allosteric site and its evolutionary role in modulating function. First presented are the chemical shift perturbations between human BLVRB bound to both reduced (NADPH) and oxidized coenzyme (NADP+) that reveal global conformational changes to BLVRB secondary structure, dynamics, and the coenzyme itself. These global changes include active site residues such as S111, and H153, but also distally coupled networks that include T164. The second set of studies presented illustrates how this dynamic human BLVRB T164 site distal is surprisingly quenched in other eukaryotic homologues, such as lemur and hyrax, which comprise a S164 instead. Both through mutations and through studies of these homologues, the motions of this 164 site appear to predict coenzyme binding. Thus, our studies have begun to reveal the hidden roles of dynamics and allostery in BLVRB function and pinpoint even conservative mutations that impart surprisingly specific changes.

ABS#448

Structure Prediction and Design (July 15, AM)

Masking T-Cell Engaging Bispecific Antibodies for Safer Cancer Immunotherapy

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Masking T-Cell Engaging Bispecific Antibodies for Safer Cancer Immunotherapy

The field of oncology continues to face a critical need for safer and more precise cancer treatment. T-cell engaging bispecific antibodies (T-bsAbs) are innovative therapeutics with the power to engage a patient's own T cells to selectively destroy cancer. Despite the success of the FDA approved T-bsAb Blincyto for acute lymphoblastic leukemia, toxicity has hindered the clinical performance of T-bsAbs for treating other malignancies and solid tumors. The goal of this project is to design a safer prodrug T-bsAb that localizes toxicity to the tumor to prevent systemic side effects, while retaining potency to destroy tumors. I have leveraged computational protein design using Rosetta with experimental biochemical binding and T-cell activation assays to identify and validate candidate prodrug T-bsAbs. This study will showcase an approach to developing T-bsAb therapeutics that prioritizes safety and has the potential to advance cancer immunotherapy treatment strategies.

ABS#449

Poster session, July 15

Systematic conformation-to-phenotype mapping via limited deep-sequencing of proteins

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Non-native conformations drive protein misfolding diseases, complicate bioengineering efforts, and fuel molecular evolution. Current experimental techniques are not well-suited for elucidating them and their phenotypic effects. Especially intractable are the transient conformations populated by intrinsically disordered proteins. The objective of this study was to develop a technology for systematically discovering, stabilizing, and purifying native and non-native conformations, generated in vitro or in vivo, and directly link conformations to molecular, organismal, or evolutionary phenotypes. I will now present this approach, which involves high-throughput disulfide scanning (HTDS) of the entire protein. Revealing which disulfides trap which chromatographically resolvable conformers requires deep sequencing of libraries of double-Cys variants of the target protein to precisely locate both Cys residues within each polypeptide molecule at the same time. That capability has also been developed in this study, by combining Cys-specific chemical backbone cleavage with library-wide mass spectrometry. HTDS of the abundant E. coli periplasmic chaperone HdeA revealed distinct classes of disordered hydrophobic conformers with variable cytotoxicity depending on where the backbone was cross-linked. HTDS can map protein conformational landscapes onto organismal phenotypic landscapes for many proteins that function in disulfide-permissive environments.

ABS#450

Poster session, July 15

A Hybrid Resolution Force Field for Accurate Simulation of Coupling between Secondary Structure and Liquid-Liquid Phase Separation

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Intrinsically disordered proteins (IDPs) frequently mediate liquid-liquid phase separation (LLPS) involved in the formation of membraneless organelles. Together with theory and experiment, coarse-grained (CG) simulations have been instrumental in modeling and understanding sequence-specific phase separation of IDPs. However, the widely-used CA-only protein models have inherent limitations in describing the peptide nature of IDPs, such as backbone-mediated interactions and effects of secondary structures in LLPS. Here, we describe a hybrid resolution (HyRes) protein force field with an atomistic backbone and coarse-grained side chains that can accurately describe the protein backbone and transient secondary structure in LLPS. We show that HyRes provides quantitative description of a wide range of local and global properties of IDPs, including secondary structure propensities and radius of gyration. Applied to pepHBP-1, HyRes is able to directly simulate its spontaneous LLPS process and fully resolve the effects of single and double charge mutations on the phase separation ability, consistent with available experimental data. Further analysis of the condense phase reveals an increased propensity of pepHBP-1 to form inter-peptide beta-sheets, as suggested by a previous CD study. We further applied HyRes to study the phase separation of TPD-43, where several disease-related mutants have been shown to affect residual helicities to modulate LLPS propensity. Our simulations successfully predict that increasing helical

propensity via 335A and 338A mutations enhances LLPS, while 326P and 337P mutations disrupt transient helices to reduce the stability of condensed phase. This is an exciting result that has never been demonstrated before with either CG or atomistic simulations. Ongoing analysis will further reveal how changing the helical propensity controls TPD-43 LLPS. We believe that the HyRes force field represents an important advance in molecular simulation of LLPS and will help elucidate the molecular basis of how the peptide backbone contributes to the regulation of LLPS in biology.

ABS#453

Protein Folding and Function in Context (July 13, AM)

Elucidating the all-α to all-β refolding of RfaH bound to transcription elongation complexes using dual-basin structure-based models

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RfaH is a two-domain bacterial elongation factor that undergoes a large structural rearrangement, in which its C-terminal domain (CTD) completely folds from an α-helical hairpin tightly bound to the N-terminal domain (NTD) into a small β-barrel. This is triggered by its association to its high-mass biological partners, namely the paused transcription elongation complexes (PEC) at a specific DNA signal (ops), thus making the computational and experimental study of its structural interconversion challenging.Here, we use cryoEM structures of the autoinhibited (α) and active states (β) of RfaH in complex with opsPEC as input to generate all-atom dual-basin structure-based models. We then use these models in molecular dynamics simulations to observe the refolding between these states while bound to opsPEC. The results from 500 independent molecular dynamics (MD) simulations using Langevin dynamics of the RfaH fold-switch from the autoinhibited to the active state provide evidence of a rugged refolding landscape consistent with previous simulations on isolated RfaH. At least 4 intermediate states are observed, which follow a precise sequence of refolding events that are dominated by the rupture of interdomain interactions between the NTD and CTD that start at the ends of the α-helical hairpin while simultaneously allowing the refolding of secondary structure elements once detached from the NTD, starting with the formation of the interactions between strands β1 and β5 of the five-stranded β-barrel conformation. Interestingly, opsPEC does not enhance refolding of RfaH CTD, with this domain being observed to refold into the active state in the absence of any interactions with the RNA polymerase, DNA or RNA.

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ABS#456

Structures of Mega-Complexes (July 13, PM)

Visualizing Massive Macromolecular Complexes in Motion with Cryo-EM, Cryo-ET, and Deep Learning

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Macromolecular machines such as the ribosome undergo massive structural changes as they assemble and function. While we have long appreciated that such structural changes exist, experimentally visualizing and analyzing large ensembles of these structures is challenging. Here, I briefly describe cryoDRGN (1-3), a software package we developed to analyze structural heterogeneity in protein complexes visualized by cryo-electron microscopy (cryo-EM). This approach, which uses a purpose-built neural network based on a variational autoencoder, maps individual particle images to a low-dimensional latent space, effectively sorting particles based on their structure. Users can then generate ensembles of hundreds to thousands of three-dimensional structures from this latent space using the trained networks, which enables a more comprehensive understanding of the structural states accessible to the protein complex of interest. Here, I detail our application of cryoDRGN to understand bacterial ribosome biogenesis generally and, specifically, how cofactors including methyltransferases and RNA helicases guide the assembly of the large and small ribosomal subunits. In analyzing a series of related cryo-EM datasets, we surprisingly uncovered that the methyltransferase KsgA 'proof-reads' the assembling ribosomes by preferentially disassembling ribosomes that have been erroneously constructed. Further, this work allowed us to

detail key assembly events that couple r-protein binding and rRNA folding, and additionally allowed us to layer rRNA helix folding/docking onto the classical r-protein association maps established by Nomura, Nierhaus, and colleagues. Finally, I describe our work deploying analogous methods aimed at analyzing structural heterogeneity in situ via cryo-electron tomography. Taken together, this work highlights new tools within the cryoDRGN framework we've recently developed and showcases their application to understanding the assembly and function of structurally heterogeneous macromolecular complexes.

ABS#457

Protein Evolution: Lessons from the Past (July 13, PM)

Pro283 Substitution on Influenza Nucleoprotein Allows Immune Escape but Also Introduces Folding Defects

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Influenza is a pathogenic RNA virus that causes acute respiratory diseases in humans. Nucleoprotein (NP) is a critical structural protein of influenza ribonucleoproteins and is central to viral RNA packing and trafficking, as well as transcription and replication. In human cells, NP is targeted by the interferon-induced Myxovirus resistance protein 1 (MxA), which binds to NP and prevents nuclear translocation of incoming viral ribonucleoproteins and newly synthesized NP. This selection pressure has caused NP to evolve a few critical MxA-resistant mutations. For example, Pro283 was one of the three MxA-resistant substitutions identified in the 1918 influenza pandemic strain, and is widely conserved in major modern human influenza strains. Interestingly, a previous work showed that Pro283 NP is extremely unfit at febrile temperature (39 °C). This effect is exacerbated when protein folding is further challenged with HSF1 inhibition, which depletes the cytosolic proteostasis network components (1). To elucidate the molecular mechanisms of how the fitness of Pro283 depends on the host proteostasis factors, we use structural and biophysical tools to compare Pro283 NP with Ser283 NP, and identify folding defects induced by Pro283 substitution. We saw that Pro283 NP exhibits lower thermal stability than Ser283, both in isolation and in cell lysate. Nevertheless, crystal structures and nuclear magnetic resonance spectra reveal that, despite site 283 being located at the center of an α-helix, the proline substitution disrupts neither the local secondary structure nor gross tertiary structure substantially. Instead, Pro283 has significantly higher aggregation propensity during guanidine hydrochloride mediated unfolding and refolding. In protein biophysics perspective, these observations suggest that Pro283 changes the folding energy landscape of NP without substantially changing the final protein conformation. In protein evolution perspective, our work suggests that hijacked host chaperones may directly promote the folding of otherwise biophysically incompetent viral protein to enable immune system escape.

ABS#458

Poster session, July 15

Photo-mediated Modulation of α-Synuclein Amyloid Formation

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Intracellular deposits of α-synuclein amyloids are found in patients suffering from Parkinson (PD), multiple system atrophy, and Lewy body dementia. Emerging data demonstrate that both in vitro and ex vivo amyloid fibrils adopt a wide range of structures, a concept known as polymorphism. Understanding the underlying driving forces in the creation of polymorphs is an important step in elucidating the structure-cytotoxicity relationship of amyloid fibrils. In this work, we investigate the effect of charged lysine residues on the aggregation kinetics and fibril morphology of α-synuclein using a photochemical approach. We chose a PD-related N-terminally truncated 66–140 variant to reduce the number of lysines from fifteen to four, facilitating a mutational scan study. Amber codon suppression was used to site-specifically incorporate a photocaged lysine analog, o-nitrobenzyl-oxycarbonyl-Nε-L-lysine (ONBK). ONBK-labeled proteins enable direct conversion of ONBK to the native positively charged amino group with precise spatiotemporal control upon light exposure (365 nm). The effects on amyloid aggregation kinetics, fibril amounts, and morphology were characterized by Thioflavin-T fluorescence, SDS-PAGE analysis, and transmission electron microscopy. We identified two critical residues, Lys96 and Lys102, that

dramatically affected aggregation propensity, which were then substituted by ONBK. Through the rapid and efficient removal of the photocage, aggregation kinetics, fibril structure, and seeding capacity of 66–140 are shown to be modulated by specific positive charges. This photochemical technique is broadly applicable and can be used to examine the role of critical Lys residues in a myriad of amyloidogenic proteins.

ABS#459

Poster session, July 15

Scanning Native Proteins for Functional Variants as an Undergraduate Teaching Project: A CURE for phosphoenolpyruvate carboxykinase

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Course-based Undergraduate Research Education (CURE) uses an experiential approach for laboratory instruction by engaging students in an active research project performed within a teaching laboratory. Biochem Project Lab adapts the CURE approach to give over 350 students per year a research laboratory experience while simultaneously having the students contribute to the investigation into the natural amino acid variation between proteins and its affect enzymatic function. Over the course of a semester, teams of students investigate genes predicted to encode proteins with hypothetical phosphoenolpyruvate carboxykinase (PEPCK) function which have been identified through metagenomic sequencing of the human microbiome. Each student team selects one genetic sequence to analyze, uses a bacterial expression system to produce recombinant protein, isolates and purifies it, and then perform a suite of biophysical and biochemical characterizations on the purified product. We will describe the educational format, explain the technological development of the procedures, and present the initial results from the first sets of student teams to complete the course. These results show that students can produce large quantities of purified, active protein and perform basic characterizations that can link enzyme activity to amino acid sequence variations. Furthermore, we will present how the format of this course could be adapted by other researchers, so that more research projects can benefit from undergraduate involvement while simultaneously giving students an interesting and educational experience in modern research tools and techniques.

ABS#462

Poster session, July 15

Quantifying a light-induced energetic change in bacteriorhodopsin by force spectroscopy

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Ligand-induced conformational changes are critical to the function of many membrane proteins, including those involved in signaling and transport, and arise from numerous intramolecular interactions. In the photocycle of the model membrane protein bacteriorhodopsin (bR), absorption of a photon by retinal triggers a conformational cascade that results in a proton being pumped across the cell membrane. While decades of laser spectroscopy and structural studies have probed this photocycle in intricate detail, changes in intramolecular energetics that drive protein motions have remained elusive to experimental quantification. Here, we measured these energetics on the millisecond time scale using atomic-force-microscopy–based single-molecule force spectroscopy. Specifically, precisely timed light pulses triggered the bR photocycle while the equilibrium unfolding and refolding of terminal 8-amino-acid region of bR's G-helix was measured. These dynamics were modulated when the EF-helix pair moves ∼9 Å away from the end of the G-helix during "open" portion of bR's photocycle which facilitates reprotoninzation of the retinal. In about half of the data, we observed a light-induced destabilization. This destabilization was 3.4 ± 0.3 kcal/mol and lasted 34 ± 2 ms. The kinetics and pH-dependence of this destabilization were consistent with prior measurements. In the other half of the data, excitation by light stabilized a longer-lived putative misfolded state that likely arises from the interplay of photoexcitation and force application. Through this work, we establish a general single-molecule approach for measuring ligand-induced energetic effects in membrane proteins.

ABS#464

Poster session, July 15

The Hemoglobin of a Piezophile and Its Response to Pressure

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The Hemoglobin of a Piezophile and Its Response to Pressure

The genome of the deep-sea psychropiezophile Shewanella benthica encodes two hemoglobins of the truncated lineage. This raises several biochemical and biophysical questions related to the response of globins and heme proteins to extreme conditions. We chose one of the S. benthica globins, UniProt A9DF82 or SbHbN, for study. SbHbN, in which we replaced two Cys with Ser (S2-SbHbN hereafter), was successfully expressed in Escherichia coli. Optical, NMR, SEC, SAXS, and X-ray crystallography data were combined to investigate the protein at atmospheric and high hydrostatic pressure. S2-SbHbN exhibits two unusual properties compared to its bacterial relatives: facile heme modification with hydrogen peroxide and self-association at micromolar concentrations. The replacement of a tyrosine pointing into the heme pocket (Tyr34) eliminated the heme reaction and facilitated structural determination. X-ray diffraction analysis of Y34F S2-SbHbN (PDB ID 7TT9) revealed the location of a potential dimer interface and suggested which amino acids may stabilize the assembly. High pressure NMR data showed that an increase in hydrostatic pressure from atmospheric to 2.5 kbar does not cause unfolding of S2-SbHbN or heme loss but favors the monomeric state. By comparison, modification of the interface with single amino acid replacements altered the response to pressure as evaluated by SAXS. The perturbation was evident for Y108A S2-SbHbN, which appeared monomeric at atmospheric pressure and exhibited crystal contacts different from those in structure 7TT9. Heme reactivity and nascent quaternary structure make SbHbN an excellent candidate for exploring the properties and evolution of extremophilic hemoglobins. Supported by NSF CHE-2003950 (JTJL) and NSF DMR-1829070 (CHEXS).

ABS#466

Poster session, July 15

Exploring the VFLIP Design Strategy to Improve Stability and Yield for Human Common Cold Coronavirus Spike Proteins

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Human coronaviruses (HCoVs), such as HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1, have been identified to contribute to human common colds [Liu et al.]. HCoVs can cause severe lower respiratory tract infections and have been intensively studied even prior to the emergence of the highly pathogenic SARS-CoV-2 strain that caused the COVID-19 pandemic. Similar to SARS-CoV-2, the spike protein is an attractive target for a vaccine against common cold HCoVs. In our hands, the expression of HCoV spike proteins was faced with challenges such as low yield or in some cases the inability to cleave off purification tags. Therefore, we designed HCoV spike protein sequences based on the VFLIP (five (V) prolines, Flexibly Linked, Inter-Protomer disulfide) design strategy. This strategy has been reported to significantly improve protein stability as well as protein yield for SARS-CoV-2 spike variants [Olmedillas et al.]. We transiently transfected mammalian Expi293F™ cells to express SARS-CoV-2-Omicron and HCoV spike protein variants stabilized by either two proline residues (2P) [Hsieh et al.] or by the VFLIP design strategy. Recombinant proteins were purified and then characterized using SDS-PAGE, Western blot, differential scanning fluorimetry (DSF), dynamic light scattering (DLS) and size exclusion chromatography multi angle light scattering (SEC-MALS) to determine protein purity, stability and higher order structure. Our preliminary data confirm that the VFLIP design strategy increases the expression yield for SARS-CoV-2 Omicron spike protein 6- to 7-fold when compared to its 2P counterpart. However, in our hands the VFLIP design strategy did not lead to increased yields for the common HcoVs spike proteins. Contrary to our expectations, the VFLIP design led to a decrease in expression of HCoV-HKU1-2 and HCoV-OC43 spike protein when compared to their 2P counterparts. Further structural analyses are needed to be able to understand the observed differences between SARS Omicron and common cold spike protein.

ABS#469

Poster session, July 15

OPA-1 Deficiency Promotes Muscle Atrophy through Upregulating Phago-MERCs

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Autophagy classically operates as a physiological process to degrade cell membrane components, protein aggregates, and damaged organelle, as a mechanism for nutrient breakdown, and as a regulator of cellular architecture. Proper autophagic flux is paramount for functional skeletal muscles and has been previously studied as a metabolic controller in muscle. The underlying molecular mechanisms that regulate autophagy in skeletal muscle have started to materialize. Notably, mitochondrial dynamics have been linked to autophagy mechanisms. Other mitochondria-associated structures, such as mitochondrial endoplasmic reticulum contact sites (MERCs) can also regulate autophagy. However, it is not well understood if OPA1 regulates autophagy through MERCs or an independent pathway. MERCs are enriched with specific proteins and lipids that aid in specialized structural rearrangements such as the formation of autophagosomes. We hypothesized that loss of OPA1 in skeletal muscle increases Phago-MERCS and serves as the platform for autophagosome formation. To investigate the role of OPA-1 on Phago-MERCs, studies were performed in OPA-1 deficient mice, flies, and OPA-1 ablated in murine myotubes. The ablation of OPA-1 in skeletal muscle showed a decrease in soleus, gastrocnemius, quadriceps, and tibialis anterior muscle weight. Loss of OPA-1 in gastrocnemius muscle showed an increase in MERC proteins that are important for Phago-MERC tethering. Loss of OPA-1 increased the Phago-MERC recruiting protein ATG5. While qPCR analysis after the deletion of OPA-1 in 40-week skeletal muscle displayed an increase in MFN-2, LC3B, ATG7, and ATG5 transcripts, also p62 transcripts were shown to decrease. Ablation of OPA-1 in myotubes increased MERC proteins. TEM Analysis of OPA-1 deficient skeletal muscle and myotubes had an increase in Phago-MERCs and lysosome structures. Loss of OPA-1 Like in drosophila muscles increases Phago-MERCs. This data suggests that loss of OPA-1 increased Phago-MERCs, which may lead to decreased muscle mass, but a mechanism of action still needs further exploration.

ABS#470

Poster session, July 13

Comparing covalent and non-covalent inhibition mechanisms of small molecules targeting the disordered transactivation of the androgen receptor

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Intrinsically disordered proteins, which do not adopt well-defined structures under physiological conditions, are implicated in many human diseases. Small molecules that target the disordered transactivation domain of the androgen receptor have entered human trials for the treatment of castration-resistant prostate cancer (CRPC). These molecules have been shown to be covalently reactive with cysteine residues of the androgen receptor under physiological condition. Here, we utilize all-atom molecular dynamics computer simulations to simulate covalent adducts of two ligands, EPI-002 and EPI-7170, bound to residue CYS404 of the disordered androgen receptor transactivation domain. We compare the conformational ensembles and protein:ligand interactions observed in simulations of the covalent adducts to the conformational ensembles and protein:ligand interactions observed in non-covalent binding simulations. Our simulations reveal that covalent adducts of EPI-002 and EPI-7170 bound to disordered androgen receptor transactivation domain remain heterogenous and disordered, and covalent attachment of these ligands drive androgen receptor to sample more collapsed helical states compared to non-covalent complexes. We utilize a t-distributed stochastic neighbor embedding (t-SNE) clustering approach to compare androgen receptor conformational ensembles in the presence of each ligand and identify several common ligand binding modes that are present in simulations of both ligands. Our results suggest strategies for developing more potent covalent inhibitors of the disordered androgen receptor transactivation domain.

ABS#471

Poster session, July 13

Negative Cooperativity And The Atypical Kinetics Of hUGDH

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Human UDP-Glucose Dehydrogenase (hUGDH) catalyzes the formation of UDP-glucuronic acid (UDP-GlcA), the essential substrate in Phase II metabolism of drugs.

Attaching GlcA to drugs promotes the excretion of the resulting glucuronides. Glucuronidation is also a common drug resistance mechanism found in some cancers. Thus, regulating the availability of UDP-GlcA is a promising strategy in cancer treatment. Key to this goal is a detailed understanding of the reaction mechanism of hUGDH. In the hUGDH mechanism, UDG-Glc is converted to UDP-GlcA through a four electron transfer reaction, in which two molecules of NAD oxidize UDP-Glc in a stepwise fashion. Kinetic analysis reveals several atypical features, the most striking being negative cooperativity with respect to NAD+. Negative cooperativity typically arises from molecular asymmetry or a mixture of folded and misfolded proteins, and either can result in two or more active sites with different NAD+ affinities. However, we found no evidence supporting either mechanism despite numerous crystal structures and biophysical studies. We hypothesize that negative cooperativity in hUGDH originates from the unique kinetic mechanism of the enzyme. Specifically, if the second NAD+ binding step is rate limiting at low [NAD+], then it is possible for a subsequent kinetic step to become rate limiting at high [NAD+]. This NAD+-dependent shifting of rate limiting steps could be observed as a change in KM which could produce negative cooperativity in a substrate saturation curve. To test this, we have simulated a kinetic model which reproduces the negative cooperativity seen in hUGDH. This model also predicts several other unusual kinetic features of hUGDH, including: (i) apparent substrate inhibition by UDP-Glc at sub-saturating [NAD+]; (ii) hyperbolic binding of UDG at saturating [NAD+]; and (iii) biphasic progress curves. Current work is focused on collecting experimental data of each step in the mechanism to refine the accuracy of the model.

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ABS#472

Aggregates, Amyloids, or Condensates? (July 16, AM)

Protein Stability, Folding, and Confinement in CAHS Hydrogels

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One function of disordered proteins is to assist organisms in rapidly responding to external stresses including freezing, osmotic stress, high temperature, and desiccation. A family of tardigrade-specific cytoplasmic abundant heat soluble (CAHS) intrinsically disordered proteins protect and preserve cellular components while tardigrades are in a desiccated state. However, there remains uncertainty surrounding how these disordered proteins assist in keeping tardigrades viable. The goal of this project is to uncover how CAHS D hydrogel formation induced by hypertonic stress modulates protein stability and folding kinetics in vitro and in cellulo. Here we use fast relaxation imaging (FReI) of two FRET-labelled model proteins, a crowding sensor protein (CrH2) and phosphoglycerate kinase (PGK), to probe local environment effects as a function of CAHS D concentration. CrH2 is used to quantify the confinement effect of cross-linked hydrogels in vitro: 12.5 mg/ml CAHS is similar to aqueous solution (no confinement), while 50 mg/ml CAHS is highly confined. PGK undergoes cooperative unfolding that is sensitive to environmental influences. In vitro PGK measurements reveal that both noncovalent interactions and confinement modulate PGK properties in CAHS D. Low concentrations of CAHS D had no effect on PGK stability, promoted a more extended unfolded PGK state, and prevented aggregation. Confining concentrations of CAHS D stabilized PGK, suggesting that confinement by CAHS hydrogels is broadly correlated with an increase in protein stability. In cellulo measurements of CrH2 in HEK293 cells reveal that CAHS D expression does not inherently increase crowding in the cellular environment. Upon hypertonic shock CrH2 is confined, consistent with CAHS D hydrogel formation in vitro. Our results support a mechanism where noncovalent protein-CAHS interactions prevent against aggregation and confinement by CAHS hydrogels promotes protein stabilization. Understanding how CAHS D hydrogels interact with and preserve proteins will ultimately benefit applications in biology, medicine and materials science.

ABS#473

Poster session, July 15

Unravelling Paralog-Specific Notch Signaling through Thermodynamic Analysis of Ternary Complex Formation and Transcriptional Activation of Chimeric Receptors

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The Notch signaling pathway is a highly conserved juxtacrine signaling pathway in metazoans that regulates context-dependent cellular differentiation and plays a

central role in development of most animal organs and tissues. The Notch receptor is a large transmembrane protein composed of a ligand-binding extracellular domain (NECD), a short transmembrane helix, and an intracellular domain (NICD). The four paralogous Notch receptors in humans (Notch1-4) have distinct functions yet, interestingly, share a conserved architecture and the same canonical activation pathway: a Notch ligand on an adjacent cell binds NECD, inducing a series of extra- and intracellular proteolytic cleavages which ultimately liberate NICD from the membrane, allowing its nuclear translocation. Once in the nucleus, NICD binds to transcription factor CSL via its disordered RAM and tandem ankyrin repeat (ANK) domains and recruits co-activator MAML to form the active ternary complex on DNA, upregulating transcription of Notch-responsive genes.

Though the shared mechanistic features of canonical signaling are well understood, it is unclear how the four human paralogs leverage this same mechanism to elicit drastically different outcomes such as oncogenesis and tumor suppression by different paralogs in the same context. To better understand the origins of paralog-specific differences, we have used isothermal titration calorimetry to characterize the thermodynamics of each human Notch paralogs' RAM-ANK domains binding to CSL, and their thermodynamics of MAML recruitment. We extended this analysis to chimeric constructs to understand the individual contributions of each domain in formation of the Notch transcriptional complex (NTC). Further, we have devised a model to quantitatively describe the role of the RAM, ANK, and C-terminal domains of each NICD in activating transcription in cells and used this model to analyze transcriptional activation strength of the four Notch paralogs and their chimeras. These data constitute a significant advancement in the quantitative understanding of paralog-specific Notch signaling.

ABS#474

Protein Folding and Function in Context (July 13, AM)

Using Hydrodynamics, Crowders and Kosmotropes to Turn a kcan't into a kcat

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The intracellular environment is extremely crowded and filled with compatible osmolytes, both of which are important features that favor the folded state of proteins. This environment exerts a ubiquitous selective pressure in the evolution of protein function that is often ignored or neglected in in vitro studies. As a result, many proteins appear unstable or exhibit poor kinetic parameters in assays. Here, we use hydrodynamics to identify a folding defect in the enzyme N-acetylglucosaminyltransferase II (MGAT2), and show how it can be rescued with a combination of molecular crowding and kosmotropes. MGAT2 is an essential UDP-GlcNAc transferase in the synthesis of complex N-glycans. Using high-resolution sedimentation velocity analysis, we show that the purified enzyme sediments more slowly than predicted based on crystal structures, which indicates an expanded hydrodynamic radius most likely due to local unfolding. The addition of the kosmotropic Hoffmeister salt ammonium sulfate restores the sedimentation rate of MGAT2 to the expected value, supporting the local unfolding hypothesis. Additional evidence comes from six unique crystal structures of MGAT2, which reveal a surprising heterogeneity in the packing of the protein core near the active site reminiscent of nuts-and-bolts-in-a-jar behavior. It is likely that this region is locally unfolded in dilute solutions, and the observed folded conformation is selected by the crystal lattice. Guided by these observations, we increased the specific activity of MGAT2 100-fold through the systematic addition of molecular crowders and the physiological kosmotrope glycine betaine. This work demonstrates the power of high-resolution sedimentation velocity to identify folding defects and serves as an apt reminder of the importance of considering solvent conditions beyond simple pH and ionic strength in the study of proteins at infinite dilution.

ABS#475

Structures of Mega-Complexes (July 13, PM)

Visualizing the AAA+ protease ClpXP with a fully engaged substrate

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ClpXP is a AAA+ protease that is important for protein homeostasis in bacteria and mammalian mitochondria. The ClpXP complex consists of ClpX, an ATP-dependent hexameric protein unfoldase and polypeptide translocase, and ClpP, a double-ring tetradecameric peptidase. ClpX initially binds to an unstructured degron sequence, typically at the N- or C-terminus of a native protein, and then unfolds the substrate and translocates the denatured polypeptide into ClpP for degradation. In bacteria, ssrA tags or degrons added by tmRNA-mediated ribosome rescue mark cytoplasmic proteins for ClpXP degradation. Recent cryo-EM structures of ClpXP have determined structures of the substrate-free enzyme, a recognition complex in which the ssrA tag binds to the upper portion of a closed axial channel of ClpX, and an intermediate complex in which the degron is translocated six residues deeper into an open channel. A very important missing structure was one in which a folded portion of a substrate was pulled tightly against ClpX, which should allow a subsequent power stroke to exert an unfolding force. Here, we present cryo-EM structures that visualize this substrate-engaged state at near-atomic resolution. Importantly, these structures reveal significant conformational changes within ClpX and across the ClpX-ClpP interface. These new structures also provide insight into the degradation of branched and disulfide-bonded substrates, which require the concomitant translocation of multiple peptide chains through the axial channel of ClpX. In combination, these structures illuminate the major steps in the action of a AAA+ protease for the first time.

ABS#476

Poster session, July 13

Exploring the Druggability of Chikungunya Virus Protease nsP2 using Biomolecular NMR

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Chikungunya virus is an arboviral infection which has infected more than 2 million people across 60 countries in the past 10 years alone. CDC suspects that it may result in a widespread outbreak across the globe in the coming years owing to the absence of any drug or preventive vaccine. Pathogens causing these infections are enveloped positive-sense single-stranded RNA viruses which encode a protease that plays a critical role in viral replication and maturation. Since proteases are essential to the viral life cycle, they have been successfully exploited as drug targets for treating viral diseases such as Hepatitis-C and HIV. These findings strengthen the motivation for exploring non-structural protein 2 (nsP2) as a significant antiviral drug target. In this work, we have screened a 500 compound fragment library against chikungunya virus protease nsP2 to validate binding using biomolecular NMR 19F, 1H and STD-NMR experiments. 1H- 15N HSQC (2D)- NMR experiments were further carried out on the identified hits and labelled protein to study specific ligand binding and then determine dissociation constant (Kd) using chemical shift perturbation experiments. Our next steps involve identifying the regions of compound binding in the protein through a combination of triple resonance (3D)-NMR experiments for backbone assignments. We will use the knowledge thus gained to synthesize molecules with higher binding affinity and ultimately develop an effective inhibitor against this disease.

ABS#477

Poster session, July 15

The Thermodynamic Cost of Domain-Swapping in Long-Lived Eye Lens γ-Crystallins

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Maintaining the transparency of the eye lens and thereby proper vision throughout adulthood is achieved by the high kinetic and thermodynamic stabilities of the long-lived crystallin proteins. Vertebrate βγ-crystallins adopt two homologous domains connected via a linking peptide which is considered a key determinant of their tertiary and quaternary arrangements. In vitro, γ-crystallins are monomeric whereas β-crystallins are thought to form mainly compact 'face en face' dimers [1] that associate via domain-swapping to form larger oligomers

(e.g., tetramers, octamers) [2]. Domain-swapped dimers of either γ- and β-crystallin are yet to be unambiguously observed in solution. To better understand their stability and aggregation properties, we engineered domain-swapped homodimers of γD- and γS-crystallin analogous to the crystallographic dimer of βB2-crystallin by shortening their interdomain linker. The domain-swapped dimeric conformation of the γ-crystallin mutants was predicted by AlphaFold Multimer, solved by X-ray crystallography and verified in solution by small-angle X-ray scattering. While preserving their native domain structures, both domain-swapped dimers exhibit reduced thermodynamic stability relative to their wildtype monomeric counterparts, suggesting that this arrangement is unfavorable in an aging lens where the risk of protein misfolding and aggregation is pronounced due to cumulative post-translational modifications. Accordingly, linker sequences in vertebrate lens βγ-crystallins show a narrow length distribution (typically 6-8 residues) despite a low residue conservation. Our findings suggest that linker length has been optimized in βγ-crystallins to facilitate interdomain interactions while avoiding opportunities for domain-swapped dimerization and thereby susceptibility to protein aggregation in the lens, the primary cause of cataract.

ABS#480

Poster session, July 13

Solid-State NMR Assignment of Rigid Segment of Chemoreceptor in Functional Chemotaxis Signaling Complexes

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Membrane protein complexes are important for cellular signaling and are major drug targets. Bacterial chemoreceptor complexes are responsible for the signaling that controls cellular swimming during chemotaxis by binding attractants and repellants in the cell's environment. These complexes are made up of the chemoreceptor, inserted in the bacterial cell membrane, associated with a histidine kinase CheA and a coupling protein CheW, in the cytoplasm. These protein complexes are unique to chemotactic bacteria making them ideal targets for new antibiotics. However, the mechanism of signal propagation through the chemoreceptor to control the kinase is unknown. Solid state NMR data collected by our lab shows that only a small fraction of the chemoreceptor residues are rigid enough to have nonzero 13C-15N dipolar couplings and the number of rigid residues changes between signaling states.1 Hydrogen-deuterium exchange mass spectrometry data collected by our lab has identified the protein interaction region (PIR) of the chemoreceptor as the most well-ordered region with the lowest exchange rate.2 2D and 3D 13C-15N dipolar coupling experiments will allow for detection of only the most rigid residues of the chemoreceptor. We propose that the PIR will be a part of the visible segment in these experiments. Assignment of this portion of the chemoreceptors in functional homogeneous complexes with defined signaling states has the potential to identify residues involved in changes in dynamics and protein interactions between the signaling states. This work will help to understand the mechanism of signaling in chemoreceptor complexes.

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ABS#481

Membrane Proteins: From Natural to Designed (July 14, PM)

Insights into Intercellular Bridging via TAM Receptor Structures

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TAM receptors are a unique receptor tyrosine kinase (RTK) subfamily with a vital role in maintaining cellular homeostasis through the clearance of apoptotic cells and control of inflammatory and immune responses. Due to these and other important regulatory roles, dysregulation of TAM receptors is implicated in numerous disease states including cardiovascular disease, autoimmune disorders, chronic inflammation and cancer. TAM receptor mediated signaling pathways can also be hijacked by viruses to gain entry into host cells, and this includes a suspected role in SARS-COV-2 infection. As such, there is growing interest in developing therapies that target TAM receptors, but a lack of clarity on their multiple regulatory roles create challenges for developing therapeutic strategies. Understanding TAM receptor activation mechanisms is important not only for understanding their roles in disease but for increasing our understanding of the complexity through which RTKs are activated. It's become increasingly clear that the classic understanding

of RTK activation via ligand induced dimerization is not able to fully explain the complexity of signaling modulated by RTKs. My preliminary work with TAM receptors suggests that they are capable of ligand independent dimerization and I present the first structures of an intact TAM receptor extracellular domain, which gives some insight into potential activation mechanisms. In addition, I will introduce the audience to assays under development in my new lab to study the structural basis for TAM receptor activation and signaling. These studies aim to increase our basic understanding of how TAM receptors form bridging complexes between cells to activate signaling responses and facilitate apoptotic clearance.

ABS#482

Poster session, July 14

Spatiotemporal Heterogeneity of De Novo Lipogenesis in Huh7 Cells

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Proteins play crucial roles in modulating the metabolic pathways by up or down regulating in response to external stressors. De novo lipogenesis (DNL) is a highly regulated and vital metabolic pathway that is responsible for the production of the majority of lipids in liver and adipose tissue. The dysregulation of DNL has been associated with metabolic syndrome, cancer, and other pathological conditions. Thus, understanding rates and cellular control of DNL in living systems is necessary for tracking protein function as well as developing treatment methods and early stage detection of these diseases. However, DNL is challenging to track and label in vivo, and current techniques do not provide high spatial resolution. Optical photothermal infrared (OPTIR) microscopy addresses these obstacles by providing a label free and nonperturbative technique to obtain submicron resolution infrared imaging. Here, by feeding cancerous liver cells isotopically labeled glucose and free fatty acids, we track the incorporation of 13C carbons throughout DNL in both live and fixed cells using OPTIR. This allows us to observe the composition and morphology of the resulting lipid droplets and calculate rates of DNL. Comparison of DNL in live and fixed cells shows similar rates, yet differing morphology of the lipid droplets. The introduction of free fatty acids and disruptors to DNL and their effect can also be observed using OPTIR. With this work, we provide a thorough method of tracking this intricate metabolic process to make advancements in understanding altered protein function in cancer.

ABS#483

Poster session, July 13

Structural Insights into the Conformational Transition of a DNA Polymerase Clamp Loader from an Inactive to the Active State

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Processive chromosomal replication relies on DNA polymerases that attach to sliding DNA clamps, which are loaded onto primed DNA by clamp loader complexes. Clamp loaders are AAA+ ATPases, and the clamp loading process relies on synergistic interactions among the clamp loader, the clamp, and primed DNA to trigger ATP hydrolysis and conformational changes of the clamp loader complex.

Current structural information on the T4 bacteriophage clamp loading system is quite limited, partly due to the challenge of crystallizing such a dynamic molecular machine. In particular, capturing the T4 clamp loader complex in multiple conformational states has been unsuccessful so far.

To gain structural insights into the transition between different conformational states of the clamp loader complex, we used cryo-EM to investigate the T4 clamp-loader:sliding-clamp complex in the presence and absence of primer-template DNA. We presented a series of cryo-EM reconstructions of the clamp loader alone, clamp-loader:sliding-clamp complex, and clamp-loader:sliding-clamp:DNA complexes.

Unexpectedly, these results show that the clamp loader adopts a distorted and previously undefined inactive conformation when it is alone, or in a complex with the sliding clamp in the absence of DNA. Through comparison to the DNA-triggered active conformation of the clamp-loader:sliding-camp complex, we uncovered the molecular mechanism of the clamp loader complex in transition from the inactive to active state, which involves both global changes in the assembly of the complex as well as conformational changes in critical switches within the AAA+ modules.

ABS#484

Poster session, July 13

Enzyme Catalyzed Repair of Peptide Hormones

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GLP-1 is an essential peptide hormone that maintains glucose homeostasis by acting on its cognate receptor (GLP-1R) and promotes insulin release in a glucose-dependent manner. The hormone suffers from an extremely short half-life (≤ 2 mins) as it is cleaved by the ubiquitous serine protease dipeptidyl peptidase-4 (DDP-4). The removal of a dipeptide from the N-terminus (His-Ala) produces a shorter fragment that is a million fold less active than native GLP-1. We here report a series of small molecules that are capable of restoring the function of the deactivated endogenous hormone by running the protease in reverse. Judicious molecular design makes this restoration possible and paves the way for the design of orally bioavailable molecules that can restore DDP-4 inactivated substrates.

ABS#488

Poster session, July 15

Structure-Function Relationship of Human La-Related Proteins 1 and 6

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La-related proteins (LARP)s are a superfamily of RNA-binding proteins whose membership is dictated by the presence of a highly conserved La domain.(Maraia et al., 2017) In the vast majority of LARPs, this La domain forms the La module in tandem with a downstream ribonucleotide recognition motif (RRM). (Maraia et al., 2017) LARP1 plays a role in the posttranscriptional regulation of terminal oligopyrimidine (TOP) containing mRNAs. (Al-Ashtal et al., 2019) LARP6 marks a delineation from canonical RNA binding by recognizing a structured stem-loop in the 5' untranslated region of both type 1 collagen peptides A1 and A2.(Martino et al., 2015) hairpin structures in their respective mRNA targets, rather than single-stranded mono- or di-nucleotide repeats.(Eichhorn et al., 2018)
Here, we present the structural characterization of LARP1 and LARP6. It was previously suggested that only the entire La module consisting of La and downstream RRM domains was capable of RNA recognition. While this holds true for the majority of La modules, we show that both LARP1 and LARP6 deviate from this common principle and bind their cognate RNA targets in a non-canonical fashion using solution NMR spectroscopy.

ABS#489

Poster session, July 13

Specificity of Peptide Binding of Nontypeable Haemophilus influenzae Sensitivity to Antimicrobial Peptides Substrate-Binding Protein (SapA)

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Nontypeable Haemophilus influenzae (NTHi), commensal bacteria of the respiratory tract, are opportunistic pathogens that cause diseases including otitis media, chronic bronchitis, and community-acquired pneumonia. The sensitivity to antimicrobial peptide (Sap) ATP binding cassette (ABC) transporter, a large factor in NTHi virulence, is involved in resistance to antimicrobial peptides (AMPs) and heme transport, but its mechanism of substrate binding, selectivity, transport, and regulation are not well understood. Here we investigate the mechanism and specificity of peptide binding for the substrate binding protein SapA. Using surface plasmon resonance (SPR), the residues involved in AMP binding and the affinity for full length and truncated AMPs were determined. SapA was demonstrated to have high affinity for defensin AMPs. The conserved loop regions of hNP1, hBD2 and hBD3 were sufficient for SapA recognition and binding was independent of the loop structure. Spectral-shift binding assays revealed a minimal fragment in the loop region with comparable affinity to full length AMPs through comparison of the affinities of AMP fragments and single and double substitutions in the sequence. Overall, these studies reveal how SapA can establish specific interactions with charged regions in defensin AMPs while having lower affinity for other peptide substrates. Other peptide substrate binding proteins in NTHi, such as OppA and HbpA which have high sequence similarity

with SapA, bind peptides more indiscriminately (1). Comparison of the peptide binding residues in the binding cavity reveals how SapA but not related proteins bind AMPs. Understanding the mechanism of substrate binding in AMPs provides essential information for exploring how SapA evolved peptide specificity.

ABS#490

Poster session, July 14

An In-Vitro Investigation of Tau Protein Auto-acetylation

Andra-Elena Coşoreanu (1); Manuela-Diana Ene (2); Ioana-Mădălina Cărăuş (1); Ștefan-Eugen Szedlacsek (1)
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Tau proteins modified through pathological posttranslational modifications (hyperphosphorylation and more recently proposed, acetylation) represent well-known biomarkers for the detection of neurodegenerative diseases (such as Alzheimer's Disease).

The aim of this study is the evaluation of Tau protein auto-acetylation under in vitro conditions. As a main analytical tool for detecting Lys acetylation we used LC-MS/MS on Tau produced in a typical BL21(DE3)RIL E. Coli strain as well as an acetyltransferase-deficient one, BL21(DE3)ΔpatZ E. Coli.

Experiments employed eight Tau proteins: a wild-type protein, two simple mutants and one double mutant (Cys-to-Ala mutations at two Cys considered critical for auto-acetylation) for the full-length Tau and for a truncated Tau respectively, containing only the essential repetitive regions. We expressed all proteins in a prokaryotic system and purified using a boiling step and ion exchange chromatography.

A key question regarding auto-acetylation of Tau protein is whether it interacts with acetylcoenzyme A (AcCoA), the main acetyl group donor. To answer this question, we performed a pull-down assay using immobilized Coenzyme A (CoA), the backbone of AcCoA. The experiment evidenced that Tau protein is pulled down by CoA, thus supporting the preliminary asumption that Tau protein can auto-acetylate.

To comparatively explore the time dependence of global auto-acetylation of Tau protein in wild-type and Cys-to-Ala mutants, we developed an enzyme-coupled reaction assay. Our results show that the double mutation reduces tau auto-acetylation but it does not abolish it completely.

This study suggests that Tau auto-acetylation pattern is a complex process and the two Cys residues contribute to the outcome of Tau protein auto-acetylation.

Our results reported here represent a step forward in understanding the detailed molecular mechanism behind Tau protein autoacetylation.

ABS#492

Poster session, July 14

Extensive Sampling of Rigid-Body Protein Docking Methods Reveals Current Shortcomings in Protein-Protein Interaction Scoring Methods

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With the success of deep learning methods in monomeric protein structure prediction, protein-protein interfaces have now become the latest grand challenge in protein structure prediction. The simplest scenario in protein binding is that of rigid body docking, where the monomers do not change conformations significantly upon binding. While efficient computational methods exist to generate rigid-body interface models, it is unclear whether the current state-of-the-art interface scoring functions can accurately predict native structure. In this work, we consider a small set of high-quality heterodimers, generate a large number of rigid-body docking models for each heterodimer, and score them using several state-of-the-art scoring metrics. First, we find that a large number of diverse docking models need to be generated to properly quantify the accuracy of the interface scoring metrics. Second, after sampling a sufficient number of docking models, we find that the performance varies widely from target to target. Some targets are essentially ‘solved’ in the sense that there is a strong correlation betwen the scoring of the models and ground truth. In contrast, for other targets both high and low scoring models can be far from the native structure. We find that the difficulty of the target strongly correlates with the flatness of the target's interface. Identifying targets that remain challenging in the simplest case of

rigid body docking will allow us to develop key physical features that can distinguish true protein structures from incorrect computational models.

ABS#493

Poster session, July 13

Investigating the Structure, Function, and Regulation of Clp Proteases in Corynebacterium glutamicum

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Actinomycetota is an exceptionally diverse bacterial phylum, encompassing wide-ranging environmental species, as well as the industrial microbe Corynebacterum glutamicum and the human pathogen Mycobacterium tuberculosis. Actinobacteria possess conserved ATP-dependent Clp proteases that modulate cellular pathways through regulated protein destruction. Foundational studies in M. tuberculosis revealed unusual complexity in Clp protease architecture and regulation. However, actinobacterial Clp proteases are underexplored outside of mycobacteria, and it has been uncertain whether the M. tuberculosis enzymes are representative of the entire phylum. We sought to address this gap in our knowledge by interrogating the structure, function, and regulation of Clp proteases from C. glutamicum, which share >90% sequence identity with their mycobacterial orthologs. Using in vitro enzymatic assays, we found that C. glutamicum Clp proteases have broadly similar assembly and regulatory characteristics to those of mycobacteria. The ClpP1 and ClpP2 peptidase subunits are inactive individually, but form an enzymatically active ClpP1P2 tetradecamer in the presence of stabilizing antibiotics that mimic unfoldase contacts and N-benzyl peptide agonists that mimic substrates. Formation of active protease requires Clp unfoldases along with both ClpP1 and ClpP2. Notably, inclusion of peptide agonists did not increase the activity of the full protease, in contrast to the behavior of M. tuberculosis enzymes, suggesting higher stability of the C. glutamicum protease. Surprisingly, we also found that the ClpC1 unfoldase interacts with ClpP2 alone to form a proteolytically inactive complex, which may have implications on our understanding of Clp protease regulation within the cell. Our data suggest that the major functional paradigms of these enzymes are preserved across Actinomycetota, and that C. glutamicum is a tractable model for studying actinobacterial Clp protease regulation. It may ultimately be possible to harness our knowledge of Clp proteases to develop novel proteolytic circuits, useful for tuning C. glutamicum biosynthetic pathways to improve biomanufacturing efficiency and yield.

ABS#494

Undergraduate Research Session

Investigating the Role of Conserved Proline Residues in Human DNA Primase

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The C-terminal domain of human DNA primase (p58C) coordinates a redox-active [4Fe4S] cluster that is essential for DNA replication. The redox state of the [4Fe4S] cluster in p58C controls the binding affinity of primase for DNA, despite it being 25 Å away from the DNA binding site. Given this distance, some form of allosteric mechanism is required to communicate information between the two sites. We have sought to understand how the [4Fe4S] cluster's specific protein environment influences its redox properties. Of interest are three well-conserved proline residues, P285, P286, and P385, which, due to their proximity to protein elements required for DNA binding, we hypothesize may be involved in a proline "switch" mechanism. To investigate this, we have expressed and purified several site-directed mutants of p58C and have characterized their structure and DNA binding capabilities. We first demonstrate that P285 is likely required only for the structural stability of p58C, whereas P286 and P385 are not; rather, these residues appear to influence the overall electronic properties of the cluster. Finally, we discuss how mutation of P286 and P385 affects the substrate-dependent redox behavior of the [4Fe4S] cluster in p58C.

ABS#496

Poster session, July 14

Unraveling The Structure, Interactions And Dynamics Of Phosphorylated Bcl-xL Using Genetic Code Expansion

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Bcl-xL is an anti-apoptotic protein in the Bcl-2 family that interacts with pro-apoptotic proteins to regulate cytochrome-C release from the mitochondrial membrane. Dysregulation of this interaction network protects cancer cells from death signals and cytotoxic agents thereby promoting chemoresistance, a hallmark of cancer. The intrinsically disordered region (IDR) of Bcl-xL contains several post-translational modifications (PTMs) including a principle regulatory phosphorylation site serine 62 (pSer62). Cell-based studies suggest phosphorylation of pSer62 initiates apoptosis, but the underlying biochemical and functional mechanisms are not clear, largely stemming from our inability to produce pure Bcl-xL with authentic phosphoserine at this site. In this work, we overcome this challenge by employing genetic code expansion (GCE) to produce Bcl-xL with genetically encoded pSer62. Moreover, we use our recently developed methodology to generate wild-type and phosphorylated Bcl-xL isotopically labeled with 15N/13C and collected 2D and 3D nuclear magnetic resonance (NMR) analyses. Through complementary binding assays with known partner proteins and characterizations of changes in Bcl-xL protein dynamics induced by phosphorylation, we gain important insights into the regulatory role(s) and the mechanism through which phosphorylation regulates Bcl-xL and apoptosis. These insights will help development of therapeutic strategies that selectively target phosphorylated Bcl-family proteins.

ABS#498

Poster session, July 13

Structural implications of an HIV-1 second strand reverse transcription initiation complex +/- NNRTI for slow nucleotide incorporation and high sensitivity to NNRTI inhibition

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A major target of antiretroviral therapy for HIV-1 is the reverse transcriptase (RT) enzyme, which converts viral RNA into the DNA provirus, with nearly 50% of FDA approved HIV treatments targeting RT. Of these, non-nucleoside reverse transcriptase inhibitors (NNRTIs) are potent allosteric inhibitors of RT, but questions remain open about where and how they act in different stages of the viral lifecycle and reverse transcription.

Biochemical studies have shown that plus-strand initiation of reverse transcription (initiating "second strand" DNA synthesis) is particularly sensitive to inhibition by NNRTIs, with more modest inhibition occurring in minus-strand synthesis*. As such, our work investigates the structural features of a simplified plus-strand RT initiation complex (+miniRTIC) with and without an NNRTI (rilpivirine) to explore potential mechanisms that underlie these biochemical observations. Accordingly, we determined the first crystal structure of HIV-1 RT with a DNA template and a polypurine tract RNA primer, representing the +0 state of plus-strand initiation. Preliminary results show the +miniRTIC in the absence of an NNRTI shows hyperextended thumb and finger domains and a partially open NNRTI-binding pocket (NNIBP), with a displaced primer 3'-OH terminus, in a state poised for catalysis. The rilpivirine-bound structure captures a non-productive state with canonical NNRTI binding, and similarly hyperextended thumb and finger domains and displaced primer terminus. These structures provide new insights into the initiation state of RT, whereby the NNIBP more closely resembles an NNRTI-bound state, even in the absence of NNRTI, thus explaining increased susceptibility to inhibition. Further understanding of subsequent incorporation states may reveal new avenues for targeted therapy development.

ABS#499

Poster session, July 15

Gene therapy without the genes: Intracellular delivery of therapeutic proteins via traceless bioreversible esterification Strategy

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While the biological roles of proteins are being discovered at a remarkable pace, the number of FDA-approved biological drugs is significantly lower than that of small molecules. The power and impact of protein therapeutics are substantially undermined because of the fundamental limitation: proteins cannot spontaneously cross the membrane. Conventional delivery techniques fail to address this fundamental problem in that protein cargo is predominantly delivered into cells via endocytosis, leading to degradation pathways. Thus, the ability to modulate protein surface to interrogate factors important for cell

permeability is highly desired in both biological research and protein therapeutics. Addressing the fundamental limitation, we developed a bioreversible esterification strategy to endow proteins with the ability to directly enter the cytosol of human cells. Specifically, we show that the library of α-aryl-α-diazoacetamides can esterify carboxyl groups [1] in proteins, enabling their delivery across cellular membranes. The ensuing esters are substrates for intracellular esterases.

To further expand the utility of α-aryl-α-diazoacetamides in protein delivery applications, we developed a more general and modular probe for reversible protein modification [2]. Our probe consists of a diazo moiety for protein conjugation, a thiol-reactive group for late-stage functional diversification, and a self-immolative carbonate group to promote traceless release from the protein. We showed that our probe can generate a diverse set of protein conjugates modified with cell-penetrating peptides, targeting ligands, or PEG under mild conditions [3]. Our strategy represents a significant advance over previous reversible strategies because protein mutagenesis is not required, modifications are done under mild conditions, the probe is synthetically accessible, and most importantly, our strategy is potentially compatible with virtually any protein of interest. Therefore, this strategy implements a traceless means to deliver native proteins into the cytosol of live cells for applications in the laboratory and clinic.

ABS#501

Poster session, July 15

Rationally Designed Peptides Improve Cognition in Rats

Horea Stefan Szedlacsek (1); Dávid Bajusz (2); Rodica Aura Badea (1); Andreea Pop (1); Constantin Cătălin Bică (1); Lilla Ravasz (3); Dániel Mittli (3); Dominik Mátyás (3); Georgiana Necula-Petrăreanu (1); Cristian V.a. Munteanu (4); Ildikó Papp (3); Gábo
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Cognitive impairment and learning ability of the brain are directly linked to synaptic plasticity as measured in changes of long-term potentiation (LTP) and long-term depression (LTD) in animal models of brain diseases. LTD reflects a sustained reduction of the synaptic AMPA receptor content based on targeted clathrin-mediated endocytosis. AMPA receptor endocytosis is initiated by dephosphorylation of Tyr876 on the C-terminus of the AMPAR subunit GluA2. The brain-specific striatal-enriched protein tyrosine phosphatase (STEP) is responsible for this process. To identify new, highly effective inhibitors of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) internalization, we performed structure-based design of peptides able to inhibit STEP−GluA2-CT complex formation. Two short peptide derivatives were found as efficient in vitro inhibitors. Our in vivo experiments evidenced that both peptides restore the memory deficits and display anxiolytic and antidepressant effects in a scopolamine-treated rat model. The interference peptides identified and characterized here represent promising lead compounds for novel cognitive enhancers and/or behavioral modulators.

ABS#504

Poster session, July 15

Direct Determination of Hierarchical Organization of Membrane Proteins and Lipids Using a Tunable Lipid Bilayer NativeMS Platform

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The organization of membrane proteins (MP) and lipids in the cell membrane is crucial for all living organisms. Detecting and analyzing these complexes is challenging and demands a platform that can directly identify them from their lipid bilayer environment. To address this need, we have developed a tunable in-vitro nativeMS platform that allows detection of MPs and bound lipid complexes directly from membranes. This platform can be customized to mimic any target physiological membrane, including its lipid composition and biophysical properties, such as curvature, fluidity, and tension. The platform's general feasibility has been established by detecting a range of oligomeric MPs directly from membranes mimicking bacterial membrane, plasma membrane, mitochondrial, endoplasmic reticulum, Golgi-like

membranes, and lipid bilayer with varying degrees of membrane curvature and tension. We demonstrate broad applicability of the platform by targeting a range of MPs, with different masses, oligomeric states, and numbers of transmembrane helices. Next, we applied this in-vitronativeMSplatform to understand how VAMP2-lipid interactions regulate neurotransmitter release from synaptic vesicles (SV). Direct high-resolution nativeMS and top-down MS/MS analysis of the SV membrane revealed that VAMP2 specifically binds to phosphatidylcholine (PC) and cholesterol. The same vesicles which were used for nativeMS were then used in a fluorescence-based fusion assay to demonstrate how the specific binding of PC regulates the timescale of neurotransmitter release at neuronal synapses. We subsequently used this platform to study the bacterial sugar transporter protein, semiSWEET. Leveraging on the tunability of the platform, we show that an increase in cardiolipin percentage in the membrane leads to a direct increase in the functional oligomeric population of the protein. Overall, the in-vitro bilayer nativeMS platform enables the direct determination of molecular organization of proteins and lipids in membranes and further enables quantitative evaluation of how specific membrane components and properties regulate these observed organizations.

ABS#505

Poster session, July 15

Applying ProteinMPNN, RFDiffusion & ColabFold tools for the generation & optimisation of useful protein-tools

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Protein-based tools require proteins that are thermostable & functional. Here, we focus on applying recently-released machine-learning-based protein design tools (including ProteinMPNN, AlphaFold Design and RFDiffusion) to generate new-to-nature protein sequences that are experimentally characterised in the lab using biophysical & structural (e.g. X-ray crystallography) approaches. We assess the applicability of these open-access tools for the generation of well-behaving protein-based tools by applying them to several common protein engineering problems (e.g. the design of a protein-binder, generation of hetero-oligomers, small-molecule binding proteins) using model proteins from diverse protein families (inc. Protein A for binding of IgGs, periplasmic binding proteins, and enzymes). This work is further complemented with computational modelling studies (e.g. Schrodinger, AF2) to further investigate the structure, function & properties of the designed proteins.

ABS#506

Poster session, July 15

Dynamically driven allosteric inhibition of flavivirus protease

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Zika virus (ZIKV) is a mosquito-borne virus which belong to the flavivirus family. It was found to be associated with congenital abnormalities in the developing fetus and newborns. ZIKV expresses a virally encoded protease which has been the target of antivirals because of its role in the viral life cycle. Structure of Zika virus protease (ZVP) consists of the NS3 protease (NS3pro) region and the NS2B region which is required for stability and proteolytic activity of NS3pro. NS2B region, in solution, has been shown to be dynamic and exist in open and close conformation. From high-throughput screening, we have discovered compound MH1 which inhibits Zika virus protease (ZVP) in low micromolar range (IC50= 0.45μM). Inhibition assay shows that MH1 binds allosterically and has a unique ability to distinguish among flavivirus proteases including Dengue (IC50= 22μM) and West Nile virus (IC50= 8μM). MH1 is also observed to inhibit cytopathic effects in Zika virus infected cells. Differential scanning fluorimetry and inhibition assays of MH1 against hybrid enzymes such as ZVP (DVP2 NS2B 48-100) and DVP2 (ZVP NS2B 48-100), where we paired up NS2B region of one flavivirus protease with another NS3pro, suggests that our inhibitor binds mainly at the NS3pro region but requires NS2B for its full potency. To further probe into this, we collected NMR HSQC spectra of WT ZVP and several hybrid constructs that locks the protease in close and open conformation. Our NMR data shows that MH1 binds to the NS3pro region, and the accessibility of the binding site is dictated by the flexibility of the NS2B region. Upon binding, MH1 blocks NS2B and

NS3 interaction which is necessary for proteolytic activity.

ABS#508

Aggregates, Amyloids, or Condensates? (July 16, AM)

Cis-Gatekeeper: Isomerization of A Single Proline Governs TDP-43 Aggregation

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TDP-43, a DNA/RNA binding protein, plays broad roles in transcriptional repression, mRNA splicing and translational regulation, and its aberrant aggregation is heavily involved in neurodegenerative diseases such amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Revealing its aggregation mechanism is the key to understanding its pathological roles and developing therapies. Here, we used DAmFRET and atomistic molecular simulations to study the effects of over 100 rational mutations on TDP-43 amyloid formation in cells. We found that the conformation of a single proline at residue 363 (P363) profoundly influences amyloid nucleation by TDP-43. Specifically, any mutation to this residue --regardless of its physicochemical properties --massively accelerated aggregation. This suggests that it is proline's unique ability to populate a cis isoform that prevents aggregation, and further, that that isoform does so in a dominant fashion as the great majority (~90%) of molecules instead contain the highly amyloidogenic trans isoform. By conducting the largest atomistic simulations yet undertaken for a disordered protein, we confirmed that the cis isoform of P363 profoundly changes the global conformational ensemble of TDP-43, while prolines at other positions had only minor effects. This finding is exciting because it provides a promising mechanistic link between TDP-43 aggregation and the greatest genetic risk factor for ALS: a hexanucleotide repeat expansion at C9ORF72. The latter causes an aberrant accumulation of proline-arginine dipeptide repeat polypeptides, which have been shown to inactivate the major prolyl isomerase, PP1A, which functions to accelerate trans-to-cis isomerization of proline residues. Genetic ablation of PP1A was independently shown to induce TDP-43 pathology in mice. Together with our new findings, these observations allow us to propose that TDP-43 pathology is intimately linked to the cis/trans equilibrium of P363.

ABS#513

Peptide Modalities: Size Doesn't Matter (July 14, AM)

More contacts, copies, and stability - optimising efficacy of a PCSK9 peptide inhibitor

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Like many therapeutic targets, design of inhibitory compounds is challenging because the target binding sites are flat and featureless. This is certainly the case for the serum protein PCSK9, a validated targeted for lowering cholesterol levels [1]. Thus, strategies are needed to design and increase efficacy from in vitro to in vivo stages of investigation.
Here, we investigated three strategies for optimisation of peptidic drug leads of PCSK9, including: (i) bioactive cyclisation (to increase stability and activity by increasing the contact surface) [2], (ii) increasing valency (to capture effects of avidity) [3], and (iii) increase in vivo half-life (to provide a long-lasting reservoir of inhibitor peptide and thus extend the active window) [4]. Peptides were designed using a combination of computational, phage library screening and chemical synthesis. Subsequently, structures were characterized by NMR and MD, binding characterized by SPR and ITC, inhibition of PCSK9:LDLR interaction by competition ELISAs and on-cell Westerns, effect on cellular metabolism of cholesterol by using dye-labelled lipids, and in vivo activity using a model of hypercholesterolemia. Peptides were in the pico- to nanomolar range.
The success of the strategies employed in this work suggest they could be broadly applicable to other protein targets, which we identified using a bioinformatic screen of all protein structures, that remain underexplored due to their challenging topological nature.

ABS#514

Poster session, July 15

Structure of a Pentavalent Ubiquitin Ligase Complex

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Cullin3-Ring-Ligases (CRL3s) are a large, modular class of E3 ligases that include BTB domain substrate receptors. Notably, BTB domains can self-associate into stable dimers, pentamers and oligomers and thus drive the multimerization of CRL3 complexes. Proteomic studies have identified KCTD5, a pentameric BTB CRL3 substrate receptor, as an interactor of Gβγ, suggesting that this substrate receptor can promote the ubiquitylation of G protein subunits via a CRL3(KCTD5) complex. Here, we report the structure and functional characterization of a CRL3(KCTD5)/Gβγ E3 ligase complex.
Methods: We used in vitro binding and ubiqutination assays to characterize the interactions between CRL3(KCTD5) and Gβγ and cryo-electron microscopy to determine the structure of the CRL3(KCTD5)/Gβγ complex.
Results: We show that KCTD5 modulates Gβγ levels in cells and that a CLR3(KCTD5) complex ubiquitinates a Gβγ heterodimer in vitro. The cryo-EM structure of a KCTD5/CUL3/Gβγ complex reveals that KCTD5 forms a central pentameric scaffold that simultaneously engages five CUL3 subunits via its N-terminal BTB domain and five Gβγ substrates via its C-terminal domain (CTD) into a 560 kDa 15-chain assembly. Internal flexibility is a common feature of E3 ligases, and large-scale rotational dynamics between the BTB/CUL3 and CTD/Gβγ moieties of the 5:5:5 supercomplex generate multiple poses of Gβγ relative to CUL3. We used the experimental structure to model the full reaction complex and show that the internal rotations in KCTD5 generate conformers in which specific lysines of Gβγ are positioned near activated E2∼ubiquitin held by the neddylated CUL3/RBX1 domain, and likely represent the priming complex.
Conclusions: The cryo-em structure of a pentameric CRL3KCTD5/Gßγ complex demonstrates the role of multivalency in the CRL3 ligases and reveals how the architecture and dynamics of an E3 ligase can position a structured target for ubiquitylation.

ABS#517

Modern Anti-viral Strategies (July 13, AM)

Wastewater viral surveillance using modified porous protein microcrystals

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Current gold standard assays for nucleic acid surveillance are predominantly quantitative PCR (qPCR) based, the best of which demonstrate limits of detection (LoD) as low as ∼100 copies of nucleic acid per milliliter of sample. Nevertheless, considerable variation in the limits of detection for approved assays can result in a substantial increase in false negative rates for every 10-fold increase in LoD. Therefore, any upstream method that can concentrate very small quantities of virus from a large volume of sample can dramatically reduce the rate of false negatives, while simultaneously increasing the limits of detection for less sensitive assays. Porous protein microcrystals grown from a Campylobacter jejuni protein ("CJ") possess a number of unique structural characteristics which make them amenable as a scaffold for capturing viruses and environmental nucleic acids; these include the feasibility of attaching affinity peptides at specific locations within the crystal, along with their capacity to strongly adsorb guest RNA and DNA. As a proof of concept for using the surfaces of crystals for virus capture, we have shown that biotinylated CJ crystals are able to selectively capture streptavidin-coated microspheres. We have further demonstrated successful installation of an ACE2 peptide mimic within CJ microcrystals. In vitro testing of capture of a fluorescently-labeled SARS-CoV-2 spike receptor binding domain (RBD) is ongoing. This platform will be integrated into a pump housing and upstream tangential flow filter to allow continuous scavenging and concentration of potentially very small quantities of virus or environmental nucleic acids, from several liters or more of commercial wastewater, over a 24-hour period.

ABS#519

Poster session, July 13

Improved Protein Crystal Production via Microfluidic Flow Cells

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The design of microfluidic devices for protein crystallization involves precise engineering of flow channel geometry, which allows optimization of protein crystallization conditions. Varying flow channel design motifs were tested in pursuit of a device to facilitate the highly

reproducible growth of protein crystals with a tight size distribution. Reproducible protein microcrystal growth serves to increase the efficiency of protein crystal manufacturing. Here, we tested the devices for the growth of protein crystals with unusually large solvent channels (13 nm diameter). The resulting crystals have advantageous properties as scaffold materials such as the uptake and storage of synthetic information-bearing DNA to create edible barcode particles. [1] Similar crystals could be optimized for the capture of specific partner molecules from the environment for biosurveillance applications. In both cases, reliably producing protein crystals with uniform, tailorable size is important.

As a nucleation and growth phenomena, protein crystal growth is highly sensitive to solution conditions. Increasing Reynolds number results in increased turbulence and enhanced mixing, leading to improved mass transfer. The flow channels within the microfluidic device were designed to induce specific nucleation and growth conditions along the length of the channel by altering the Reynolds number with different channel geometries. Simple laminate microfluidic devices were designed in Fusion360 and cut with a commercial Glowforge laser cutter. Assembling devices from thin acrylic sheets provided a flexible and durable platform that is easy and relatively cheap to manufacture. This construction method enabled complex 2-dimensional geometries and simple 3-dimensional geometries in single and multilayer devices, providing fine tunable control over the protein crystallization process.

ABS#520

Poster session, July 13

Elucidation of the Photoinduced Hydrogen Evolution Reaction of Metalloprotein Co-cytochrome b562 by Employing Directed Evolution

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Metalloproteins are proteins with a two-spherical system that contain a metal ion cofactor at the center. The surrounding protein shell when accommodating a metal cofactor shows profound effects on the catalytic activity of the center. Co-cytochrome b562, a metalloprotein, shows catalytic activities such as photoinduced proton reduction to form hydrogen, and CO2 reduction. However, the preparation of Co-cytochrome b562 typically requires isolation of the apoprotein, and in vitro incorporation of the cobalt-substituted cofactor. Very recently, it has been demonstrated that the direct expression of non-natural metal cobalt (Co) substituted metalloproteins (Myoglobin) is possible using E. coli BL21 (DE3) grown in an iron-limited but CoCl2-supplemented minimal medium (ref 1). In this study, the biosynthesis of Co-cytochrome b562 is observed using similar bacterial cells and growth conditions. While the substitution of the metal center can be very useful to expand the functionality of metalloproteins, the most expansion can be achieved from the modulation of amino acids of the apoprotein counterpart. Previously our research group showed that hydrogen production increases 2.5 folds in Co-cytochrome b562 (reconstituted in vitro by swapping Fe with Co) by interfering with the protein coordination sites of the cofactor CoPPIX (ref 2). In the current study, we will utilize directed evolution to explore the role of amino acid manipulation of the outer sphere (apoprotein) in the photoinduced hydrogen evolution reaction. We made a cytochrome b562 library by random mutagenesis and developed an assay (Pd/WO3 sensor) to screen the library to observe its hydrogen production capabilities. High-yielding variants of the library will be further assessed by gas chromatography. Debriefing the interactions between cofactor and protein by employing directed evolution will provide insights into the Co-cytochrome b562 structure, catalytic activity, and spectroscopic properties. We expect to understand metalloprotein's intrinsic activity due to changes in the long-range interactions by protein engineering.

ABS#521

Poster session, July 15

Using Martini 3, Alphafold2 Multimer and solution NMR to Understand the Structural Basis of EphA2 Dimerization and Domain-Membrane Interactions of the Transmembrane- and Membrane Proximal Regions

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EphA2 plays a critical role in cellular growth, differentiation and motility. EphA2 overexpression is reported in several different cancer types, making it a biomarker as well as target for therapeutic approaches for this group of diseases. In EphA2 ligand binding shifts the monomer-

dimer equilibrium thought stabilization of the dimeric state following a conformational change in the extracellular domains. For the signal to cross the lipid bilayer, however, dimerization of the single transmembrane (TM) region is a key regulatory step [1,2]. In EphA2, the juxtamembrane (JM) region follows the TM domain at one end and connects to a catalytic domain at the other end on the intracellular side of the protein. Other signaling molecules specifically PIP2 and PIP3 utilize basic amino acids in the JM domain for binding and occlude the nearby region from phosphorylation. Similar protein-membrane interactions were reported for the first two extracellular fibronectin domains of EphA2.

While working with membrane proteins is known to be extremely challenging, the recent developments in molecular modeling (Alphafold2) and dynamics simulation (coarse grained Martini 3) have used these powerful in silico tools to study membrane proteins and their interactions [3].

We have now applied the same two computational approaches to longer protein segments which encompass the two membrane proximal fibronectin domains (FN1+2), the TM as well as the JM region. Very recently we have been successful in obtaining complete solution NMR spectra of such a protein construct in presence of detergents or in bicelles. To our knowledge this is one of the first NMR studies of a single membrane crossing protein with an extensive intra- and extra- cellular segment. Both approaches point to the presence of significant contacts between not just the TM, but also the FN and the JM domains.

ABS#522

Poster session, July 14

Deciphering the Interaction Mechanics of Aβ (1-42) Amyloid Precursors and Cell Membrane in the presence of Insulin using Dielectric Relaxation Spectroscopy

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Recent research suggest that Alzheimer's disease (AD) and Type II Diabetes (TD2) are linked, and insulin resistance has been implicated as a risk factor for developing AD. There is evidence that both diseases are initiated by the production, aggregation, and deposition of amyloid fibrils in the brain and respectively pancreatic tissue. Prior to amyloid fibril formation, the amyloid precursors, such as the monomers, dimers, oligomers, and the protofibrils perturb the cell membrane integrity leading to its death; however, the molecular mechanism by which the amyloid precursors interact with the cell membrane is not deciphered. We used Dielectric Relaxation Spectroscopy to investigate (1) the aggregation of the amyloid Aβ (1-42) proteins responsible for AD in the presence of insulin and (2) the interaction of the oligomeric-insulin incubated Aβ (1-42) with the cell membrane of neuronal cells. We initially incubated the Aβ (1-42) amyloid monomers and oligomers with insulin and further the neuronal cells were incubated with the Aβ (1-42) solution of monomers, oligomers, and fibrils in the presence and absence of insulin. We measured the dielectric response of the insulin-incubated amyloid precursors and cells at different concentrations over a wide range of frequencies (from 10-2 Hz to 107 Hz). Two dispersion processes (α, β) can be observed for all concentrations of the cell suspensions. Further analysis of this dispersion processes suggested that the interaction of Aβ (1-42) oligomers and fibrils with neuronal cells induces a change in the cell membrane permittivity and the conductivity of cytoplasm. When Aβ (1-42) fibrils were incubated with insulin the changes in the cell membrane permittivity and conductivity were suppressed. This suggests the toxicity of the precursors causing marked alterations in the electrical properties of the cell membrane. Our results are consistent with previous microscopy and computational studies of amyloid interaction with cell membrane.

ABS#523

Poster session, July 15

A Scale-dependent Definition of an Atomic Resolution Criterion (ARC) for CryoEM

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A Scale-dependent Definition of an Atomic Resolution Criterion (ARC) for CryoEM

Recent technological and computational advances in cryogenic electron microscopy (cryoEM) methodology

have significantly furthered our ability to determine the atomic structures of proteins and other biomolecular complexes. These advances have been driven by an increase in the quality of experimental cryoEM density maps, which consequently have increased the precision and accuracy with which the atomic coordinates comprising molecular models may be determined from those maps. This improvement in the quality of density maps has been hailed as the cryoEM 'resolution revolution.' Yet, these advances have also been coupled with debate about the 'resolution' of the cryoEM density maps, and how the reported resolution relates to the actual spatial information concerning the individual atoms in these maps. Related to this question of resolution, researchers are concerned with what is the quality of the structural model that is inferred from such experiments, and how grounded are the assignments of atomic coordinates in the experimental evidence. Here, we develop an atomic resolution criterion (ARC) based upon the hierarchy of biomolecular structure (e.g., primary, secondary, etc.) to show how these two questions are intimately related. By using this ARC to determine the length-scales at which different structural features are best described in a cryoEM density map, we use probabilistic inference to develop a machine learning approach capable of perceiving local atomic resolution within the experimental cryoEM data. Using this approach, we have investigated the local atomic resolution for all of the cryoEM density maps and structures available in public databases, and performed a global analysis of common factors affecting biomolecular structure quality.

ABS#527

Poster session, July 14

Structural And Dynamical Basis for the Interaction of HSP70-EEVD with JDP Sis1

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Protein refolding and disaggregation are facilitated by the binding and releasing activity of HSP70, which is aided by J-domain proteins (JDPs, DNAJs, or HSP40s) that stimulate the ATPase activity of HSP70 and stabilize complexes between HSP70 and nonnative proteins. We investigated by NMR the essential interaction of the C-terminus EEVD motif of HSP70 with the class B JDP (Sis1). We demonstrated multiple, specific, and transient interactions of a-synuclein (client) and EEVD with the full lenght Sis1 (70 % of the resonances assigned). The unique dynamical feature of Sis1 combined with EEVD/client interaction at multiple sites created the necessary environment for the transfer of the client to HSP70. We proposed models taking into consideration the competing and simultaneous binding of the client protein and EEVD to the globular domain CTDI and the intrinsically disordered region GF/GM, which may generate entropic forces to orient the transfer of the client protein to HSP70. The new understanding arose from the dynamics and multiple interaction of EEVD with CTDI, competing and displacing the client (site II) and anchoring the ternary complex HSP70/Sis1/client (site I). EEVD participated in the autoinhibition of J-domain by a-helix 6, through the interaction with GF and J domain, possibly regulating the recognition of the J-domain to HSP70 stimulating ATPase activity.

ABS#529

Poster session, July 13

Characterization of TRPM8 Ligand Binding and Temperature Sensitivity Using 19F-NMR

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The transient receptor potential melastatin member 8 (hTRPM8) is a polymodal ion channel that functions as a primary cold sensor in humans and is also activated by endogenous ligands and exogenous cooling compounds such as menthol and icilin. TRPM8 is an attractive drug target that has been implicated in various diseases, including chronic pain, obesity, and cancer progression. However, no drugs have passed clinical trials because of severe on-target side effects such as hypothermia. Developing effective TRPM8 targeting drugs requires understanding the underlying molecular mechanism for ligand and temperature activation and the allosteric coupling

between activation modes. Structural and functional have shown TRPM8-VSLD is important for chemical binding, while regions important for temperature sensing remain controversial. In this study, we use 19F NMR to study isolated human TRPM8-VSLD to characterize ligand binding, temperature sensitivity, and allosteric coupling. Our data shows that isolated hM8-VSLD recapitulates full-length binding affinity and contributes significantly to the overall full-length channel thermosensitivity. Importantly, our results show that conformational dynamics of ligand bound protein state, as assessed from 19F labeled hM8-VSLD, can be leveraged to provide chemometric analysis which also correlates with cellular functional studies.

ABS#531

Poster session, July 15

Directing Protein Design Choices by Per-Residue Energy Breakdown Analysis With an Interactive Web Application

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Recent developments in machine learning have greatly facilitated the design of proteins with improved properties. However, accurately assessing the contributions of an individual or multiple amino acid mutations to overall protein stability to select the most promising mutants remains a challenge. Knowing the specific types of amino acid interactions that improve energetic stability is crucial for finding favorable combinations of mutations and deciding which mutants to test experimentally. In this work, we present an interactive workflow for assessing the energetic contributions of single and multi-mutant designs of proteins. The energy breakdown guided protein design (ENDURE) workflow includes several key algorithms, including per-residue energy analysis and the sum of interaction energies calculations, which are performed using the Rosetta energy function, as well as a residue depth analysis, which enables tracking the energetic contributions of mutations occurring in different spatial layers of the protein structure. ENDURE is available as a web application that integrates easy-to-read summary reports and interactive visualizations of the automated energy calculations and helps users selecting protein mutants for further experimental characterization. We demonstrate the effectiveness of the tool in identifying the mutations in a designed polyethylene terephthalate (PET)-degrading enzyme that adds up to an improved thermodynamic stability. We expect that ENDURE can be a valuable resource for researchers and practitioners working in the field of protein design and optimization. ENDURE is freely available for academic use at:www.endure.kuenzelab.org

ABS#532

Structure Prediction and Design (July 15, AM)

Towards Structure Prediction and Design of Disordered Proteins

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Intrinsically disordered proteins (IDPs) are pervasive across proteomes but defy the sequence-structure-function relationships that we have come to learn for folded proteins. By populating a diverse set of transiently formed structures, IDPs help shape biological functions and are involved in numerous diseases. Structural studies of disordered proteins generally rely on combining molecular simulations with experimental data to correct for intrinsic biases in simulation methods. Such studies have provided a wealth of insights for individual IDPs, but do not provide information about the structural properties for proteins that have not been characterized experimentally. As a supplement to these one-by-one studies of IDRs, we have recently taken a different approach where we used experimental data on more than 50 different proteins to learn a coarse-grained molecular energy function to predict conformational properties of IDPs. By globally optimizing a transferable model, called CALVADOS, we can study the conformational ensemble of an IDP in the absence of experimental data. I will describe the Bayesian formalism we developed and applied to parameterize CALVADOS by targeting data from small-angle X-ray scattering experiments and nuclear magnetic resonance spectroscopy on IDPs in solution. I will describe how this model enables us to study interactions within and between IDPs in biomolecular condensates. As examples of applications, I will first describe how we can perform large-scale simulations to explore the relationship between sequence, structure, and function of IDPs. I will then describe results showing how we can use the information encoded in CALVADOS to design disordered proteins with desired conformational properties.

ABS#536

Proteins in Motion (July 15, AM)

Perturbing Protein and Ligand Conformational Landscapes to Link Dynamics and Function in Protein Tyrosine Phosphatases

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Protein function hinges on dynamic shifts of tertiary structure, but it remains challenging to map protein conformational landscapes at high resolution using traditional approaches in structural biology. Our lab leverages avant-garde computational and experimental methods to illuminate protein structural excursions to excited states, and explores their fundamental connections to biological functions including ligand binding, enzyme catalysis, and allostery. First, for the archetypal protein tyrosine phosphatase (PTP), PTP1B, we report the largest crystallographic ligand screen to date for any protein at room temperature, in contrast to >94% of protein-ligand crystal structures which were solved at cryogenic temperature. This trove of structural data reveals that temperature modulates ligand binding pose, solvation, location, and allosteric protein response (Mehlman, eLife, 2023). Having shown that temperature modulates protein and ligand conformational ensembles, we next sought to contrast its effects to those of a complementary biophysical perturbation that has been under-explored by crystallography: pressure. Using a relative of PTP1B, the idiosyncratic PTP STEP, we quantitatively explore the temperature and pressure axes with respect to protein structure. Our results reveal that high temperature vs. high pressure have different effects on global unit cell and protein volumes, ordered solvation patterns, and protein conformational ensembles, with each perturbation stabilizing distinct aspects of active-like STEP states. Finally, building on fragment-bound structures of PTP1B, we thoroughly characterize AI-designed novel allosteric small-molecule modulators targeting a previously underexplored but validated allosteric site. Our results reveal distinct classes of new ligands, including both high-affinity benign binders and allosteric inhibitors. Using advanced high-resolution local HDX-MS experiments, we demonstrate that different PTP1B ligands have surprisingly far-ranging and distinct patterns of allosteric effects on protein conformational dynamics. Overall, our lab's research highlights the importance of moving beyond the single-structure paradigm to build a more fundamental understanding of dynamic protein function.

ABS#537

Poster session, July 13

Impact of the Leucine Zipper on the DNA Binding in the Human Transcription Factor FoxP1

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Transcription factors (TF) are complex, multidomain proteins with specific DNA binding and regulatory domains. For the case of the human Forkhead box P subfamily (FoxP) of transcription factors, this becomes relevant, as they possess a bifunctional domain (Forkhead, FKH) that allows DNA-binding and dimerization that is connected to a canonical Leucine Zipper (ZIP) by a large, disordered region (linker). However, the role of the ZIP domain in the DNA-binding process is unknown. We employed ensemble and single-molecule fluorescence spectroscopy to analyze the binding affinity and impact of the DNA on the ZIP-linker-FKH and FKH domains. Even when our ensemble findings indicate that the DNA affinity does not change with the addition of the ZIP domain, single-molecule anisotropy shows that the structural impact of the DNA on the FKH domain significantly varies when the ZIP domain is present. We showed that DNA promotes structural alterations on both FKH and ZIP domains that promote the monomeric FKH while allosterically stabilizing the dimeric ZIP domain.

ABS#539

Poster session, July 13

Structure studies of IMP-specific phosphatase ISN1 from Saccharomyces cerevisiae

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Nucleotide metabolism involves purine nucleotide degradation and produces urate via formation of the

nucleobase xanthine. The end product, nitrogen-rich urate, is excreted by human and primates, but is fully catabolized to glyoxylate, carbon dioxide, and ammonia by the ureide pathway, which is conserved in plants, many bacteria, and fungi. Inosine 5'-monophosphate (IMP) is an intermediate in the catabolism. In parallel to the catabolic fate of IMP, IMP production from inosine by the salvage reaction was identified in Escherichia coli and A. thaliana. Accordingly, an interconversion between IMP and inosine might be metabolic process involved in the regulation of nucleotide metabolism. Currently, IMP-specific phosphatases, which are responsible for inosine production in catabolism, are unknown, except for IMP-specific 5'-nucleotidase 1 (ISN1). ISN1 from Saccharomyces cerevisiae (ScISN1) catalyzes the dephosphorylation of IMP to inosine. In this presentation, we will report our progresses on the structural and biochemical studies of ScISN1. These regulatory and catalytic features of ScISN1 are unusual, mainly due to sequence and structural variations. Our findings provide structural and biochemical insight into the allosteric regulation of enzymes of the ISN1 family.

ABS#540

Poster session, July 13

Conformational restriction shapes the inhibition of a multidrug efflux adaptor protein

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Increasing antimicrobial resistance is one of the biggest, current threats to global human health. Tripartite membrane efflux pumps play a major role in bacterial multidrug resistance, thus finding approaches to inhibit these efflux pumps is critical to 'revive' current classes of antibiotics and restore efficacy, decreasing the burden of discovery for new drugs. Understanding the structural dynamics of protein systems is essential for the design of novel inhibitors against medically relevant targets. In this work, we deploy a range of chemical biology techniques to uncover the first mechanism of inhibition against periplasmic adapter protein AcrA, which is part of the AcrAB-TolC multidrug efflux pump. The AcrAB-TolC pump is a member of the resistance nodulation and cell division (RND) superfamily and is native to Escherichia coli with homologs across other ESKAPE bacteria. Hydrogen deuterium exchange mass spectrometry of AcrA allows us to define its intrinsic dynamics and reveals that the NSC 60339 inhibitor can inflict a long-range stabilisation across all four domains. Alongside molecular dynamics simulations and cellular efflux assays, our results suggest a model where NSC 60339 binds to AcrA in a cleft between the lipoyl and αβ domains and acts as a molecular wedge to restrict AcrA's structural dynamics to affect the efficiency of efflux. This work reveals a new approach to targeting periplasmic adaptor proteins in multidrug efflux systems.

ABS#541

Poster session, July 13

Ribosomal protein engineering enables previously inaccessible single-molecule fluorescence studies of translation

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During protein synthesis, the ribosome translates the triplet-nucleotide codon sequence of a messenger RNA (mRNA) into the encoded protein product. To do so, the ribosome, a multi-megadalton ribonucleoprotein machine consisting of a large and small subunit, undergoes large-scale structural rearrangements that drive and regulate translation. One such rearrangement is a rotation of the small subunit 'head' domain with respect to the 'body' domain termed head rotation. Comparative structural analyses of ribosomal complexes suggest that head rotation plays an essential role during all stages of translation. Consequently, mutations and small-molecule inhibitors that disrupt head rotation are frequently lethal to cells. Despite the importance of this structural rearrangement, it has not yet been possible to

determine how the timing and kinetics of head rotation contribute to the various mechanistic steps of translation in which it plays a role. Indeed, the results of previous single-molecule fluorescence resonance energy transfer (smFRET) studies of head rotation have been difficult to interpret due to the limited residue positions within the ribosome that could be specifically labeled with FRET donor and acceptor fluorophores using conventional approaches. Thus, existing smFRET signals simultaneously report on two or more structural rearrangements, preventing the unambiguous characterization of head rotation. To overcome these limitations and ambiguities, we have used genome engineering, non-canonical amino acid mutagenesis, and bioorthogonal chemistry to label the small subunit with donor and acceptor fluorophores at residue positions expected to report directly on head rotation independent of other structural rearrangements. Using such a dual-labeled small subunit, we have obtained proof-of-principle ensemble FRET and smFRET results consistent with structural studies. Having developed and validated this fluorophore labeling strategy, we are currently generating a series of dual-labeled small subunit constructs to identify those that yield optimal smFRET signals, in terms of signal-to-background and photophysical properties, while retaining full biochemical function.

ABS#543

Poster session, July 14

Key Role of an Alpha-helical Lid in Driving the Target DNA toward Catalysis in CRISPR-Cas12a

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CRISPR-Cas12a is a powerful RNA-guided genome-editing system that came to the limelight not only as a genome-editing scissor, but also as a robust nucleic acid detection tool. It generates double-strand DNA breaks using its single RuvC nuclease domain by a sequential mechanism in which the initial cleavage of the non-target strand is followed by target strand cleavage. How the spatially distant DNA target strand (TS) traverses toward the RuvC catalytic core is presently not understood. Here, continuous tens of microsecond-long molecular dynamics and free-energy simulations reveal that an α-helical lid, located within the RuvC domain, plays a pivotal role in the traversal of the TS. It anchors the crRNA:TS hybrid and elegantly guides the TS toward the RuvC core, as also corroborated by DNA cleavage experiments. In this mechanism, the Rec2 domain drives the DNA target strand toward the RuvC catalytic cleft, owing to concerted motions with the Nuc domain. While the REC2 domain pushes the TS inward into the core of the complex with its short alpha helices, the Nuc domain aids the bending and accommodation of the TS within the RuvC core by bending inward. Alchemical free energy simulations revealed F1010 as the most significant residue responsible for the accommodation of the TS at the catalytic site, which mutating into alanine led to a precipitous drop in the DNA cleavage assays. Taken together, our findings along with the available structural and biochemical data, we propose a model for the traversal of the DNA TS strand toward the RuvC core for cleavage. The identified intermediates provide information on the critical residues involved in the biophysical process, holding promises for future engineering strategies aimed at improving the overall Cas12a activity.

ABS#544

Poster session, July 13

Determining Mechanisms of Membrane Recruitment of Peripheral Membrane Proteins through Top-down Native Mass Spectrometry Analysis

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Transient association of proteins with cellular membranes play a central role in regulating a myriad of signaling pathways in biology. Pathways such as the Ras-MAPK, B-Cell receptor signaling, and JNK, central to human health, all include peripheral membrane proteins (PMPs) as key regulators. Increasing evidence suggests that lipids play a pivotal role in regulating signaling of PMPs, including membrane recruitment, modulating membrane bound conformations, and facilitating oligomerization. Nevertheless, it remains an experimental

challenge to detect these lipid interactions and their effects directly from native-like membrane environments. We have recently established a method to study integral membrane proteins from a tunable lipid bilayer and investigate membrane-associated oligomeric states and lipid interactors (Panda et. al. Nature Methods 2023). Here, we extend this platform to PMPs and introduce top-down fragmentation using electron capture dissociation (ECD). This enables us to directly study target PMPs from lipid membranes with customizable lipid compositions and biophysical properties such as curvature, fluidity, and tension. Native mass spectrometry (nMS) analysis reveals the membrane-bound oligomeric states, identity of specifically bound lipids, and top-down ECD allows precise fingerprinting of the binding site. We establish this using Bruton's Tyrosine Kinase (Btk), which is a non-receptor tyrosine kinase involved in B-Cell growth, proliferation, and activation. B-Cell receptor activation generates signaling lipid phosphatidylinositol – 3,4,5 – triphosphate (PIP3), which is recognized by the Pleckstrin Homology (PH) domain of Btk, triggering Btk auto-phosphorylation and kinase activity. Using lipid-vesicle nMS, we detect a novel lower-affinity binding of phosphatidylserine (PS) to membrane-associated Btk. This PS binding is outcompeted by high-affinity binding to PIP3. Subsequent ECD top-down analysis of the lipid bound Btk confirms the lipid binding site. To summarize, using Btk as an example, we present a platform to investigate membrane-bound oligomeric states, lipid binding specificity and stoichiometry, and lipid-binding regions in a streamlined process for various PMPs.

ABS#545

Aggregates, Amyloids, or Condensates? (July 16, AM)

Biochemical Characterization of New Archaeal Intramembrane Aspartyl Proteases (IAPs)

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Intramembrane aspartyl proteases (IAPs) cleave peptide bonds within the hydrophobic lipid membrane. The best characterized IAP is presenilin, the catalytic subunit of γ-secretase, which is known for cleaving amyloid precursor protein into the amyloid-β peptide that aggregates in the brains of Alzheimer's patients. More than 100 substrates have been documented for γ-secretase, yet no consensus recognition sequence has emerged, at least in part due to the technical complexities of studying this membrane-embedded proteolytic system. To date, the presenilin homolog from Methanoculleus marisnigri JR1 (MCMJR1) has been the sole microbial model of non-eukaryotic IAPs for in vitro molecular studies. A recent bioinformatic study uncovered over 1000 putative IAP sequences lurking in archaeal and bacterial organisms. Here, we report recombinant expression, purification, solution characterization by small angle neutron scattering (SANS), and enzymatic activity of new IAP(s). These new IAPs express well and are from noted archaea such as H. volcanii and Lokiarchaeota. These putative IAP orthologs share key structural and cleavage preference similarities with MCMJR1 IAP and presenilin. By studying the molecular biochemistry of more IAP family members, additional trends and insights regarding cleavage preferences and peculiarities will emerge. Such knowledge will further illuminate the fascinating fundamental and complex chemistry occurring within the lipid membrane.

Key words: intramembrane proteolysis, Presenilin, Gamma-Secretase, Haloferax volcanii, Alzheimer's Disease, small angle neutron scattering, proteomics, intramembrane aspartyl proteases

ABS#550

RNA-Protein Machines: Ancient Synergies (July 14, AM)

How snRNAs and Proteins Collaborate to Recognize Introns during pre-mRNA Splicing

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pre-mRNA splicing in eukaryotes is catalyzed by a large machine composed of 5 small nuclear RNAs (snRNAs) and nearly 100 proteins called the spliceosome. These RNAs and proteins work together to efficiently recognize introns and remove them while ligating the flanking exons together. Even though the catalytic core of the spliceosome is (like the ribosome) a ribozyme, proteins can have a tremendous influence on this reaction. In this presentation, I will provide recent examples from my lab of proteins that can influence the splicing fate of pre-

mRNAs. I will then give an in-depth look at how one particular splicing factor, the U1 small nuclear ribonucleoprotein (snRNP), uses both RNA-RNA base pairing interactions and proteins to rapidly and reversibly surveil RNAs for potential 5′ splice site sequences. Using single molecule fluorescence microscopy, we have been able to visualize this process in real time and derive kinetic models for splice site recognition by U1. I will also discuss similarities and differences between the yeast and human U1 splicing factors as well as the relevance of these results to how drugs that change U1/RNA interactions function.

ABS#551

Poster session, July 13

Unveiling cas8 dynamics and regulation of a transposon-encoded Cascade-TniQ complex

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Unveiling Cas8 Dynamics And Regulation of A Transposon-Encoded Cascade-TniQ Complex
Many disease states are caused by misfolded or absent proteins and therefore typically have genetic origins. To combat deleterious genetic mutations, researchers have successfully repurposed the CRISPR/Cas9 bacterial adaptive immune system for use in targeted genome editing. Since this revolution, researchers have gone on to discover a whole family of CRISPR/Cas systems. One system, known as Type 1F-3 or 'Cascade,' has been naturally evolved to associate with Transposition protein TniQ to conduct targeted insertion of transposons. A better understanding of the mechanism of action of this system may help us re-engineer it for use in mammalian cells, allowing us to conduct gene insertion in a targeted and highly accurate manner. To this end, we used molecular simulations to study the dynamics of Cascade-TniQ and particularly a crucial subunit within the complex known as 'cas8.' CRISPR systems' specificity comes from the sensitivity of their fully DNA-bound states. Typically, conformational changes occur upon complete target DNA binding to the CRISPR RNA, which prepares the system for its downstream functionality – in this case, transposase recruitment. We hypothesize that a helical 'bundle' of cas8 is responsible for interacting with and sequestering the non-target DNA strand away from the target strand – promoting complete target strand/CRISPR RNA binding. Thus, we concentrated on the dynamics of this bundle itself. To study the relevant molecular details of Cascade-TniQ, we first had to model the complex, which was done via an integrative approach using AlphaFold V2.1 (Figure, inset). We then ran Umbrella Sampling and classical Molecular Dynamics simulations. Our findings suggest that Cascade systems which cannot fully bind target DNA have a more restricted cas8 bundle than systems which do fully bind their target (Figure, attached). We also comment on the basis of this difference, demonstrating the importance of the other proteins within Cascade in bundle dynamics.

ABS#552

Poster session, July 15

A Conserved Centrosomin Domain Forms a Dimer That Stimulates the Activity of the Universal Microtubule Nucleating Complex, the Gamma-Tubulin Ring Complex (γTuRC)

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During the cell cycle the underlying microtubule (MT) cytoskeleton is dynamically remodeled, such that the cell-spanning interphase MT network can switch during mitosis to form the mitotic spindle. To accomplish this dynamic structural rearrangement, the cell must spatially and temporally control where microtubules are nucleated. At a fundamental level, this control involves stimulating the universal MT nucleation template, the γ-tubulin ring complex (γTuRC). Although γTuRC is present throughout the cytoplasm, its activity is preferentially stimulated at specific sites including the centrosome and Golgi. The well-conserved γ-tubulin nucleation activator (γTuNA) domain has been reported to increase the number of microtubules generated by γTuRCs. However, direct real-time observation of this stimulating interaction has been lacking, and prior confounding results made it unclear if the γTuNA domain was a true γTuRC activator. Here, we report a minimal in vitro, real-time reconstitution of γTuRC MT nucleation in the presence of γTuNA, observed via total internal reflection (TIRF) microscopy. With this, we report the

first real-time observation that γTuNA directly increases γTuRC activity in vitro, which is thus a bona fide γTuRC activator. We further validate this effect in Xenopus egg extract. By assaying different mutants, we also find that the core γTuNA domain forms an obligate dimer. Critically, efficient dimerization as well as γTuNA's L70, F75, and L77 residues are required for binding to and stimulation of γTuRC's MT nucleation activity. Finally, we find that γTuNA's activating effect opposes inhibitory regulation by stathmin, a tubulin sequestering protein. In sum, we now show that direct γTuNA binding strongly activates γTuRCs, explaining previously observed effects of γTuNA over-expression in cells and illuminating, at least in part, how γTuRC-mediated microtubule nucleation is regulated.

ABS#553

Poster session, July 15

Neutralizing monoclonal antibodies elicited by mosaic RBD nanoparticles bind conserved sarbecovirus epitopes

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Increased immune evasion by SARS-CoV-2 variants of concern highlights the need for new therapeutic neutralizing antibodies. Immunization with nanoparticles co-displaying spike receptor-binding domains (RBDs) from eight sarbecoviruses (mosaic-8 RBD-nanoparticles) efficiently elicits cross-reactive polyclonal antibodies against conserved sarbecovirus RBD epitopes. Here, we identified monoclonal antibodies (mAbs) capable of cross-reactive binding and neutralization of animal sarbecoviruses and SARS-CoV-2 variants by screening single mouse B-cells secreting IgGs that bind two or more sarbecovirus RBDs. Single-particle cryo-EM structures of antibody-–spike complexes, including a Fab-Omicron complex, mapped neutralizing mAbs to conserved class 1/4 RBD epitopes. Structural analyses revealed neutralization mechanisms, potentials for intra-spike trimer crosslinking by IgGs, and induced changes in trimer upon Fab binding. In addition, we identified a mAb resembling Bebtelovimab, an EUA-approved human class 3 anti-RBD mAb. These results support using mosaic RBD-nanoparticle vaccination to generate and identify therapeutic pan-sarbecovirus and pan-variant mAbs.

ABS#555

Poster session, July 15

Structure and regulation of human leucine-rich repeat kinase 1

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The two human leucine-rich repeat kinases (LRRKs), LRRK1 and LRRK2 are large and unusually complex multi-domain kinases which serve to regulate fundamental cellular processes. Near-uniquely, the LRRKs contain both a kinase domain and Ras-like GTPase domain in the same polypeptide chain, along with several scaffolding domains. Both LRRKs are implicated in human disease. Specifically, LRRK1 is implicated in bone development, with mutations in LRRK1 causing a bone disease, osteosclerotic metaphyseal dysplasia (OSMD), while mutations in LRRK2 are associated with Parkinson's disease. Despite their biological significance, both LRRKs have proved to be challenging structural targets, due to their size (∼250 kDa), heterogeneity, flexibility, and low recombinant expression levels. Recent experimental structures of LRRK2 in a variety of states have begun to provide structural detail into this family of proteins, however the structure and exact molecular mechanisms regulating the activity of LRRK1 remain unclear.
Here, we report a cryo-EM structure of the LRRK1 monomer, and a lower-resolution cryo-EM map of the LRRK1 dimer. The monomer structure, in which the kinase is in an inactive conformation, reveals key interdomain interfaces which serve to control kinase activity through linking the kinase domain and the Ras-like GTPase domain, which we have further validated experimentally. LRRK1 is structurally distinct compared to LRRK2, particularly in the position of the leucine-rich repeats relative to the kinase domain. Through analysis of the experimental cryo-EM data, we observed structural dynamics in the leucine-rich repeats in LRRK1, which we speculate may have a role in controlling substrate access to the LRRK1

kinase. Overall, our results provide new structural insights into the human LRRKs for understanding the physiology and pathology of these proteins.
A preprint is available describing these results: doi.org/10.1101/2022.12.21.521433.

ABS#557

Poster session, July 13

Decoding the central role of E2 conjugating enzymes at the cellular level by designing potent genetically encodable inhibitors

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Ubiquitination plays a critical role in maintaining cellular homeostasis by regulating a wide range of cellular processes like cell cycle progression, DNA repair, protein trafficking, immune response, and synaptic functions. Central to the enzymatic cascade that orchestrates this activity is the E2 conjugating enzyme that plays a fundamental role in deciding the fate of the protein by regulating the type of ubiquitin chain formed. Due to the lack of E2-conjugating enzyme-specific cellular tools, understanding the role of E2-conjugating enzymes at the cellular level has always been a challenge.
Using a protein engineering strategy, where we mimicked the multivalent interaction of E2 conjugating enzyme in nature, we designed a protein-based inhibitor with less than 10-9 M affinity and high specificity for the most promiscuous E2 conjugating enzyme: Ube2D. Quantitative mass spectrometry and global proteome analysis of transfected HeLa cells revealed the varied role Ube2D had on the cellular proteome. A comprehensive analysis of various databases further revealed the central role of Ube2D in the cytosol, nucleus, and mitochondria, impacting pathways involved in translation, transcription, protein trafficking, protein folding and protein degradation thereby underscoring the importance of cellular permeable inhibitors for E2 conjugating enzymes.
The strategy to design genetically encodable inhibitors has been extended to other E2 conjugating enzymes thereby helping unravel the various intertwined biological pathways at the cellular level. The inhibitors will also allow an in-depth understanding of functional coordination of E2 enzymes at the cellular level.

ABS#558

Poster session, July 15

Formation of Metallothionein-3 Cu(I)4-Thiolate Cluster Proceeds through a Long-Lived, Cu-Coupled, and Oxygen-Inert Disulfide Radical Anion

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Mammalian metallothionein-3 (MT-3) is a small (6-7 kDa), cysteine-rich metal-binding protein widely recognized for its critical roles in mediating copper neurochemistry. Despite copper's inherent instability in the oxidizing extracellular environment that collectively promotes its deleterious redox cycling, MT-3 is known for its ability to bind this redox active metal and form metal-thiolate clusters within its N-terminal domain that are unusually inert to oxidation in the presence of molecular oxygen. Using a combination of biophysical and spectroscopic characterization techniques, we investigated the chemistry and explored the pathway of assembly behind the formation of this uniquely redox-stable Cu(I)4-thiolate cluster in the final Cu(I)4Zn(II) 4MT-3 product that is formed upon the reaction of Zn7MT-3 with Cu(II). Interestingly, the cysteine thiolate residues of MT-3 can reduce Cu(II) to Cu(I) in a concerted electron transfer process concomitant with the formation of intramolecular disulfide bonds. Stopped-flow electronic absorption spectroscopy revealed the rapid formation and consumption of transient disulfide radical anion (DRA) species with their absorption centered at 430-450 nm and have lifetimes in the seconds regime. By rapidly freeze quenching the reaction intermediates, the DRA nature of the sulfur-centered species was confirmed by both Raman and electron paramagnetic resonance (EPR) spectroscopies, with the EPR simulations revealing that the DRA intermediates are coupled to Cu(I). Overall, we were able to dissect and provide evidence that the pathway of assembly and formation of this unique Cu(I)4-thiolate MT-3 cluster with short Cu-Cu distances (<2.8 Å), as verified by absorption and low-temperature luminescence spectroscopies, proceeds through the formation of long-lived, Cu-coupled, and oxygen-stable disulfide radical anion intermediates.

ABS#559

Poster session, July 13

Catechol 1,2-Dioxygenase with Improved Thermostability

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Catechol 1,2-dioxygenase (C12O) is involved in the catabolism of aromatic compounds and biosynthesis of cis, cis-muconic acid, an important precursor chemical and a compound of potential relevance to petrochemical replacement. However, the low thermostability of C12O is a hindrance to industrial application performance. In this study, the thermostability of a mesophilic C12O from Rhodococcus opacus was enhanced by the rational design and engineering of disulphide bonds in the protein structure. A computational pipeline of existing algorithms was used to identify possible locations for the introduction of stabilizing disulphide bonds. A set of selection criteria were used to narrow down the list of candidates, and molecular dynamic simulations were performed for each of the selected candidates under simulated thermal denaturing conditions. Subsequent wet bench testing of the selected candidates showed that two disulfide locations in particular (including an intra-subunit bond and an inter-subunit bond) yielded significant increases to protein thermostability. These two mutants showed greater than 5°C rises in melting temperature and T50, as well as significant increases in half-life at 40°C. Current work on a double mutant of these is ongoing to determine whether the effects of increased stability are additive. Overall, we conclude that the introduction of disulfide bonds resulted in significantly improved thermostability of C12O compared to wild type.

ABS#561

Poster session, July 13

Structural studies of the C-terminal domain of Major ampullate Spidroin 1 present alpha-helical to coil/beta-sheet transition upon fiber formation

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Developing high-performance soft materials is strategic to tackle the challenges of our era, such as biomedical advancements, climate change, technological lead, and industrial competitiveness. In nature, proteins are the building blocks of many materials with intriguing properties, with spider silk as a prominent example. Developing high-performance soft materials is strategic to tackle the challenges of our era, such as biomedical advancements, climate change, technological lead, and industrial competitiveness. These materials have served as inspiration for human-made structures for a long time, but the challenge to produce synthetic materials with comparable properties from biobased resources remains. Recent advances allow us to produce recombinant spider silk with properties that are close the natural. This It also enablesprovides for further investigation of the structure and formation mechanisms. Spider silk proteins (spidroins) are modular structures. A highly repetitive region is flanked by globular N- and C-terminal domains that are involved in the assembly process. Here, we study the C-terminal (CT) domain of recombinant major ampullate spidroin 1 (MaSp1) and exploit the factre that CT can form silk-like fibers independently to gain new knowledge about its role in the assembly of silk as the transition from a-helical/coil to β-sheets structure. The process is driven by a specific segment within the domain that has the intrinsic ability to undergo a pH-induced structural change. By integrating structural characterization methods, we are able to propose a model for the role of molecular show that we obtained thean initial explanationunderstanding reorganization of the C-terminal domain molecular organizationin the transition of spider silk transition from soluble protein to fiber. and its importance for trigging silk fibrillation.

ABS#562

Poster session, July 13

Investigating the Cell Penetration of Proteins and Lipid Nanoparticles (LNPs) with the Chloroalkane Penetration Assay (CAPA)

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Chloroakane Penetration Assay (CAPA) could provide a relatively quantitively cytosolic concentration of delivered molecules. In this study, CAPA was applied in investigating the cell penetration of proteins and lipid nanoparticles (LNPs). Cre-(-30)GFP was labeled with chloroalkane tag (CT) for CAPA assay to get cytosolic delivery efficiency. Fluorescence of GFP was used to evaluate the total cellular uptake and fluorescence DsRed was used to measure the functional delivery of Cre. Different chloroalkane tag (CT) and labeling levels' affection on protein interacting with cells and LNPs was studied. Protein with more CT has higher cell uptake, but not functional delivery. 4 CT per protein was selected for performing CAPA because both too much and less CT negatively affected protein and LNPs interaction. 72 different unformulated LNP was screened by CAPA. CAPA results varied from one time to three times of positive cells to the cells only treated with CT-protein. 80-O18 had the best cytosolic delivery ability based on CAPA and was approved by the GFP biodistribution screen and Cre functional delivery.

ABS#563

Poster session, July 13

The Homer1 EVH1 Domain is Primed for Interfamily Competition

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Intrinsically disordered regions play key roles in regulating the biological functions of their encompassing proteins. Within these regions, short linear motifs (SLiMs) are short sequences of 3-10 sequential residues that mediate binding to globular domains. Each SLiM-binding domain can recognize many SLiM-containing partners, which can be predicted by their inclusion of a pattern of residues, or motif, shared by validated binding partners. Due to the sparse nature of these motifs, a single sequence may match the motif of multiple unique domains. In theory, the lack of specificity inherent to overlapping motifs may give rise to competitive interference with critical SLiM-mediated processes, such as signaling cascades, complex assembly, and protein localization. In this study, we explore whether the predicted motif overlap of representative Enabled/VASP homology 1 (EVH1) SLiM-binding domains from the proteins ENAH and Homer1 is due to a failure of the established motifs to fully capture each domain's binding preferences. To do so, we utilized an unbiased screen of 36-mer peptides from the human proteome to identify binders of the Homer1 EVH1 domain to better define its poorly characterized motif and to identify motif-external sequence elements that modulate binding. Counterintuitively, we discover that the Homer1 EVH1 domain preferentially binds to overlapping sequences containing an ENAH motif, despite lacking the structural features thought to confer ENAH binding preferences. Furthermore, we show that the singular EVH1 domain from the amoeba Dictyostelium discoideum also shows ENAH-like binding preferences despite lacking ENAH structural features, suggesting that ENAH and Homer1 arose via incomplete divergence from an ENAH-like ancestor. Given the co-expression of ENAH and Homer1 in neurons and the prevalence of overlapping EVH1 motifs within the proteome, our results suggest that the cell must rely on external regulatory mechanisms to maintain EVH1 network integrity.

ABS#564

Poster session, July 15

Unique molecular recognition mechanisms of β-hairpin CDR3 of an anti-HigB2 VHH antibody

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The β-hairpin structure is a common motif to involve and control multiple protein-protein interactions. Single-domain VHH antibodies are regarded as potential therapeutic and diagnostic tools, and the third complementarity-determining regions of the heavy chains (CDR3s) have critical roles in antigen recognition. Although the frequency of β-hairpin structures in CDR3 is relatively low, VHH antibodies possessing β-hairpin-like CDR3 conformations would sometimes prefer to recognize unique antigen epitopes. However, specific characteristics and interaction mechanisms of β-hairpin CDR3s were not fully clarified due to the structural rarity. We reasoned that if conformational characteristics of a β-hairpin CDR3 were elucidated, it would facilitate the development of artificial VHH library designs and more accurate structural prediction.

Here, we investigated the molecular recognition of the anti-HigB2 VHH antibody Nb8, which has a CDR3 that adopts a β-hairpin-like conformation. The role of each residue was analyzed by alanine-scanning experiments and molecular dynamics simulations. Curiously, these experiments demonstrated the significance of positions 93 and 94 in framework region 3 (FR3), which is right outside CDR3 in the Chothia numbering scheme. Even though the FR3 residues had few direct interactions with the antigen, the alanine mutants showed seriously declined binding affinities. Also, the affinity influences would be larger than these of hotspot residues at CDR3. These residues formed intramolecular interactions, and maintained the antibody conformation that would be preferable for the antigen recognition. We anticipate that these findings will lead to the design and optimization of single-domain antibodies.

ABS#565

Poster session, July 15

Characterization of NixA: A Ni(II) Transporter from the NiCoT Family

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Nickel is essential for the survival and virulence of several pathogenic bacteria in humans including Helicobacter pylori (H. pylori), a pathogen that continues to be the main cause of gastric ulcers. To survive the harsh acidic conditions present in the human stomach, H. pylori utilizes Ni(II) as a metal cofactor that activates the enzyme urease. This enzyme catalyzes the hydrolysis of urea and ultimately releases ammonia and bicarbonate which are involved in creating a buffered environment that allow H. pylori to tolerate the acidic environment of the stomach. As regulation of Ni(II) homeostasis inside the cell proves to be a vital process for the growth and pathogenicity of H. pylori, unique transmembrane (TM) transporters that permit the selective translocation of Ni(II) across the cellular membranes evolved to control cellular Ni(II) uptake and efflux. NixA is a prominent Ni(II) transporter expressed by H. pylori when the bacteria experiences a drastic drop in pH in the extracellular environment. NixA is a Class I Nickel-Cobalt transporter (NiCoT) that strictly allows the transport of Ni(II) across lipid bilayers. In this study, we characterized NixA by using a platform where recombinantly expressed and purified NixA in detergent micelles could be reconstituted in artificial lipid bilayer vesicles (proteoliposomes). Several fluorescent sensors responsive to diverse stimuli (Fluozin-3-Zn(II), pyranine, and oxonol VI) were then encapsulated in the proteoliposomes to monitor in vitro real-time Ni(II) transport, lumenal pH changes and membrane potential, respectively. Kinetic transport assay analysis revealed that NixA is highly selective for Ni(II) and showed low substrate promiscuity towards other metal ion substrates of the NiCoT family. We also provided evidence that Ni(II) transport by NixA did not require proton counter-transport, thus generating a membrane potential inside the proteoliposome lumen and indicating that substrate transport is electrogenic. Mutational studies revealed conserved motifs that essential for substrate recognition, selectivity, and transport. Overall, this work provides an approach to characterize these novel types of transporters responsible for Ni(II) acquisition in prokaryotes.

ABS#566

Poster session, July 15

Structural and Functional Characterization of Triketone Dioxygenase from Oryza Sativa

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The transgenic expression of rice triketone dioxygenase (TDO; also known as HIS1) can provide protection from triketone herbicides to susceptible dicot crops such as soybean. Triketones are phytotoxic inhibitors of plant hydroxyphenylpyruvate dioxygenases (HPPD). The TDO gene codes for an iron/2-oxoglutarate-dependent oxidoreductase. A combination of mass spectrometry, nuclear magnetic resonance and enzyme activity studies indicate that rice TDO oxidizes mesotrione in a series of steps, first producing hydroxy-mesotrione and then oxy-mesotrione. Evidence suggests that hydroxy-mesotrione is a much weaker inhibitor of HPPD than mesotrione, and oxy-mesotrione has virtually no inhibitory activity. Of the close homologues which have been tested, only

corn and rice TDO have enzymatic activity and the ability to protect plants from mesotrione. Correlating sequence and structure has identified four amino acids necessary for TDO activity. Introducing these four amino acids imparts activity to a mesotrione-inactive TDO from sorghum, which may expand triketone herbicide resistance in new crop species.

ABS#569

Poster session, July 15

Structural basis for recognition of unfolded proteins by the ER stress sensor ERN1/IRE1a

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IRE1a is an endoplasmic reticulum sensor that recognizes misfolded proteins to activate the unfolded protein response (UPR). We used cholera toxin (CTx), which activates IRE1a in cells, to understand how unfolded proteins are recognized. In vitro, the A1 subunit of CTx (CTxA1) bound IRE1a lumenal domain (IRE1aLD). Global unfolding was not required. Instead, IRE1aLD recognized a 7-residue motif within a metastable region of CTxA1 that was also found in microbial and host proteins involved in IRE1a activation. Binding mapped to a pocket on IRE1aLD normally occupied by a segment of the IRE1a C-terminal flexible loop implicated in IRE1a regulation. Mutation of the recognition motif blocked CTx-induced IRE1a activation in live cells. These findings describe a mechanism for substrate recognition by IRE1a that induces the UPR.

ABS#570

Poster session, July 13

General Features of Transmembrane Beta Barrels From a Large Database

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Large datasets contribute new insights to subjects formerly investigated by exemplars. We used co-evolution data to create a large, high-quality database of transmembrane β-barrels (TMBB). By applying simple feature detection on generated evolutionary contact maps, our method (IsItABarrel) achieves 95.88% balanced accuracy when discriminating among protein classes. Moreover, comparison with IsItABarrel revealed a high rate of false positives in previous TMBB algorithms. In addition to being more accurate than previous datasets, our database (available online) contains 1,938,936 bacterial TMBB proteins from 38 phyla respectively 17 and 2.2 times larger than the previous sets TMBB-DB and OMPdb. We anticipate that due to its quality and size the database will serve as a useful resource where high quality TMBB sequence data is required. We found that TMBBs can be divided into 11 types, three of which have not been previously reported. We find tremendous variance in proteome percentage among TMBB-containing organisms with some using 6.79% of their proteome for TMBBs and others using as little as 0.27% of their proteome. The distribution of the lengths of the TMBBs is suggestive of previously hypothesized duplication events. In addition, we find that the C-terminal β-signal varies among different classes of bacteria though it is most commonly LGLGYRF. However, this β-signal is only characteristic of prototypical TMBBs. The nine non-prototypical barrel types have other C-terminal motifs, and it remains to be determined if these alternative motifs facilitate TMBB insertion or perform any other signaling function.

ABS#572

Poster session, July 15

The Impacts of Thiaproline on Collagen Triple Helices

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(2R)-4-thiaproline (Thp) is an analog of proline, in which Cγ in the pyrrolidine ring is replaced with sulfur. Its thiazolidine ring can easily interconvert between endo and exo puckers because of a small energy barrier, which leads to destabilize both polyproline I and II helices. Collagen, derived from three polyproline II helices, mainly consists of X-Y-Gly triplets, where X is often proline and

Y is frequently (2S,4R)-hydroxyproline. In this work, we incorporated Thp into either the X-position or the Y-position to study the consequences of such a substitution on the collagen triple helix. Circular dichroism and differential scanning calorimetry analyses showed that the Thp-containing collagen-mimetic peptides (CMPs) can fold into stable triple helices, in which the incorporation into the Y-position induces a larger destabilization effect. In addition, we further prepared the derivative CMPs by oxidizing Thp in the peptide to N-formylcysteine or S,S-dioxide Thp. The results indicated that the oxidized derivatives at the X-position only slightly affect the triple helix stability, but those at the Y-position induce a significant destabilization effect. In particular, the introduction of S,S-dioxide Thp at the Y-position makes CMPs fail to fold into triple helices. The impacts of incorporating Thp and its oxidized derivatives into CMPs are position dependent. We further used density functional theory calculation to reveal that the ease of interconversion between exo and endo puckers for Thp and the twist conformation of S,S-dioxide Thp in solution might cause the great destabilization effect at the Y-position. Together, we provided new insights into the impacts of Thp and its oxidized derivatives on collagen and demonstrated that Thp can be used to design collagen-related biomaterials.

ABS#573

Poster session, July 13

Impact of West African Variants on the Structure and Ligand Binding of the Novel Kinase Oncogene STYK1

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Ovarian carcinoma (OvCa) is a deadly gynecological cancer that disproportionately affects African Americans (AAs), who are more often diagnosed at advanced stages. Genetic variations (SNPs) across ancestries (African, European, Asian) such as the methylenetetrahydrofolate reductase SNP rs1801133 impact tumor progression, prognosis, and treatment. The understudied oncogene STYK1 (NOK) promotes cell growth and invasion in several cancers, including OvCa. However, STYK1 structure and function remain poorly understood: there are no available crystal structures for STYK1 or even its kinase domain and STYK1 homology to its closest relatives (members of the FGFR/PDGFR family) is only 20-30% percent. This study explores the predicted structure and ligand binding interactions of STYK1 and three STYK1 variants most prevalent among individuals with West African ancestry (WAA variants). We hypothesize that structural changes in STYK1 WAA variants may contribute to OvCa disparities in African American women.
We used a combination of computational databases to predict the impact of WAA variants on the secondary and tertiary structures of the STYK1 320-aa kinase domain. Unique secondary structural differences were predicted among each WAA STYK1 variant and the canonical domain. Somewhat surprisingly, WAA variant tertiary structures were more like one another than to canonical STYK1. We employed molecular docking to model molecular interactions between a library of small molecule ligands and the four STYK1 domains. Unique binding pocket-ligand interactions were predicted among all domains, and, as with tertiary structures, the preferred ligands predicted were similar among the three WAA variants and different from canonical STYK1. The molecular docking results provide a starting point 1) for the development of precision drugs that target the STYK1 oncogene specifically for African American women suffering from OvCa and 2) for future work exploring the functional impact of structural differences among STYK1 and its WAA variants.

ABS#574

Poster session, July 13

Deciphering mechanisms of misfolded protein targeting by ERAD

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Misfolded proteins in the endoplasmic reticulum (ER) are retro-translocated into the cytosol and degraded by the ubiquitin-proteasome system, a pathway termed ER-associated degradation (ERAD). ERAD plays important roles in the degradation of endogenous proteins and in many diseases in which mutant proteins are misfolded. Misfolded proteins in the ER lumen are handled by the ubiquitin ligase Hrd1. I used disulfide crosslinking to map the path of a substrate through the Hrd1 complex during ERAD-L (ERAD of luminal proteins). My results

and available cryo-EM structures suggest that substrates traverse from lumen to cytosol through a thinned membrane region near the lateral gate of Hrd1, rather than through a conventional pore.

Hrd1 can also handle membrane-embedded substrates – a process called ERAD-M. Current models for ERAD-M suggest that the membrane-spanning substrate enters the lateral gate of Hrd1, and ubiquitination then occurs on suitable substrate residues exposed to the cytosol. But how Hrd1 recognizes substrates inside the membrane is not well understood.

Here, we show that the thinner membrane region near Hrd1 lateral gate serves as a 'sink' to trap hydrophilic transmembrane segments – a hallmark of ERAD-M substrates. Hydrophilic TM segments are less stable in normal lipid bilayer and will preferentially partition into thinner membrane regions. Proteins that do not contain hydrophilic segments will be unlikely to remain in this thinned membrane region for long enough to be ubiquitinated and will be spared. This model would explain how Hrd1 selects a broad range of substrates that contain diverse polar as well as positively and negatively charged residues in their transmembrane segments. Our results clarify important biological questions, including how proteins cross a membrane, a particular mystery for misfolded polypeptides in the case of ERAD.

ABS#575

Poster session, July 13

Investigating the Non-Electrostatic Component of Substrate Positioning Dynamics

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Substrate positioning dynamics (SPD), which orients the substrate to a reactive conformation in the active site, is critical in mediating enzyme catalysis. However, given that conformational changes often accompany variations in the enzyme interior electrostatics, it remains unknown whether SPD contains a non-electrostatic component that independently mediates catalysis, or originates primarily from perturbation of enzyme interior electrostatics. This study integrated computational and experimental approaches to investigate the non-electrostatic component of SPD using Kemp eliminase (KE) as a model enzyme. A molecular dynamics-derived descriptor, substrate positioning index (SPI), was used to quantify the impact of protein dynamics on substrate positioning. Using high throughput enzyme modeling, we selected 7 KE variants for kinetic assessment – these variants involved significantly different SPD but similar interior enzyme electrostatics. We observed a valley-shaped, two-segment piecewise linear correlation between the experimentally characterized activation free energies and SPI values. The trend is further validated using previously reported kinetic data. An optimal SPI value, corresponding to the lowest activation free energy, was observed for R154W, a surface mutation located distantly from the active site. Compared to the wild type, R154W involves favorable SPD that increases the proportion of reactive conformations for substrate deprotonation. These results indicate the presence of the non-electrostatic component of SPD, a concrete factor that mediates catalysis by tuning the population of reactive conformation.

ABS#576

Poster session, July 14

Semi-rational Design of a Cytochrome P450 BM3 Library for p-Coumaric Acid Hydroxylation

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Cytochrome P450 monooxygenases are a superfamily of enzymes that catalyze a wide range of reactions, such as hydroxylation, and can functionalize inert C-H bonds with high regioselectivity and stereoselectivity under mild conditions, a challenge in organic chemistry [1]. Due to its high expression, solubility and self-sufficiency, the cytochrome P450 monooxygenase from Bacillus megaterium (P450BM3) has been the target of several engineering studies to expand the substrate beyond the C12-C20 fatty acids that are its natural substrates [2]. The main objective of this project was to design a semi-rational library of P450BM3 variants using protein engineering approaches to shift the substrate specificity of the enzyme towards aromatic compounds such as p-coumaric acid for the production of caffeic acid. The selection of a set of seven amino acids as targets for mutagenesis was based on structural analysis and docking studies of p-coumaric acid in the active site of P450BM3 and supported by information reported in the literature. An effective screening strategy is required to identify P450BM3 variants with p-coumaric acid hydroxylation activity and potential caffeic acid formation in a library of 20000 variants. A screening assay based on the

antioxidant capacity of caffeic acid complexed with ferric ferrozine (Fe(III)-FZ) under acidic conditions was established. The Fe(III)-FZ assay was validated for use in high-throughput screening of the library, a detection limit (0.4 mM) and a linear detection range (0.4 to 4 mM) was obtained. Combinatorial mutagenesis was performed, resulting in a diversity of approximately 20000 variants. To date, a total of 2,500 variants of P450BM3 have been screened and four variants have been selected for further analysis of their reaction products and kinetic assays. The results suggest that semi-rational library design can be an effective strategy to obtain a reduced number of variants, but still full screening requires optimization of screening efforts.

ABS#577

Poster session, July 15

Incorporation of Virus-Like Particles into Ordered Mesoporous Silica

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The development of safe and effective vaccines recruits a number of different approaches, such as the use of inactivated and/or attenuated virus, the use of virus-like particles (VLPs), and also the incorporation of antigens into nanostructured silicas. The VLPs are widely accepted because the immune system responds to them in a similar way to the response against vaccines of inactivated complete virus.

In this work, several biophysical techniques (CD, SAXS, DLS, NAI, fluorescence, etc) are used to ensure the chemical and structural integrity of the VLPs at different pHs, temperatures, environmental conditions and in time, and specially after incorporation into silica particles. The ordered mesoporous silicas were able to act as a protective vehicle for the antigens, increasing their thermal and pH stability, optimizing immune response, and acting as an adjuvant in the preparation of oral vaccines. A detailed knowledge of the morphological and structural properties of the VLPs and the characterization of the factors capable of affecting their integrity are fundamental to collaborate in their use as a vaccine strategy.

ABS#578

Poster session, July 15

Assessing and Enhancing Foldability in Designed Proteins

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Recent advances in protein-design methodology have led to a dramatic increase in reliability and scale. Nevertheless, the success rate, particularly in design of new protein function, is low. Failure often stems from inaccuracy and misfolding relative to the design conception. To address this challenge, we developed methods to diagnose and ameliorate suboptimal regions in designed proteins. Using Rosetta atomistic computational mutation scanning, we detect energetically suboptimal positions in designs (available at https://pSUFER.weizmann.ac.il) and then focus FuncLib design calculations on these suboptimal positions to alleviate the strain. Applying this strategy to a previously designed low-efficiency enzyme, resulted in 330-fold improvement in catalytic efficiency. Furthermore, in a case of a de novo designed protein that exhibited limited stability, the same approach markedly improved stability and expressibility. Based on these results, we revisited the problem of de novo design of Kemp eliminates. Of 54 designed proteins, 30 showed cooperative melting curves in unfolding experiments with 54°C <Tm< 87°C, and 19 were functional, albeit, exhibiting a low level of activity. These are starting points for further computational optimisation. We conclude that foldability analysis and enhancement may dramatically increase the success rate in design of functional proteins.

ABS#579

Poster session, July 14

Synaptic Turnover of CaMKII

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Human capacity to learn and recall information depends on synaptic strength. Ca2+calmodulin dependent protein kinase II (CaMKII) is an oligomeric kinase concentrated in dendritic spines and required for synaptic plasticity. Maintaining precise control over the levels of CaMKII is critical for synaptic plasticity. Here, we seek to understand how CaMKII is regulated and how a growing number of missense mutations in the CAMK2A gene cause neurological and psychiatric disorders. Our preliminary results showed that the ubiquitin-proteasome system (UPS) specifically regulates the levels of constitutively active CaMKII along with one of well characterized disease mutant E183V. Using the genome wide CRISPR screening, we will not only identify the cellular factors regulating CaMKII stability but also potential accessory proteins that could contribute to CaMKII specificity.

ABS#580

Poster session, July 14

Conservation of Binding Hot Spots in AlphaFold Models

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The artificial intelligence program AlphaFold (AF) has revolutionized structural bioinformatics through its great accuracy in protein structure prediction. Though many examples exist in the direct usage of AF models in biological research, literature on robustly quantifying the reliability of AF models is limited. In this work, we find high conservation of binding hot spots, or regions on a protein largely contributing to the binding free energy, in AF models relative to corresponding X-ray structures.
The computational hot spot mapping program FTMap was used to identify binding hot spots on AF models and X-ray structures. FTMap identifies binding hot spots by docking molecular probes of varying polarity and size on protein surfaces, retaining the most energetically favorable docked probes, and clustering together different types of probes within a predetermined distance threshold. Cross-clusters of different probe types identify the binding hot spots. Mapping results have revealed high degree of conservation in ligand binding sites, located by binding hot spots in AF models, using ligand-bound and unbound X-ray structures as reference states. Out of the 62 proteins studied, 66.1% of AF models demonstrated a conserved druggable binding hot spot, compared to 70.9% of corresponding ligand-bound X-ray structures and 62.5% of unbound X-ray structures. Outliers reflect shortcomings of the AF algorithm in structural predictions for multidomain proteins, with especially striking local discrepancies in ligand binding sites. Running the AF algorithm on truncated, single chain sequences rather than entire multidomains improves local structural determination and hence binding hot spot recovery. Additionally, using multiple random seeds for non-binding hot spot conserving AF models to generate a greater number of multiple sequence alignments aids in predicting improved models that successfully conserve binding hot spots. Our results confirm that AF models are sufficient for determining binding hot spots with comparable performance to that of corresponding X-ray structures.

ABS#581

Poster session, July 15

Targeting the Peripheral Myelin Protein 22 using Fragment Based Drug Discovery

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Charcot-Marie-Tooth Disease (CMTD) is a hereditary disorder of the peripheral nervous system that affects 1 in 2500 humans, for which there is no available treatment. The most common forms of CMTD are associated with genetic aberrations in the peripheral myelin protein 22 (PMP22), a tetraspan membrane protein that is abundant in compact myelin. PMP22 overexpression, underexpression, or missense mutations result in defective myelin in the peripheral nerves. This is mainly due to misfolding and aggregation of PMP22. In this work, we use NMR spectroscopy to screen a fragment library in search of compounds that bind to PMP22 in vitro and potentially impact protein folding and function. We present a description of the methodological development and optimization to apply NMR-based fragment screening to an integral membrane protein target. After screening PMP22 in dodecyl-b-maltopyranoside (DDM) micelles against a subset of the library, we found a number of compounds that bind to the protein in DDM

micelles but not to a negative control membrane protein. The binding affinity of the hits were then determined by NMR. Ongoing assessment of analogs of fragment hits demonstrates the feasibility of NMR-based fragment screening to identify chemical matter that binds a tetraspan membrane protein.

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ABS#582

Poster session, July 13

Allosteric Interactions in RhoA: Insights from Molecular Dynamics Simulations Coupled with GTP Hydrolysis Experiments

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RhoA, a member of the Rho-subfamily of small GTPases, undergoes dynamic conformational changes that regulate its cellular functions. This study investigates the role of allosteric linkages, dynamics, and hydrolysis rate constants in RhoA. Crystal structures of wild-type RhoA, R70A, and E102A mutants were solved by Dr. Kendra Marcus to elucidate the consequences of removing the R70-E102 salt bridge that anchors switch II to helix 3. Here we present molecular dynamics simulations, including both classical (cMD) and accelerated (aMD) methodologies with reweighting using the Boltzmann method to correct for the boost potential in the case of aMD. Community network analysis is applied to demonstrate changes in allosteric networks connecting distant parts of the protein. HRas, the canonical small GTPase, has H-bonding connections between the upper portion of Switch II and the top of helix 3/loop 7. In RhoA these connections appear to be further stailized by the salt bridge, which is altered in both the R70A and E102A mutants. RMSF analysis shows that R70A has a more dynamic Switch II than either WT or E102A. Community network analysis shows that switch II is in the same community as helix3/loop7 in the wild type RhoA and is disconnected in both mutants. Interestingly the R70A mutant shows stronger allosteric connections between helices 3 and 4 than does the wild type, while in E102A both switch II and helix 4 are disconnected from helix 3. While hydrolysis rate constants is expected to decrease for both mutants, subtle differences may be due to the different patterns of allosteric connections. This work highlights the role of the R70-E102 interaction and allosteric connections between helix 3 and Switch II in RhoA dynamics and function. Further investigations can build upon these insights to explore the functional significance of RhoA dynamics and its potential as a therapeutic target.

ABS#583

Poster session, July 13

Investigating the utility of protein language models for modeling isoforms

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Protein language models (pLM) have emerged as potent tools for predicting protein structures. Yet, the degree to which these models truly understand the inherent biophysics of protein structures remains a subject of investigation. In this study, we investigated if pLMs were capable of finding alternate predictions for isoforms, protein fragments that share high coevolutionary information with their full-length isoforms yet many of which are predicted to be disordered. We find that AlphaFold2, OmegaFold, ESMFold fall prey to predicting these isoform fragments identically to the structure the fragment would adopt within the context of the full-length protein. Motivated by this, we conducted a more thorough interpretability analysis, subjecting models to artificially modified sequences and subsequently evaluating the biophysical plausibility of the resulting protein structure predictions. More specifically, we find the deletion of secondary structure elements (SSE) such as beta-strands results in compensatory actions, where the sequence shifts and the neighboring strands take their place. If the SSEs are rearranged with enough linker sequence between them, they adopt similar structure. Finally, replacing the SSEs with glycine does not affect the prediction. Though these experiments suggest that pLM could potentially model sequence rearrangements (as seen in isoform) that change the fold without affecting the

architecutre, this is likely performed via non-sequential homology detection without biophysical consideration. Our investigation reveals a general susceptibility in current language models to the error of predicting structures of modified sequences within the context of the full-length protein. This finding accentuates the imperative to enhance the contextual awareness of these models for more accurate and biophysically consistent protein structure predictions.

ABS#587

Poster session, July 14

Chemically inducible self-cleaving protein tagging system for regulated protein export to media or plasma membrane from eukaryotic cells

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Extracellular proteins such as growth factors, cytokines, antibodies, and enzymes, initiate vital signaling pathways in multicellular organisms. Tools for manipulating secretory transit of those effector proteins to control downstream physiology could prove useful for basic and applied biological research. Reagents exist to block protein secretion at the post-translational level; however, we lack corresponding tools for bioorthogonal activation of native protein export, particularly in multicellular organisms. In this poster, we describe our ongoing feasibility studies of a one construct/one molecule protein tagging approach called SHERPA (Sonic HhC ER Protein Autoprocessing). Translational fusion of a protein of interest (POI) to SHERPA (i.e., POI-SHERPA) is found to enable chemically inducible POI secretion from HEK293 cells. The small, bright nanoluciferase is used as a test POI. In the presence of inducer, SHERPA cleaves itself from nanoluciferase in the ER. Subsequent transit through the secretory pathway for display on the plasma membrane or release into the media is controlled by the structure of the SHERPA inducer. Steroidal SHERPA inducers result in membrane display; non-steroidal inducers result in extracellular secretion. In the absence of inducer, the POI-SHERPA fusion is retained in the ER and eventually degraded by ERAD. For added control in this chemically inducible tagging system, SHERPA inducers are prepared for photo-uncaging. Based on preliminary experiments, SHERPA seems poised to provide a novel chemically inducible protein tag for traceless POI export from eukaryotic cells.

ABS#589

Poster session, July 13

Mechanisms of SARS-CoV-2 Nsp1 mediated host mRNA nuclear export inhibition

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Viruses use a variety of mechanisms to inhibit host gene expression to promote viral replication. The nonstructural protein 1 (Nsp1) of SARS-CoV-2 is a crucial virulence factor that inhibits host gene expression using multiple strategies. Nsp1 has been known to block host translation by obstructing mRNA entry into the ribosome. We discovered that Nsp1 inhibits host mRNA nuclear export via a previously unknown interaction with the major mRNA export receptor, NXF1-NXT1 heterodimer. During SARS-CoV-2 infection, Nsp1 disrupts the interaction between NXF1-NXT1 and other mRNA nuclear export machinery and causes an accumulation of mRNAs in the nucleus. The Nsp1-NXF1 interaction and the Nsp1-mediated mRNA nuclear export inhibition are crucial to SARS-CoV-2 virulence as increased levels of NXF1 rescue the Nsp1-induced nuclear accumulation of mRNAs and inhibit SARS-CoV-2 infection. Targeting the Nsp1-NXF1 interaction represents a potential new strategy to restore host immunity against SARS-CoV-2 infections. We are currently dissecting the details of the recognition mechanisms of this interaction using a combination of biochemical and biophysical approaches.

ABS#590

Poster session, July 14

Additive energetic contributions of multiple peptide positions determine the relative promiscuity of viral and human sequences for PDZ domain targets

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PDZ domains are a category of small peptide binding domains, identified by their well-conserved structure of a five-stranded anti-parallel beta-sheet and 1-2 alpha helices. They are prevalent in multicellular organisms and play an integral role in cell signaling and trafficking, protein-protein interaction, cellular scaffolding, and more. The human proteome contains 200+ PDZ domains, which can be targeted by various diseases and viruses which often disrupt cellular networks for their own benefit. This process may be related to higher pathogenicity in said diseases, which therefore makes PDZ domains a target for therapeutic research.

This study focuses on human PDZ domains which are bound by the C-terminal sequences of HPV16 E6 oncoprotein and cystic fibrosis transmembrane conductance regulator (CFTR). These Class I PDZ domains most notably recognize 2 key motif residues, which HPV16 and CFTR have in common (Lysine and Threonine, where CFTR: VQDTRL and HPV16: RRETQL). Despite this, HPV binds to significantly more PDZs, making it a more "promiscuous" binding domain. We have investigated the impact that the other non-motif residues have on overall binding affinity and whether they may cause this difference in promiscuity. To investigate this, we expressed and purified human PDZ domains which exhibit preferential binding to either CFTR or HPV16 and tested their binding affinity via fluorescence polarization assays through the use of substituted peptides. These peptides mimic the C-terminal sequences of CFTR and HPV16E6 with single point amino acid substitutions to that of the other sequence. We saw little to no variation between the binding of the variant peptides and the original sequences to the PDZ domains which we tested. Our results indicate that there is not a single residue which is controlling the increased binding of HPV16 vs CFTR, but rather a compounded effect of them together as a whole.

ABS#592

Poster session, July 14

Cholesterol Binding Regulates Membrane Bending by Prominin-Family Proteins

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Prominin-1 (Prom1, CD133) is a pentaspan transmembrane (TM) protein that regulates plasma membrane curvature and induces secretion of extracellular vesicles (EVs) during stem cell and cancer stem cell differentiation. Prom1 EVs form at the tips of microvilli and cilia as well as through bulk blebbing from cholesterol-rich domains of the plasma membrane. Patient mutations in Prom1 cause retinal degeneration due to failure of membrane homeostasis. Despite its importance in EV biogenesis during differentiation, the molecular mechanisms underlying EV formation by Prom1 are unclear.

We mutate several sites in the TM of Prom1 to reduce stable cholesterol binding. We reconstitute Prom1 EV formation by transient transfection in Expi293 cells. EVs are purified using ultracentrifugation, gradient separation, and size-exclusion chromatography. We characterize EV morphology and composition using electron microscopy and mass spectrometry. We use immunopurification to quantify cholesterol binding and bifunctional crosslinkers and limited proteolysis to study Prom1 orientation and conformation in purified EVs.

Wild-type (WT) Prom1 and mutants all promote EV formation, but mutant EVs have more deformed membranes. All Prom1 EV membranes are less deformed than those produced by a homolog (Ttyh1) that lacks all cholesterol binding. Structure-function studies indicate that WT and mutant Prom1 in EVs assume different oligomeric states and conformations.

Cholesterol binding modulates the oligomerization and conformation of Prom1, and is thermodynamically linked to membrane bending and release of Prom1 EVs. We suggest a model where stable interaction of Prom1 with cholesterol inhibits membrane bending and EV release. This mechanism may regulate Prom1 EV release during stem cell differentiation.

ABS#593

Poster session, July 14

ExploreTurns and BetaMapper: Tools for the Exploration, Analysis, Mapping and Retrieval of Beta Turns and Their Contexts

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Four-residue (beta) turns embody about a quarter of all residues in proteins and play crucial roles in structure and function. Previous work has developed beta-turn classification systems that operate in the Ramachandran space of backbone (BB) dihedral angles [1,2], and an existing tool [3] supports the study of small hydrogen-bonded motifs including turns. This work presents two new tools which are specialized for the study of beta turns with or without hydrogen bonds and combine the advantages of Ramachandran- and Euclidean-space representations, utilizing a new, Euclidean-space turn coordinate system and global alignment. The ExploreTurns tool supports the comprehensive exploration, analysis, geometric "tuning" and retrieval of beta turns and the wide variety of local contexts in which they appear. ExploreTurns takes the form of a database selection screen, structural profiler and 3D viewer, integrated onto a single web page, that renders a redundancy-screened, PDB-derived beta-turn dataset structurally addressable by a wide range of criteria describing the turns and their BB neighborhoods. Criteria include a newly derived set of geometric parameters for the bulk BB shape of turns that describes modes of structural variation not explicitly captured by Ramachandran-space classifications. The second tool, BetaMapper, uses 3-stage hierarchical clustering to generate 3D interactive conformational heatmaps of the distributions of BB structure, side-chain (SC) structure, BB context and BB/SC H-bonding associated with each sequence motif in beta turns. ExploreTurns is applied here to the identification of new types of beta-bulge loops in support of a proposed generalization of the loop's definition, and these tools, which complement each other, should prove useful in research, education, and in applications such as protein design, in which an enhanced Euclidean-space turn description with geometric tuning, together with a comprehensive picture of structure and interaction in turns, may improve understanding or performance.

ABS#594

Poster session, July 15

Inhibiting Human Neutrophil Serine Protease 4 with mRNA display-derived Macrocyclic Peptides

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Neutrophil serine protease 4 (NSP4) is the recently discovered 4th member of the NSP family of serine proteases, which includes neutrophil elastase, proteinase 3 and cathepsin G. Expression of NSP4 during the early stages of mast cell development regulates cellular histamine and serotonin levels, which effects vascular leakage. NSP4 is an Arg-specific protease with a non-canonical active site conformation featuring a shallow S1 specificity site that is well-suited for macrocyclic peptide inhibitors. Therefore, we performed mRNA display against human NSP4 using 4 codon tables encoding 10-14-mer cyclic peptide scaffolds containing both natural and non-natural amino acids. The top 20 hits were synthesized as cyclic peptides and tested in enzymatic assays. We identified 7 macrocyclic peptides (12-14-mers) that showed complete inhibition of NSP4 activity with IC50 values of 4 – 200 nM and corresponding KD values in the same range as determined by SPR. The macrocyclic peptides are highly selective to human NSP4 over other serine proteases, including NSP family members. All 7 macrocyclic peptides inhibited NSP4 labeling by an activity-based probe. In addition, SPR experiments demonstrated that the peptides no longer bound to active site-blocked human NSP4, suggesting that they bind to the active site region. Structural studies are ongoing to elucidate the molecular details of the inhibitory mechanism. The herein identified macrocyclic peptide inhibitors may serve as valuable tools for studying NSP4's biological functions and highlight mRNA-display as a powerful technology for generating potent and specific serine protease inhibitors.

ABS#598

Poster session, July 14

Progress Toward the Design and Evaluation of Buforin-II Peptide Variants with Increased Antimicrobial Potency

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Antimicrobial peptides (AMPs) act by targeting generic bacterial structures, thereby requiring high barriers for

resistance and making them promising alternatives to traditional antibiotics. Buforin-II (BF2) is a naturally-occurring AMP that acts by first interacting with and translocating through the bacterial cell membrane and subsequently targeting intracellular targets such as DNA. The interactions between BF2, which is cationic, and both the membrane and DNA are likely mediated in large part by electrostatics. In this work, we use a combination of molecular dynamics simulations, electrostatic charge optimization, and continuum electrostatic calculations to identify neutral BF2 residues that, when mutated to arginine, lead to a range of increased binding affinities to either the cellular membrane or to DNA. By experimentally measuring both the binding affinities and the antimicrobial potency of these variants, we aim to understand the relationship between membrane binding affinity, DNA binding affinity, and potency in this translocating AMP system. Thus far, our work has led to the identification of a BF2 T1R variant with both increased DNA binding and antimicrobial potency, with computational models providing evidence for the structural basis of potential increased interactions with nucleic acids. Through computationally and experimentally comparing T1R and L8R variants, we have also demonstrated that BF2/DNA binding affinity is dependent not only on overall peptide monopole but also on its particular sequence. Preliminary ongoing work suggests that the structural determinants of tight membrane binding may be different than those for tight binding to DNA. Ultimately, we hope to build on our growing understanding of the relationship between multi-target binding and potency in this system to design and experimentally characterize BF2 variants with both membrane and DNA binding optimized for maximal potency.

ABS#600

Poster session, July 15

Biomimetic membrane fabrication using biologically derived nanochannels

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Development of biomimetic separation membranes which harness the separation phenomenon existing in nature has been in development for over a decade with varying degrees of success in design and performance. Biologically derived channel proteins incorporated into liposomes and nanosheets have been used to fabricate active layers to achieve increased permeability and a certain degree of salt rejection. However, the stability of lipid-based nanoparticles and the scalability of nanosheets has been a rate limiting step in achieving superior performance. We present a novel approach which uses polymersomes incorporated with porin channels producing a biomimetic membrane embedded within an active block copolymer layer crosslinked to a support layer. We show intact and functional protein molecules embedded in the layer as detected by fluorescence imaging. Additionally, we have also measured pure water permeability and salt rejection. A proteopolymersome coverage greater than 80% has been achieved. We believe that this approach is a stepping-stone to the development of a new class of biomimetic membranes which will revolutionize the separation technology and further push the boundaries in making target specific membranes using various types of porin protein channels with pore sizes from ~1nm - ~3 nm thus enabling the precise separation of water, salt, sugars, mRNA of different lengths and secondary structures to name a few.

ABS#602

Poster session, July 14

Practical Course in Macromolecular Crystallography

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As x-ray crystallography becomes more automated and the plethora of programs available increases, the new user to the field can be overwhelmed by the sheer number of choices available for any task from data processing, data analysis, structure solution and refinement. In order to make crystallography more accessible, NE-CAT offers a course in the practical aspects of crystallography, guiding users at all levels: undergraduate student, graduate student, postdoc or new investigator. The course covers

the programs needed to take a crystal from an initial dataset to a final publication-ready structure.

ABS#603

Poster session, July 15

Refining the Understanding of Sortase Enzymes via Molecular Dynamics Simulations and Experimental Analysis

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Characterization of Sortase enzymes through a combination of molecular dynamics simulations and experimental assays has proven to be an invaluable tool for unraveling the structural dynamics of enzymatic catalysis. These methods have enabled significant progress in our understanding of sortase B enzymes, which suffer from a lack of experimental and structural data. Sortase enzymes are cysteine transpeptidases expressed on the cell surface of gram-positive bacteria and play vital roles in cell adhesion, immune evasion, housekeeping, and various other functions across different classes (A, B, C, D, and E). However, class B sortases remain poorly understood both in terms of their mechanics and structure, despite their potential applications beyond their native bacterial context.

One promising application is Sortase-mediated ligation (SML), which exploits the specific recognition sequence of sortase enzymes. Through enzymatic cleavage, SML enables the anchoring of proteins to solid supports, antibody labeling, and other diverse applications. However, the utility of these sortase-based applications is hindered by our limited knowledge of their underlying structure.

By combining molecular dynamics simulations with experimental assays, we have made significant strides in enhancing our understanding of sortase B enzymes. These investigations reveal the intricate mechanisms of enzymatic catalysis and offer insights into their dynamic behavior. By exploring the structural details of sortase enzymes, we can expand the scope of their applications and exploit their potential beyond their conventional role in bacteria. These advancements hold promise for the development of novel techniques and technologies, paving the way for innovative applications in biotechnology and other fields. These findings contribute to the broader understanding of enzymatic catalysis and open doors to exciting opportunities in various practical applications.

ABS#604

Poster session, July 13

A biophysical framework for the double-drugging of kinases

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Drugging kinases with orthosteric inhibitors, while being immensely successful, has encountered significant challenges. Selectivity issues leading to side effects and the emergence of resistance mutations have rendered treatments targeting the active site of kinases ineffective. Recently, simultaneous inhibition of distant orthosteric and allosteric sites, which we refer to as "double-drugging", has been shown to be effective in overcoming drug resistance. However, a comprehensive biophysical characterization of the cooperative interplay between orthosteric and allosteric modulators has not been undertaken. In this study, we present a quantitative framework for double-drugging kinases utilizing isothermal titration calorimetry, Förster resonance energy transfer, coupled-enzyme assays, and X-ray crystallography. We discern positive and negative cooperativity for Aurora A kinase (AurA) and Abelson kinase (Abl) with different combinations of orthosteric and allosteric modulators and find that a shift in the conformational equilibrium is the main principle governing cooperativity. Notably, we observe a synergistic decrease in the required dosages of orthosteric and allosteric drugs when used together to inhibit kinase activities, achieving clinically relevant inhibition levels for both AurA and Abl. X-ray crystal structures of the double-drugged kinase complexes provide molecular insights into the cooperative nature of double-drugging AurA and Abl with orthosteric and allosteric inhibitors. Finally, we observe the first fully-closed conformation of Abl when bound to a pair of positively cooperative orthosteric and allosteric modulators, shedding light on the puzzling abnormality of previously solved closed Abl structures. Collectively, our data provide mechanistic and structural insights into the rational design and evaluation of future double-drugging strategies.

ABS#605

Poster session, July 14

Biophysical-Mechanical Correlation for Engineered Recombinant Hybrid Spider Silks

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Spider silks are natural protein-based biomaterials produced by spiders for purposes such as web construction, locomotion, wrapping of prey and protection of eggs.. They are renowned for their mechanical properties and hold great promise for applications ranging from high-performance textiles to the regenerative medicine. Our lab has been focused on two less characterized spider silks: aciniform and pyriform silks. Each silk has distinctive mechanical behaviour and physicochemical properties, with materials produced using combinations of these silks currently unexplored. This study focuses on the development and comparative characterization of hybrid silk fibres spun from fused proteins consisting of repeat units of aciniform (W) and pyriform (Py) silks. Two recombinant silk fusion proteins of the same composition but differing architecture (Py-Py-W-W and Py-W-Py-W) were expressed in Escherichia coli and purified by Ni2+-NTA affinity chromatography. Far-UV circular dichroism spectroscopy demonstrated α-helicity for fused proteins in a fluorinated acid- and alcohol-based solution used to form a dope for wet-spinning. Building on methods previously introduced in our lab, wet-spinning enabled continuous fibre production. For Py-Py-W-W, post-spin stretching of the resulting wet-spun fibres (i.e., stretching from the "as-spun" state) in air or ethanol significantly improved the extensibility (∼30-fold more extensible) and the tensile strength (∼4-fold stronger), respectively. Polarized light microscopy revealed that the anisotropy of hybrid fibres increased upon post-spin stretching, indicating increased molecular alignment along the fibre axis. Fibre-state secondary structuring was evaluated by Fourier transform infrared spectromicroscopy which demonstrated differences in the proportions of α-helix, β-sheet, and other structures as a function of post-stretching conditions. Comparison of the morphology, structuring, and mechanical properties of Py-Py-W-W and Py-W-Py-W will allow us to make inferences about the underlying influence of positioning of repeat units upon mechanical properties. Understanding the properties of hybrid fibres will determine their suitability and tunability for disperate applications.

ABS#606

Poster session, July 13

Unraveling the Structure of the Circadian Clock Late Repressive Complex

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The late repressive complex (LRC) containing Brain and Muscle ARNT-Like 1 (BMAL1), Circadian Locomotor Output Cycles Kaput (CLOCK), and Cryptochrome 1 (CRY1) is an indispensable transcriptional protein structure involved with maintaining circadian rhythmicity in mammals [1-3] (Fig. 1A). Given that numerous studies have demonstrated the formation of this complex [2-9], in this study we aim to utilize cryogenic electron microscopy (Cryo-EM) to resolve the structure of the LRC. We first utilized the AlphaFold-Multimer to predict a putative model of this complex [10,11]. Next, we leveraged the baculoviral expression system to co-express BMAL1, CLOCK and CRY1 containing different tags [5]. The expressed LRC was then subjected to affinity chromatography (AC), ion exchange chromatography (IEX), and size exclusion chromatography (SEC). The purified protein sample was validated for the presence of the LRC using negative-staining electron microscopy (EM). Subsequent cryo-EM imaging will be performed of this sample. Although the AlphaFold-Multimer failed to predict a reasonable structure of the LRC (Fig. 2A & B), the results of AC and SEC indicated that CRY1 can be co-purified and co-migrate with BMAL1 and CLOCK, suggesting the formation of a stable complex (Fig. 3A & B). Images from the negative-staining EM revealed the presence of the complex (Fig. 4A). Additionally, we aim to knock out BMAL1 with CRISPR/Cas9 in U2OS cells to evaluate residues located at critical interfaces of BMAL1 and CLOCK/CRY1 binding after reconstructing the LRC structure. BMAL1 knock out was validated by Sanger sequencing (Fig. 5A & B), and subsequent single cell isolation and validation by Western Blot are ongoing. In conclusion, our current work is paving the way for solving the structure of the LRC using cryo-EM. The structure of the LRC and understanding of BMAL1 interactions with the other components of the LRC can deepen our knowledge on how the circadian rhythm is maintained from a mechanistic perspective and benefit structure-based discovery of circadian drugs to treat diseases such as cancer.

ABS#607

Poster session, July 13

No Granules, No Problem: Stress Granules Are Not Required For mRNA Condensation Or Translational Regulation During Stress

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When cells experience stress, they undergo a number of conserved molecular changes including the formation of biomolecular condensates, clusters of protein and RNA. When these clusters become visible by microscopy, they are called stress granules, and their presence is conserved across species and stresses. While it has long been speculated that stress granules may play an adaptive role in a cell's ability to respond to stress, their function has remained unclear. Out of the many proposed functions for stress granules, most do not require the large size of stress granules, raising the question of whether stress granules themselves are functionally important or merely easy to see.
Here, our investigations of stress-induced mRNA condensation reveal that submicroscopic molecular clusters form in functionally coherent ways even when stress granules are not visible or are pharmacologically blocked. Using sedimentation, we have identified RNA molecules that condense during stress. Additionally, we found that perturbing the translational state of transcripts affects the magnitude of condensation, connecting RNA condensation to translation initiation. Our results suggest a model in which translationally-blocked RNA condenses, while translationally-competent transcripts escape condensation. Condensation may function to sequester most mRNA during stress, promoting the translation of specific stress transcripts.

ABS#610

Poster session, July 15

Optimizing a Synthetic Carbon Capture Cycle by Increasing the Activity of a Thermophilic Carboxylase at Room Temperature

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The exponential growth of atmospheric carbon dioxide is one of the main driving forces of global warming Synthetic biology has the potential to contribute to reducing atmospheric CO2. A synthetic carbon-fixing module was developed at NCState based on the reductive TCA (rTCA) cycle to improve the yield of biofuel feedstocks. The synthetic cycle utilizes two thermophilic enzymes from Aquificales: 2-Oxoglutarate Carboxylase (OGC) and Oxalosuccinate Reductase (OSR) that carboxylates and reduces 2-oxoglutarate to isocitrate. In order to optimize the synthetic rTCA cycle, we are engineering these enzymes to increase their activity at room temperature. OGC is a biotin-dependent carboxylase homologous with pyruvate carboxylase. All biotin-dependent carboxylases are composed of three domains. The first biotin carboxylase (BC) domain is conserved in all members of this family and utilizes ATP to capture CO2 and carboxylate the biotin cofactor. We determined the crystal structure of the OGC BC domain with and without substrates. ATP binding results in a large conformational change in the BC domain, stabilizing the “lid” in a closed conformation. We propose that product release is rate-limiting for enzyme turnover. Mutations that facilitate lid opening would be expected to decrease the temperature of activity of OGC BC. The product of OGC, oxalosuccinate, is reduced by OSR to isocitrate. OSR is an ancestral non-decarboxylating isocitrate dehydrogenase. The structure of OSR will elucidate the evolution of isocitrate dehydrogenase and facilitate the design of a mesophilic OSR. The resulting mesophilic OGC and OSR will promote carbon capture by the 5-enzyme synthetic rTCA cycle, catalyzing the conversion of 2-oxoglutarte to isocitrate in the reductive direction at room temperature.

ABS#611

Poster session, July 15

Capturing Transient Oligomers in Peptide Aggregation

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Alzheimer's disease (AD) is associated with aggregation of the amyloid-β (Aβ) peptide into amyloid fibrils which accumulates as neuritic plaques in the brain. The initial steps in Aβ aggregation include the formation of small pre-nucleation oligomers, which exhibit high toxicity and are thus interesting species in AD pathology. However, studying Aβ oligomers is challenging due to their polydispersity, instability, and low abundance. We here employ native ion mobility-mass spectrometry (IM-MS), all-atom molecular dynamics (MD) simulations, and gas phase infrared (IR) spectroscopy to gain mechanistic insights into the structures and assembly pathways of these transient species. IM-MS enables sensitive detection of the entire peptide-ensemble and reports on the relative abundance of oligomers. This is complemented by IR and MD which in contrast provide in-depth structural and dynamic information on selected states. The studies are conducted on several sequence variants to systematically modulate the folding and aggregation state of the peptide.

The unstructured Aβ peptide gradually adopts β-sheet structure as the oligomeric state (n) increases, through folding into a β-hairpin conformation (Figure 1A). The oligomers grow spherically until reaching n = 4, at which point they transition to linear growth. Introducing an intramolecular disulfide bond enhances the formation of linear oligomers by pre-arranging the peptide into the hairpin fold. Truncating the C-terminal end of the Aβ peptide on the other hand reduces the hairpin propensity and eliminates the linear oligomers (Figure 1B). This highlights the importance of the hairpin motif, as modulating the presence or absence of this fold greatly influences the oligomer population. Such modulation could potentially be employed therapeutically to prevent oligomer formation and mitigate disease-related effects. Moreover, membrane-mimicking micelles are found to stabilize Aβ oligomers, inhibiting the transition into extended structures and nucleation into fibrils. This exemplifies a possible in vivo mechanism for enrichment of toxic oligomers.

ABS#612

Poster session, July 15

Use of Computationally Designed LOO-GFP to Make Fluorescent Biosensors in Fibrous Protein Biomaterials

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Green Fluorescence Protein (GFP) has been used as a template for the "leave-one-out" (LOO-GFP) biosensor design method in which GFP is engineered to omit a segment from the middle of the sequence by circular permutation and truncation. The empty site is computationally designed to accommodate a peptide from a protein target. In our current design, we have used a highly stable and fluorescent mutant of GFP called OPT, with the 11th strand left out, immobilized on fibers by a fusion protein named SGMU [Sortase A (SrtA)- LOO-GFP- Maltose Binding Protein (MBP)- Ultrabithorax (Ubx)]. Ubx is a protein that forms fibers by spontaneous cross-linking of tyrosine side chains. The protease activity of Sortase A is activated by calcium to cleave a linker that connects LOO-GFP and the left-out strand, allowing the latter to diffuse away when LOO-GFP is unfolded by dropping pH. MBP is a bulky protein used to separate LOO-GFP from Ubx, fixing a problem observed with the previous construct where LOO-GFP sticks electrostatically to Ubx. GFP strand 11, the target peptide, was fused N-terminally before SrtA. SGMU was expressed well in Escherichia coli bacterial system at low temperature and was purified using Ni-NTA chromatography. SGMU polymerized upon overnight exposure to air in a wide pan, producing a protein skin. Fibers are produced by pulling, using a paper clip. We show that SrtA is activated by calcium, efficiently cleaves the linker, and that the target peptide with SrtA attached efficiently diffuses quickly out of the fibers. LOO-GFP must be pH unfolded to remove the "priming" peptide that allows the chromophore to form. Upon returning to folding conditions without the peptide present, not all the LOO-GFP returns to a state that can bind the peptide.

ABS#613

Modern Anti-viral Strategies (July 13, AM)

Structural insights into the development of pan-Coronavirus immunotherapies

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Human monoclonal antibodies from convalescent individuals that target the SARS-CoV-2 spike protein have been used as SARS-CoV-2 therapeutics. However, SARS-CoV-2 variants that evolved to resist similar, naturally occurring antibodies have rendered nearly all of these first-generation antibodies obsolete. Furthermore, most SARS-CoV-2 antibodies are ineffective against other sarbecoviruses and the larger Orthocoronavirinae subfamily. Thus, the development of broadly neutralizing anti-Spike antibodies against SARS-CoV-2 variants of concern as well as future coronaviruses with pandemic potential is needed. We recently discovered a set of human monoclonal antibodies that widely neutralize emerging SARS-CoV-2 variants with the same potency as the most potent spike binding therapeutic antibodies, while also cross-reacting with many spike glycoproteins from the four human coronavirus genera. Structural and biochemical investigations revealed that these antibodies target conserved epitopes, suggesting that they could be valuable prophylactic and therapeutic agents against any current and future SARS-CoV-2 variants, as well as zoonotic coronaviruses that could lead to future pandemic threats.

ABS#614

Poster session, July 13

Interactions of selenoprotein S with proteins in the endoplasmic reticulum-associated degradation (ERAD) pathway

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Selenoprotein S (selenos) is an endoplasmic reticulum membrane-embedded enzyme whose function remains unclear. Previous studies have indicated its involvement in endoplasmic reticulum-associated degradation (ERAD), a process responsible for recycling misfolded proteins and misassembled complexes. Malfunctions in ERAD have been associated with various pathological conditions, highlighting the importance of studying this process.

In the ERAD, selenos is known to interact with derlins which are part of the protein pore that translocate proteins from the ER lumen to the cytoplasm for degradation. However, the role of selenos or its mode of association with derlins remain unknown. Therefore, our objective was to use cryogenic electron microscopy (cryo-EM) to determine the structure of the derlin bound to selenos. To accomplish this, we expressed the derlin-2, which is known to contribute to the degradation of glycosylated proteins, with selenos in insect cells. The complex was then purified in sufficient quantities for subsequent structural characterization by cryo-EM.

ABS#617

Poster session, July 13

Experimental evidence of intrinsic disorder and amyloid formation by the Henipavirus W proteins

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The Nipah (NiV) and Hendra (HeV) viruses are zoonotic agents gathered in the Henipavirus genus within the Paramyxoviridae family. Beyond the P protein, the Henipavirus P gene also encodes the V and W proteins which share with P their N-terminal, intrinsically disordered domain (NTD) and possess a unique C-terminal domain (CTD). Henipavirus W proteins antagonize interferon (IFN) signaling through NTD-mediated binding to STAT1 and STAT4, and prevent type I IFN expression and production of chemokines. Structural and molecular information on Henipavirus W proteins, and in particular on their CTD, is lacking. By combining bioinformatic and biophysical approaches, we showed that the Henipaviruses W proteins and their CTD are intrinsically disordered. In addition, fluorimetry assays and negative-staining transmission electron microscopy showed that the W proteins form amyloid-like fibrils. Finally, by confocal and ultramicrotomy experiments the presence of the fibrils was also confirmed in a cellular context, strengthening the hypothesis of their involvement in Henipavirus pathogenesis. The present study provides an additional example, among the few reported so far, of a viral protein forming amyloid-like fibrils, therefore significantly contributing to enlarge our currently limited knowledge of viral amyloids.

ABS#618

Poster session, July 14

Characterizing the conserved NM domain pH sensor of the yeast protein Sup35

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Intrinsically disordered regions (IDRs) are flexible domains that initiate assembly changes across eukaryotic species. These proteins are associated with toxic aggregates in humans, yet some eukaryotes use these domains as environment sensors and undergo amyloid self-assembly and/or phase-separation potentially as an advantageous characteristic. An example is Sup35 in Saccharomyces cerevisiae, which is involved in translation termination in yeast and conserved across many distant yeast species. This protein has been extensively characterized in vivo but lacks basic structural data in its native (disordered) state. The extremely distant yeast S. cerevisiae and S. pombe both have Sup35 with conserved NM domains that undergo liquid-liquid phase separation upon changes in pH (Franzmann, et al., 2018), indicating these proteins can serve as environmental sensors within the cell. The conserved domains between these two species have a glutamine-rich N-terminal domain and charged middle domain with glutamic acids that are clustered, which could upshift the pKa of glutamic acid to protonate the side chain at a higher pH and serve as a pH sensor. Characterizing the switch to the assembly of intrinsically disordered proteins is invisible by traditional structural techniques, but we provide our progress using NMR spectroscopy to characterize these potential pH sensors and evaluate which particular residues participate in sensing the environmental change.

ABS#619

Poster session, July 14

Rare Crystal Form at High Resolution Offers Unique Insights Into the Conformational Heterogeneity of Apo PTP1B

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Cellular homeostasis is at the heart of normal functioning in a cell. Several processes work to maintain this balance, phosphorylation being a key mechanism. The proteins that drive this process are kinases and phosphatases, working antagonistically to maintain normal levels. In contrast to kinases, phosphatases are relatively understudied and have not been successfully targeted with clinically approved drugs, despite being well-established therapeutic targets. The challenges so far have mostly revolved around the limitations of active-site inhibition, which call for more structural work to better characterize potential new allosteric susceptibilities in phosphatases. Here, I present a structural study of the founding member of the non-receptor protein tyrosine phosphatase family, PTP1B (protein tyrosine phosphatase 1B), a validated therapeutic target for diabetes and obesity. This study uses X-ray crystallography to solve the structure of apo PTP1B in a rare crystal form. This dataset is among the highest-resolution out of >250 crystal structures of PTP1B and is the highest-resolution WT apo structure to date. Moreover, unlike most crystal structures of PTP1B, our crystal form features two chains in the asymmetric unit that are conformationally distinct. This new structure enables a controlled comparison of conformational heterogeneity between chains at the residue level and comparisons to other previous structures, using various computational tools focused on side-chain rotamer sampling, hierarchical B-factors, and more. Overall, our new structure opens doors to better characterizing the conformational landscape of PTP1B.

ABS#620

Poster session, July 14

NRas Q61 oncogenic mutations and dimer formation in the presence of BRaf-RBD

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The Ras proteins are small GTPases that regulate cell growth and proliferation. They act as binary switches in the MAPK/ERK pathway, shifting between active (GTP-bound) and inactive (GDP-bound) states. NRasQ61 oncogenic mutations of NRas at residue 61 are aggressive drivers of melanomas through molecular mechanisms that are not fully understood. Even though the NRas Q61X mutants (X= H, K, L, R) are all oncogenic, previous biochemical and biophysical characterizations showed that they can either increase or decrease GTP

hydrolysis rate constants in comparison with NRas wild-type. Therefore, the hydrolysis rate constants measured in solution for the monomeric forms of NRas do not show a clear correlation with the oncogenic phenotypes associated with these mutants. For instance, the mutant NRasQ61R shows hydrolysis rate constants nearly 3 times higher than that of the wild-type protein, suggesting that impaired hydrolysis is not a major factor. The fact that mutations in NRas are prevalent in melanomas, which also can be driven by mutations in Raf, points to possible oncogenicity in the context of the Ras/Raf/MEK/ERK pathway. Recently, our lab has shown that CRaf-RBD interaction with the G-domain of Ras changes its dynamics/allosteric effects and that Raf-RBD induces dimerization of Ras on supported lipid bilayers. Based on these findings, here we aim to explore this new perspective and study the oncogenic mutations in the context of the NRas/BRaf-RBD dimer. Using size exclusion chromatography, we have shown that the mutants NRasQ61X in complex with BRaf-RBD favor the dimer in solution, relative to the wild-type protein, for which the monomer is the prevalent species. In the future, we plan to obtain crystal structures of the NRasQ61X/BRaf dimer for molecular dynamics (MD) simulations and community network analysis, to study how allosteric pathways that promote dimerization are altered in the oncogenic mutants.

ABS#621

Poster session, July 15

Isotopic Labeling via Mammalian Cell Culture Expression of preT Cell and T Cell Receptor β Subunit for Protein NMR

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Protein NMR depends on isotopic labeling of polypeptides by utilizing heterologous expression in a variety of hosts. Most commonly E. coli cell lines are used by leveraging isotopically substituted ammonium and glucose to uniformly label proteins with 15N and 13C, respectively. Moreover, E. coli are also able to grow and express proteins in uniformly deuterium substituted water (D2O), a strategy useful in experiments targeting high molecular mass proteins. Unfortunately, many proteins cannot be expressed readily with their native post-translational modifications like disulfide bonding or glycosylation in E. coli hosts. One such class of proteins are the T cell receptors (TCRs) and their related preT cell receptors (preTCRs). As such, we have expressed the TCRβ subunit in the non-adherent human embryonic kidney cell line EXPI293F. In addition, we have specifically labeled the amino acids Ile, Leu, and Val in this system utilizing an amino acid specific labeling protocol that allows targeted incorporation at high occupancy without significant isotopic scrambling. These results are broadly applicable and will allow us to explore the dynamic interaction between these important immune receptors and their peptide-MHC ligands and bring insights into the earliest selection processes of the mechanoresponsive T lineage.

ABS#622

Poster session, July 14

Adsorption of DNA-Binding Proteins via Engineered Host DNA Crystals

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DNA tensegrity triangles are nanostructures formed by seven DNA strands that join three dsDNA blocks with three Holliday Junctions. The resulting tiles exhibit remarkable self-assembly properties, serving as versatile building blocks for constructing complex DNA-based materials. In this study, we utilized the software NUPACK to design a new DNA crystal that displays the sequence motif RCCGG within the solvent channels of the designed tensegrity triangle crystal lattice. RCCGG is the recognition sequence for c-clamp, a truncated human zinc finger from the DNA-binding domain of HDBP1. The designed DNA annealed into tiles, via slow cooling from 60 C, proceeds to grow into crystals. These DNA crystals were then stabilized via chemical ligation with 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) which joins stacked DNA within the crystal into longer contiguous DNA

strands. Ligation resulted in DNA crystals hardy to their environment, opening doors to the ability of this biomaterial to function in a plethora of environments. Ligated tensegrity triangle crystals were then incubated in solution with 5-Carboxytetramethylrhodamine (TAMRA)-tagged c-clamp protein. Soaking resulted in visible adsorption of c-clamp protein from solution. Guest protein capture was quantified by confocal microscopy, comparing TAMRA fluorescence within the crystal to the surrounding solution. This study demonstrates that it is possible to design and grow DNA lattices that are customized for the capture and retention of specific DNA-binding proteins. This targeted loading of a precisely ordered DNA biomaterial innovates on traditional tensegrity tile designs by functionalizing the DNA sequences beyond their tertiary structure formation. Fortifying these DNA crystals and demonstrating their ability to adsorb target proteins from the environment opens the door for downstream applications such as high-specificity purification, extended release delivery, or biomolecular sensing material.

ABS#623

Poster session, July 15

Identification and Characterization of Small Molecule Inhibitors of the LINE-1 Retrotransposon Endonuclease

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The long interspersed nuclear element-1 (LINE-1 or L1) retrotransposon is the only active autonomously replicating retrotransposon in the human genome. While L1 is usually repressed to protect genomic integrity, L1 expression often occurs in cases of cellular dysfunction, such as during aging. As a result of this activation, L1 can contribute to disease progression by inserting new copies, generating DNA damage, and triggering inflammation. Therefore, preventing L1 activity by targeting its proteins is a unique avenue for developing therapeutics applicable to a variety of diseases. L1 encodes the RNA-binding protein ORF1 and the enzymatic protein ORF2, which contains endonuclease (EN) and reverse transcriptase (RT) activities required for retrotransposition. Previous research has focused on inhibition of the L1 reverse transcriptase due to the prevalence of well-characterized inhibitors of related viral enzymes and demonstrated the efficacy of these inhibitors in reducing the harmful impacts of L1. However, development of RT-specific inhibitors is hampered by difficulties in biochemical characterization. On the other hand, the EN domain structure has been solved, making it amenable to computational and biochemical screening. Additionally, the EN initiates retrotransposition and L1-induced DNA damage is mediated by EN activity. Therefore, here we present the L1 endonuclease (EN) as another target for reducing L1 activity. We have discovered and characterized structurally diverse small molecule EN inhibitors using computational, biochemical, and biophysical methods. We also show that these inhibitors reduce L1 retrotransposition, L1-induced DNA damage, and inflammation reinforced by L1 in senescent cells. These inhibitors could be further used to better understand the L1 life cycle and its impact on human health, and as potential initial candidates for the design of L1-targeted therapeutics.

ABS#625

Poster session, July 14

Functionalization of shell proteins with disordered regions promotes membrane formation around condensates

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To carry out complex reactions, cells sequester enzymes and small molecules through the formation of compartments. These include organelles surrounded by lipid membranes, bacterial microcompartments (BMC) formed by shell proteins similar to viral capsids, and membraneless organelles made of dense droplets of intrinsically disordered proteins (IDP) through liquid-liquid phase separation (LLPS). The last two options are of interest to the ProteoCell project as their components are DNA encodable.

We used the RGG domain of the common IDP LAF-1 to observe LLPS. Like other IDPs, RGG droplets sequester small molecules and a wide array of proteins. The ability to restrict access has then become a recent interest. Research has shown that lipids will form a membrane around the droplets, providing both a barrier and stabilization of the dynamic system. To form a proteinaceous membrane, we expressed BMC-H shell protein 5815 with the RGG domain in varying lengths at the C-terminus.

It was observed that during LLPS, the shell proteins with RGG truncations will form a membrane at the droplet interface. Without the RGG domain, BMC proteins are sequestered entirely within the droplet, indicating that a disordered domain is necessary for shell formation in a phase separated system. Additionally, the amount of partitioning is related to the length of the truncation. Longer disordered sequences were sequestered more within the droplets suggesting an optimal length of the disordered region for membrane formation at the interface.

ABS#626

Poster session, July 14

Engineering brighter fluorescent proteins with DropSynth and machine learning methods

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Fluorescent proteins (FPs) are integral tools in the observation and investigation of biological phenomena. However, the color spectra of currently available FPs is sub-optimal, particularly in the blue and red spectral ranges, which are significantly less bright. To address this, we have used DropSyth multiplexed gene synthesis to assemble comprehensive protein libraries containing nearly all known beta-barrel FPs. We set out to synthesize 621 FPs and successfully recovered gene assemblies for 583 (94%) of these proteins. Utilizing gene shuffling, we diversified these libraries further, generating novel chimeric proteins. The observed diversity in this collection was only limited by our sequencing depth, with over 120,000 unique variants detected. To evaluate the brightness of the sorted blue and red barcode-tagged variants at scale, we used Flowseq and long-read sequencing to map barcodes to their corresponding variants. FACS sorting of the shuffled library revealed that 1.6% of sorted events emitted some blue fluorescence upon excitation with a 405 nm laser. In contrast, the original, unshuffled library contained approximately 15% blue members, which represented 5.7% of the sequenced population. This data provided a valuable training set for machine learning models, enabling us to generate novel libraries potentially filled with brighter red and blue FPs. We fine-tuned a deep unsupervised language model, ProtGP2, which had been trained on protein space, using 16,367 blue-sorted FPs, resulting in the creation of 1536 novel diverse variants. Following assembly, FACS sorting of this library revealed that 0.3% of events emitted blue fluorescence, thereby validating the model's capacity to learn key functional properties. In conclusion, our study demonstrates the potential of both DropSyth technology and machine learning in exploring and enhancing the functional sequence space of FPs. The generation of diverse mutant libraries, combined with the evaluation of brightness in the blue and red regions, lays the groundwork for the development of improved FPs.

ABS#627

Poster session, July 14

An In-gel Proteomic Investigation of Human Serum from Donors with Stage IIB Breast Cancer and Matched Controls to Identify Protein Biomarkers for Earlier Breast Cancer Detection

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Breast cancer (BC), is a leading cause of death for women globally. Invasive ductal carcinoma (IDC) is one of the most common subtypes, accounting for 80-85% of all BCs. Early diagnosis and treatment of BC is crucial. Samples were chosen on the basis of the TNM anatomic staging system: this study focuses on 8 women with stage IIB BC with tumors between 30-50mm across (T2) and invasion into 1-3 nearby lymph nodes (N1) which were age-matched to 8 controls. One way to detect BC in its early phase is through identification of proteins that are dysregulated due to the onset of BC (i.e. protein biomarkers). Mass spectrometry (MS)-based proteomic methods are ideal for the investigation of serum protein biomarkers, as serum is an accessible 'tissue' for screening studies. MS-based proteomic experiments enable quantification of protein differences for serum from women with BC compared to controls. Differentially expressed protein biomarkers could be diagnostic for BC, facilitating earlier diagnosis and treatment. We use in-gel based proteomic methods followed by nano-liquid chromatography tandem mass spectrometry(nanoLC-MS/MS) to identify proteins which are dysregulated between the matched sets but also quantify these proteins. If specific proteins are found to be consistently dysregulated in larger BC cohorts, a protein biomarker could be established to aid in the diagnosis, evaluation, and treatment of BC.

ABS#628

Poster session, July 14

Repurposing an Anti-Tumor Drug Candidate for Targeting Bacterial ATP-Dependent Proteases

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Protein turnover is a critical process for maintaining proteostasis. The bacterial AAA+ chaperones ClpX and ClpA, coupled with the peptidase ClpP form complexes (ClpXP and ClpAP, respectively) which mediate the degradation of proteins in many bacterial species by threading a substrate through the central pore of the chaperone into the proteolytic site of ClpP. Due to the essential function of these proteins in cell survival, they make for promising targets of novel antimicrobial drugs. Another AAA+ chaperone, ClpB, does not bind to ClpP but bears a similar function of threading substrates through a central channel for subsequent reactivation by other chaperones. We have previously discovered that N2,N4-dibenzylquinazoline-2,4-diamine (DBeQ), a small molecule inhibitor of the mammalian AAA+ protein p97, an anti-tumor target, inhibits ClpB and suppresses the growth of E. coli (Glaza et al., 2021). In this study, we investigated the effects of DBeQ on the engineered ClpB variant, BAP that, like ClpX and ClpA, binds to ClpP and mediates degradation of substrates, instead of their reactivation. We found that DBeQ inhibits the BAP/ClpP-mediated degradation of casein with an apparent IC50 $\sim$ 5μM, but does not affect the intrinsic peptidase activity of ClpP. The loss of BAP/ClpP activity is linked to DBeQ-induced dissociation of the BAP-ClpP complex, as determined by sedimentation velocity and gel filtration experiments. We hypothesize that DBeQ may serve as an inhibitor candidate for multiple ATP-dependent protease complexes, including ClpXP and ClpAP.

ABS#629

Poster session, July 15

In situ characterization of Opa1-dependent mitochondrial cristae ultrastructure

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The mitochondrion is a dynamic organelle that is critical for cellular processes, including apoptosis, ATP production, metabolism, and respiration [1]. Many of these functions are dependent on the shape of cristae or the folds in the inner mitochondrial membrane (IMM). Cristae architecture changes in response to a cell's functional needs, but mechanistic details of proteins that remodel cristae are not well understood. Opa1, an IMM localized protein, has a fundamental role in mitochondrial fusion and regulation of cristae architecture. IMM proteases process a portion of the Opa1 to yield two forms, an integral-membrane bound long-form (l-Opa1) and a soluble short-form (s-Opa1) [2]. Previous studies demonstrate that both forms of Opa1 are necessary for efficient mitochondria fusion [3] and loss of Opa1 dramatically affects cristae shape[4]. How each form of Opa1 influences cristae morphology is unknown. We used cryo-focused ion beam-scanning electron microscopy (FIB-SEM) and cryo-electron tomography (cryo-ET) to investigate how each form of Opa1 modulates cristae architecture in situ. We compared wild-type (WT) Mouse Embryonic Fibroblasts with cell lines where either Opa1 was over-expressed, all Opa1 remained unprocessed and in the long-form, all Opa1 is processed into the short-form, or Opa1 was knocked-out. We describe in detail cristae 3-dimensional shape and note correlations between cristae shape and impaired mitochondrial functions.

ABS#631

Poster session, July 14

Measuring the Conformational Ensemble of Caspase-9 using Ion Mobility-Mass Spectrometry

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Uncontrolled cell-death leads to neurodegeneration and cancers, underscoring the need for tight regulation. Caspase-9 (casp-9) is from a family of proteases that regulate apoptosis, and thus aberrant structures and behaviors

can disrupt cell-death. There is currently no high-resolution structure of full-length holo casp-9, and questions remain about its regulation, including via monomer:dimer equilibrium. Ion mobility-mass spectrometry (IM-MS) data obtained under native solution conditions (pH= 6.9; ionic strength= 100 mM ammonium acetate) is being used to provide insight into casp-9 structure and conformation in various maturation states. Casp-9 is composed of a pro-domain (CARD), large subunit, and small subunit, that assemble to form a monomer (C9WT). Collision cross sections (CCS) were measured for wild-type (C9WT), CARD, and the pro-domain-deleted variant (ΔCARD). Prior to the measurements, casp-9 activity in ammonium acetate was confirmed. C9WT displayed several charge state distributions associated with the monomer, suggesting multiple possible gas-phase conformations. Single distributions representative of the small subunit alone, the CARD+large, and the dimer were also observed. CCS values from the five AF2-generated solution-phase models of monomeric C9WT, which feature variations in relative CARD positioning, were calculated. Experimentally obtained CCS values for C9WT monomer 12+ through 15+ are 3320±10 Å, 3320±30 Å, 3560±60 Å, and 3600±100 Å, respectively. These are consistently smaller than those computationally obtained from solution-phase models, pointing to possible gas-phase compaction or a need for additional models. Molecular dynamics simulations in the absence of solvent are currently being conducted to assess the possibility of gas-phase compaction. We next investigated how the CCS of the CARD alone compares to C9WT and the large-small complex. We find that the CCS of the CARD alone (1480±20 Å) is about half that of C9WT, despite being responsible for only 138 of 422 residues, suggesting that the CARD compacts against the large and small subunits in gas-phase C9WT.

ABS#634

Poster session, July 14

Engineering Chimeric Receptor Histidine Kinases

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Bacteria have evolved an extensive range of sensor histidine kinases (HKs) that facilitate their interaction and response to environmental stimuli. These HKs detect a wide array of stimuli, including nutrients, pH changes, antibiotics, and various ions. Despite the existence of over a million HKs in metagenomic databases, functional characterization has only been achieved for several hundred. Moreover, the mechanisms for transmembrane signaling through these proteins remain poorly understood except in a few instances. To address these gaps, we are developing a platform for the rapid, large-scale, multiplexed characterization of HK sensors. This will help answer fundamental questions about the biology of the organisms that employ these sensors and offer valuable insights into how we can engineer these sensors for a range of biosensing applications.

We leveraged DropSynth multiplexed gene synthesis to construct a pilot library of 21,726 unique genes. These correspond to 10,863 unique protein variants of 3,061 unique HK sensor domains, which represent 18 of the most abundant sensor domain families. Our goal is to characterize their response across a myriad of stimuli and ligands. Our assay, employing either life-death or FACS selection, eliminates constitutively active HK chimeras and retrieves the corresponding DNA sequences for the sensors activated by each stimulus. This is facilitated by a toxin-based selection system and a dual-antibiotic selection system, both of which show low escape frequencies in E. coli strains that have at least one native HK gene knocked out. This multiplexed assay has the potential to scale to tens of thousands of sensors and contribute to building a map of sequence-ligand relationships for these ubiquitous systems.

Currently, we have successfully assembled, barcoded, and sequence-verified four chimeric HK libraries. We have also constructed several positive controls using previously characterized chimeras, which we utilized for initial validation of our experimental pipeline.

ABS#641

Poster session, July 15

Protein and Structural Chemistry (P&SC) at Merck

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Protein structure determination in drug discovery is one of the main drivers of structure-based drug design. At Merck, the Protein & Structural Chemistry (P&SC) group, co-located at five research sites (Boston, London, Rahway, San Francisco & West Point), is responsible for protein production and structural enablement of all targets of interest across the Merck Research Laboratories (MRL) portfolio, including soluble and integral membrane proteins. As part of the Discovery Chemistry (DC) organization within MRL, P&SC oversees all aspects of the gene-to-structure pipeline, including protein expression, purification, biophysical characterization, and structure determination. We utilize computational structure prediction for initial construct design efforts, coupled to automated small-scale expression screening and large-scale biomass production in prokaryotic and eukaryotic expression systems. Multiple automated or manual purification methods are used to isolate high-quality protein targets, which are subjected to a variety of biophysical characterization techniques to probe protein aggregation state (a-SEC/f-SEC/DLS), stability induction (nanoDSF and thermal aSEC), and ligand binding (SPR). Thorough target characterization enables our structural biologists to determine high-resolution, ligand-bound structures of MRL portfolio targets using x-ray crystallography and cryo EM. DC utilizes this structural information to enhance the physical and pharmacological properties of molecules of interest and potentially advance them as future new drugs. Additionally, P&SC delivers samples in appropriate formulations in support of a variety of MRL project portfolio requests, including antibody production, DNA encoded libraries (DEL) screening, electrophysiology (Ephys), etc. Recent advances in cryo EM and protein structure prediction are changing the way we approach projects, as we evaluate which techniques advance structural enablement at a faster and more efficient pace. Additionally, machine learning is guiding our considerations towards data capture, so that we are well-positioned for future challenges.

ABS#642

Poster session, July 15

Allostery in Single Pass Membrane Receptors Underlies Their Differential Signaling

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We use biophysical tools to study the allostery in fibroblast growth factor receptor (FGFR) signal propagation across the plasma membrane, in response to three FGFs that are co-expressed during human development. We show that the allostery results in different FGF potencies, efficacies, and bias, as well as differential FGFR ligand-induced oligomerization and downregulation. We find that FGF8 is a biased ligand, as compared to FGF4 and FGF9, as it induces differential phosphorylation of FGFR and associated proteins, as well as different functional responses. Ligand bias is a consequence of structural differences in the ligand-bound FGFR dimers, which impact the interactions of the FGFR1 transmembrane helices, leading to differential recruitment and activation of the downstream signaling adaptors. This is the first demonstration that allostery underlies the signaling specificity of the FGF-FGFR axis during development.

ABS#643

2023 Carl Brändén

Some People and Molecules I Have Known

Arthur Lesk (1)

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I am very honoured to have received this year's Carl Brändén award. I knew Carl very well. We shared many interests, including protein structure, computer graphics, and textbook writing. He was always very supportive of me, and on many occasions gave me useful – and very needed! – advice.

I first visited Uppsala in 1962, and returned on many other occasions. Also, Carl spent time at EMBL in Heidelberg, while he was working with John Tooze on their book, and we had adjacent offices. I have always had the utmost respect for him, both personally and professionally.

No discussion of my career would be complete without mentioning Cyrus Chothia. We were partners for many years, doing research on protein structure, function and evolution. His passing is a great loss for the field in general and for me personally. Like any good marriage we complemented each other: Cyrus had a background in chemistry and crystallography, and I came more from the maths/physics side. We joined forces to do biology.

We tried to deal with proteins comprehensively – I cannot say that I have a favourite molecule, although if pushed I would confess to a warm feeling for globins and immunoglobulns. But let me quote from a parody sung at an MRC Christmas party: 'My favourite, all others scorning / Is the one they solved this morning.'

ABS#644

Poster session, July 14

Unbiased real-space difference maps for X-ray crystallography

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Crystallography is a powerful tool for characterizing a protein's subtle conformational changes in response to a perturbation. Comparison of perturbed and unperturbed data sets is often performed by direct comparison of electron densities; differences in electron density are more sensitive to small changes and more resistant to model bias than differences in refined structure coordinates. The standard method for computing difference density is the "isomorphous difference map", in which a common set of structure factor phases is used for both data sets. Here, we present an improved method for calculation of difference maps by computing distinct, unbiased phases for each data set. This method presents improvements even in the standard, "isomorphous" case. Furthermore, this method can be applied to situations where an isomorphous difference map cannot be computed, such as inputs in different spacegroups or internal difference maps across non-crystallographic symmetry. This method, matchmaps, is implemented in python as an extension of classic crystallographic software suites. Matchmaps represents an important advance in the ability to extract interesting and unbiased signal from pairs of perturbed and unperturbed crystallographic data sets.

ABS#646

Poster session, July 15

Challenges For Protein Structure Prediction: Strained Geometry And Conformational Flexibility

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The use of machine learning language models in protein structure prediction has transformed structural biology with highly accurate models. Yet, there are a growing number of examples which are not accurately predicted. Here, I present two sets of examples. The first set has two crystal structures where the predictions have nearly perfect topology, but fail in regions where crystallographic experimental density supports non-preferred orientations of side chain or main chain. In the example of an unusual histidine rotamer, misprediction led to complete failure of side chain prediction in the active site. The second set, pulled from 2020 Critical Assessment of protein Structure Prediction (CASP)14 analysis, contains twd that the proteins in solution adopt distinct conformations from the crystal structure. The structure predictions are more similar to the crystal than either to the solution conformation. These results suggest that current structure predictions may be biased by their training databases, both in the PDB and in preferred orientations.

ABS#648

Poster session, July 14

Impact of metal ions on the aggregation kinetics of fragment prion 106-128 and targeting metal homeostasis as a therapeutic strategy

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The hallmark of prion diseases is defined by the structural transformation of the cellular prion protein (PrPc) to its scrapie isoform (PrPSc), which is the plausible agent in transmissible spongiform encephalopathies (TSEs). The secondary structures of the PrP106-128 are primarily influenced by various intra and extracellular

environments, such as the presence of the metal ions, Copper, zinc, and iron, which are abundant in the brain's synaptic regions, and these biometals' age-related homeostasis may be linked to prion diseases. The principal aim of this study is to understand the impact of metal ions such as Cu2+ and Zn2+ and their coordination role to the peptide fragment 106-128 aggregation using different techniques; ThT binding assay, Atomic Force Microscopy (AFM), and CD. Our findings indicate that Cu2+ and Zn2+ are effective, mild, and poor inhibitors of PrP106-128 aggregation. It is demonstrated that Cu2+ ions, but not the Zn2+ ions, are engaged in the production of dimers or wrap the Cu2+ ion inside the peptide, which prevents aggregation. Surprisingly, Cu2+, which might form a stronger complex with peptide than Zn2+, is a more potent inhibitor of peptide fibrillization—in vitro studies. Furthermore, essential trace metal ions Cu2+ and Zn2+ are aimed to be removed using metal chelators carnosine and EDTA. Interestingly, carnosine is a moderate chelator of Zn2+ but a potent inhibitor of Cu2+ and Zn2+-mediated PrP106-128 aggregation. EDTA has a more pronounced effect than carnosine on the Zn2+-mediated aggregation process. In conclusion, our aggregation studies showed the impact of metal ions on the aggregation mechanism of PrP106-128. This facilitates understanding the possible role of metal ions in the prion disease and provides new insight into the key role of metal chelators. This study may contribute to identifying a potential therapeutic target in prion disease.

ABS#649

Poster session, July 15

Crystal Structure of K-Ras in Complex with B-Raf-RBD-CRD Reveals Differences in Its Interaction with C-Raf Relevant To Ras Dimerization

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Elucidation of the complex interplay between Ras and Raf kinase, particularly the critical role played by the cysteine-rich domains (CRD) of Raf, is of vital importance in understanding the molecular mechanisms of Ras-mediated Raf activation through dimerization of the Ras/Raf complex. Here, we present a 3.2 Å crystal structure of Ras in complex with a B-Raf construct containing both the Ras-binding domain (RBD) and the CRD, revealing the interface for B-Raf-CRD binding. In addition to pointing out key differences in the B-Raf interaction with Ras relative to C-Raf, the current investigation presents binding assays done in solution with a mixture of C-Raf and B-Raf to determine the propensity of the heterodimer C-Raf/K-Ras/K-Ras/B-Raf to form relative to the homodimer. Our crystal structure lays the ground for the characterization of K-Ras/Raf dimerization, with comparative analyses of published H-Ras/C-Raf-RBD-CRD and K-Ras/C-Raf-RBD-CRD structures. In the future, we plan to gain mechanistic insight into the role of Raf-CRD in Ras/Raf dimerization. Advancing our mechanistic understanding of Ras-Raf interplay is essential for informing future therapeutic interventions aimed at modulating dysregulation of the Ras/Raf/MEK/ERK pathway in Ras-driven cancers.

ABS#650

Poster session, July 15

Engineering Cancer-Selective Protein Payload Expression Through Alternate Translation Initiation in mRNA Therapeutics

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mRNA therapeutics, where LNP-encased transcripts can be delivered to cells of interest and translated by endogenous machinery to form targeted protein products, have been used to great effect in the design of COVID-19 vaccines. Recent work has also demonstrated their applicability to a wide range of other diseases, including cancer. However, targeting these therapeutics to specific cell types through translational control is crucial for avoiding off-target effects. Here, we present several examples of mRNA 5'UTR regions that lead to altered translation of adjacent coding regions in cancer cells compared to healthy cells. Building off of observations that ribosome biogenesis and recruitment can be altered in tumorigenic cells, we hypothesized that noncanonical translation could be exploited to design custom mRNA sequences that would produce therapeutic protein in cancer but evade translation in healthy cells. The starting points for these sequences were identified through a novel high-quality ribosomal footprint sequencing dataset for

chronic myelogenous leukemia-derived K562 cells. These endogenous UTR regions contained alternative translation initiation start sites (aTIS), leading to different protein products when compared to non-cancerous cells. Furthermore, some of these differential protein products were confirmed through publicly available LC-MS/MS proteomics experiments. We then used these 5'UTRs to design mRNA constructs containing coding sequences corresponding to fluorescent reporter proteins, and tested the oncoselectivity of these aTIS candidates directly against fibroblast cells. We obtained up to 8-fold selectivity of alternate protein products in cancer vs non-cancer lines solely from the inclusion of these 5'UTRs. These results provide a proof of principle that noncanonical translation can lead to differential selection in cancer cells, and that this process can be exploited to design precisely targeted mRNA therapeutics.

ABS#651

Poster session, July 14

Filtering Artifacts from Top Ranked Docked Molecules with an Implicit Solvent Model

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In recent work ultra large library, now of several billions of compounds, has discovered potent ligands, in novel chemotypes, for multiple targets. As the libraries have grown, however, the very top of the hit-lists have become populated with artifacts that effectively cheat our scoring function. While these cheating molecules represent rare events, it is easy to imagine that as the libraries continue to grow, they will ever-more dominate the top of hit-lists, where we often most prioritize for experimental testing.
Here, we investigate post-analysis methods to identify these artifacts large scale docking (LSD) screens. We adopt the idea of principle component analysis and the fact that solvation term is less well-described in docking. We use different implicit solvent models as cross filter to identify cheating compounds. In retrospective studies, we demonstrated that this approach could identify those cheating for different receptor targets. We also show that our method is robust against different force parameter and implicit solvent models.
We then turn to prospective tests of this method against AmpC beta-lactamase. From the very top of the docking hit list, we prioritized and had synthesized de novo 128 molecules, a mixture of 39 molecules that the solvation analysis flagged as likely cheaters and another 89 molecules that the analysis considered plausible true actives. All 39 of the predicted cheating compounds did not inhibit AmpC at up to 200μM in experimental enzyme inhibition assays. Conversely, 61% for the 89 plausible true actives inhibited the enzyme with apparent Ki values < 200μM, and 20 of them inhibited AmpC with apparent Ki values <50 μM. The new method will be incorporated as a simple one-line code into pyCHARMM; it may be broadly useful to other docking methods that may struggle with the same phenomenon. More generally, this strategy of testing docking hit-lists with orthogonal scoring methods may be useful to the field.

ABS#652

Poster session, July 15

Higher-ordered Structures of Eye Lens beta-Crystallins determined by Native Mass Spectrometry

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Purpose: To determine the stoichiometry and structures of the beta-crystallin hetero-oligomers associated with the beta-high fraction. Crystallin fractions isolated from human donor lenses predict higher-ordered oligomers of octamers and higher. Yet, these oligomers have not been characterized in vitro.
Methods and Materials: Using native ion mobility-mass spectrometry (IM-MS) we investigated dimerization kinetics of β-crystallins and also molecular dynamics simulations for accurate interpretation of IM-MS collision cross-section information.
Results: Our results show that β-crystallins readily form heterodimers that can be detected by native mass spectrometry at even micromolar concentrations, and that dimers are the building blocks for larger oligomers. Ion mobility experiments and molecular dynamics simulations further reveal that all oligomers identified in these experiments are compact (as opposed to quasi-linear or planar), in agreement with previously published hydrogen-deuterium exchange data obtained by the authors.

Conclusions: While concentrations used are much lower than that found in the native lens, the detection of higher-ordered hetero-oligomers matches the size of oligomers present in the betaHigh-fraction isolated from human donor lenses. These results help to understand how the eye lens may establish a refractive gradient by tuning β-crystallin oligomer composition and lay the groundwork for future studies measuring the oligomeric structure of β-crystallins derived from both normal and cataractous lenses.

ABS#653

Poster session, July 14

Site-specific incorporation of 3-methyl-histidine into Tannase

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Tannin acyl hydrolase or tannase functions as an esterase that catalyzes the hydrolysis of ester bonds in gallo tannins producing gallic acid and its derivates. Tannase is used in a variety of different industries such as food, chemical, agriculture, and pharmaceutical and thus represents an important target for biocatalytic development. Recent studies have emphasized the potential of genetic code expansion (GCE) to modify proteins and increase catalytic activity of enzymes. GCE allows for the incorporation of non-canonical amino acids. In this study the impact of replacing the H451 in the esterase catalytic triad with its non-canonical counterpart 3-methyl-histidine(3mH) was evaluated. A decreased reliance on the Asp of the catalytic triad, while shortening the distance between the His and the Ser was proposed to create a more efficient catalytic dyad. A pyrrolysltRNA synthetase (PyIRS)/ tRNAPyl pair were used in combination with a tannase mutant encoding an amber stop codon. The activity of the derived NmH451 tannase was compared to wild-type and unexpectedly showed a decrease in activity. Mass spectrophotometry is currently being applied to analyze accuracy of incorporation

ABS#654

Poster session, July 13

Insights into Actin Assembly by Rickettsia conorii Sca2 From Single-Particle Cryo-EM Analysis

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Spotted fever group Rickettsia undergo actin-based motility inside infected eukaryotic cells using Sca2 (surface cell antigen 2): an ∼1800 amino-acid monomeric autotransporter protein that is attached to the surfacce of the bacterium. Sca2 is the only known functional mimic of eukaryotic formins, as it can assemble long unbranched actin tails, yet it shares no sequence similarities to the latter. We have previously shown, using structural and biochemical approaches, that Sca2 uses a novel actin assembly mechanism. The first ∼400 amino acids fold into helix-loop-helix repeats that form a crescent shape similar to that of a formin FH2 monomer. Additionally, the N- and C- terminal halves of Sca2 display intramolecular interaction and cooperativity for actin assembly, mimicking a formin FH2 dimer. To better understand the actin assembly mechanism of Sca2 at a structural level, we performed single-particle cryo-electron microscopy analysis of Sca2. While high-resolution structural details remain elusive, our model confirms the presence of a formin-like core: Sca2 indeed forms a doughnut shape, similar in diameter to a formin FH2 dimer, and can accommodate two actin subunits. Extra electron density covering one side is also observed, and this is thought to be contributed by the C-terminal repeat domain (CRD). This structural analysis allows us to propose an updated model where nucleation proceeds by encircling two actin subunits, and elongation proceeds either by a formin-like mechanism that necessitates conformational changes in the observed Sca2 model, or via an insertional mechanism akin to that observed in the ParMRC system.

ABS#655

Poster session, July 13

Exploring the antimalarial potential of recently synthesized novel pyrimidine inspired hybrids

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Recently, the emergence of antimalarial resistance by Plasmodium sp. has enhanced the need for the development of new novel drug targets. Using a molecular hybridization approach, we report the design and synthesis of an unique class of antiprotozoal agents; (E)-1-(4-(4,-6-diphenylpyrimidin-2-yl)piperazin-1-yl)-3-phenyl prop-2-en-1-one derivatives (8a-n). In vitro inhibitory activity for the compounds were evaluated against the NF54 chloroquine-sensitive strain of Plasmodium falciparum (P. falciparum). From the antiprotozoal screening, three compounds displayed propitious activity with IC50 (0.18-0.21μM), using quinine and chloroquine as standard drugs. Compounds 8o and 8l emerged as the most potent candidates with IC50 values of 0.18 ± 0.02 μM and 0.21 ±0.001 μM with an associated good safety index of 18.59 and 16.75 to human kidney epithelial (HEK293) cells, respectively. Furthermore, we investigated the binding affinities of the compounds against two purified P. falciparum heat shock protein 70 homologues; PfHsp70-1 and PfHsp70-z. Compound 8l exhibited the highest binding affinity for both PfHsp70s. In silico molecular docking data validated the high binding ability between PfHsp70-1 and 8l and 8o, with the highest binding affinity of 10.5kcal/mol and 10.1kcal/mol, respectively. Therefore, it could be speculated that PfHsp70-1 is one of the targets of these inhibitors.

ABS#656

Poster session, July 14

Molecular insights into the picornavirus 2C protein, an antiviral target involved in virus replication and packaging

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The poliovirus 2C protein is a crucial component of viral RNA replication and genome packaging, although functional details remain largely unknown. Despite its unique features, such as ATPase activity, membrane and RNA binding, and placement within the SF3 helicase family, the actual biochemical functions of viral protein 2C in virus replication have not yet been fully defined. Nonetheless, the 2C protein has been identified as a potential target for antiviral drugs. This study aimed to provide molecular level insights into antiviral binding mechanisms and their influence on 2C function. To begin, we developed an expression system to isotopically label 2C for NMR studies; 2C has been notoriously difficult to express and purify. Initial NMR experiments focused on 13C-isotopically labeling the methyl side chain groups of methionine residues, given that 2C exists as a 62 kDa homodimer. Our initial results identified optimal pH conditions for observing all thirteen expected 13C-methyl methionine resonances, suggesting that the two 2C monomers exists in identical chemical space. However, at higher pH levels, only 10-11 peaks were observed, indicating increased conformational dynamics and intermediate exchange. In the future, we will also generate a 15N labeled sample for backbone labeling, and map binding sites for RNA, nucleotides, and antivirals, which will further advance the understanding of drug-2C interactions. This study will aim to provide crucial insights into the mechanisms of drug binding to the 2C protein, which can help guide the development of new and improved antiviral drugs targeting this enzyme.

ABS#657

Poster session, July 14

An adaptive biomolecular condensation response is conserved across environmentally divergent species

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Cellular responses to maladaptive environmental changes—stresses—allow for organismal adaptation to diverse and dynamic conditions. Across the tree of life, cells upregulate a highly conserved transcriptional program in response to so-called proteotoxic stresses such as heat shock. Correspondingly, in eukaryotes, these stresses induce the formation of biomolecular condensates, clusters of mRNA and protein which are referred to as stress granules under severe stress. However, major questions remain about this stress-induced response. How conserved is the condensation response relative to the transcriptional response? How does it vary across environmental niches, and to what extent does it correspond with the conserved transcriptional response? To answer these fundamental questions, we studied the growth, transcriptional, and condensation heat-induced stress responses in three fungal species adapted to thrive in different thermal environments: cryophilic S. kudriavzevii, mesophilic S. cerevisiae, and thermotolerant K. marxianus. Here we show that transcriptional heat

shock responses track each species' evolved temperature range of growth. Further, orthologous proteins—including poly(A)-binding protein, Pab1, a core marker of stress granules—form condensates in vivo at temperatures systematically tuned to the temperature at which the organisms activate the transcriptional heat shock response and slow their growth. In vitro, purified Pab1 from each species condenses autonomously at niche-specific temperatures. Homologous mutations in Pab1 cause similar shifts in relative condensation temperature across species, and crucially, mutations which suppress condensation in vitro also reduce fitness during heat stress. Our findings indicate that stress-induced protein condensation is adaptive, conserved, integrated with the growth and transcriptional responses, and tuned to features of the cellular and organismal environment to initiate at niche-specific levels.

ABS#659

Poster session, July 14

Programmable Structural Heterogeneity: Experimental Datasets to Benchmark Cryo-EM Single Particle Analysis Pipelines

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Proteins and their complexes are not static but rather occupy high-dimensional conformational landscapes that influence their function and regulation. Single-particle cryo-electron microscopy (cryo-EM) has significant potential as a tool for characterizing the structure and dynamics of macromolecular complexes at the single-molecule level; however, reconstructing and quantifying structural landscapes from highly heterogeneous cryo-EM datasets remains computationally challenging. Notably, rapid advances in cryo-EM homogeneous reconstruction algorithms, which sought ever greater resolution, were enabled by the existence of well-characterized benchmark datasets such as apoferritin yet, despite the recent surge in heterogeneous classification and reconstruction algorithms, analogous heterogeneous experimental benchmark datasets are lacking. Furthermore, although heterogeneous reconstructions tools are now capable of reconstructing large numbers of volumes (500-1000s) from a single cryo-EM dataset, strategies for systematic and quantitative comparisons of the resulting volume ensembles remain elusive. To address these limitations, we have leveraged the targeting power of the RNA-guided DNA endonuclease Cas9 to generate a series of 13 related macromolecular complexes with subtly distinct structural features. We demonstrate the utility of our ground truth datasets by combining individual particle stacks in silico and challenging heterogeneous reconstruction algorithms to accurately analyze the encoded heterogeneity. We also characterize the encoded conformational and compositional heterogeneity of the resulting structural ensembles using existing tools and those we have newly developed, and identify some instances where these tools perform well, and others that highlight the need for methodological improvements.

ABS#660

Poster session, July 13

Modulation of Antibody Light Chain Aggregation by Pre-Formed Seeds and Small Molecule Stabilizers

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Accumulation of non-native proteins as amyloid fibrils is associated with multiple diseases. In systemic light chain amyloidosis, amyloid fibrils are formed from fragments of an antibody light chain protein, the sequence of which is unique to each patient. Mechanistic understanding of how light chains form amyloid is needed to improve diagnosis and develop new therapies for amyloidosis. In this study, we asked how the properties of the light chain folded state and the presence of pre-formed amyloid seeds affect the aggregation process.

We measured the concentration-dependent aggregation rates of several recombinant light chain variable domains using thioflavin T fluorescence. These proteins, which form the structured core of amyloid fibrils, readily aggregate under native-like conditions where the folded protein is the major species in solution.

Analysis of light chain aggregation kinetics revealed a complex dependence on concentration, which could not be fitted to typical models of nucleated polymerization. Addition of pre-formed fibril seeds reduced, but did not eliminate, the lag time before aggregation occurred. Unstable light chains are associated with amyloidosis and, accordingly, aggregate more rapidly than more stable light chains. Small molecules that bind to light chains

and prevent their unfolding and subsequent aggregation represent a promising therapeutic strategy for amyloidosis. Starting from screening hits, we have identified molecules that can protect light chains from unfolding-linked proteolysis. These molecules stabilize the native homodimeric structure of light chains by binding to the variable domains. Consistent with their ability to stabilize light chains, these molecules also delayed amyloid formation.

These results support a model for aggregation of light chain variable domains that involves multiple conformational transitions. Unfolding of the native state is an important pre-requisite for amyloid formation. This step can be inhibited by binding of small molecules, which supports our efforts to develop stabilizer drugs.

ABS#661

Poster session, July 14

Impact of ALS-associated mutations in a C-terminal "hot-spot" on TDP-43 phase separation and aggregation

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TDP-43 is a ribonucleoprotein involved in the metabolism of mRNA. One of the key functions of TDP-43 is to form RNP granules through liquid-liquid phase separation (LLPS). The C-terminal domain of TDP-43 is intrinsically disordered, playing a crucial role in physiological LLPS and forms cytoplasmic aggregates, a hallmark of amyotrophic lateral sclerosis (ALS). In our research, we have identified a cluster of mutations in the C-terminal domain of TDP-43 that are associated with ALS. However, the exact impact of these mutations on TDP-43 function and their contribution to the development of the disease remains unclear. We hypothesize that these mutations increase the protein's propensity to aggregate, ultimately leading to the disease state. To investigate this, we employed microscopy techniques and NMR spectroscopy to study the structural and dynamic properties of these disease-associated TDP-43 mutants. Through our experiments, we found that some mutations in fact reduce the phase separation of the protein, while others enhance its propensity to aggregate. These findings provide evidence that these mutations modify the phase behavior of the protein, shedding light on their potential role in ALS pathogenesis.

ABS#662

Poster session, July 14

Regulation of endosomal SNARE complex assembly by VPS45 and its neutropenia-associated mutants

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In eukaryotic cells, membrane-bound vesicles transport cargos between organelles and are essential for secretion, cell-cell communication, cellular growth, and survival. The Sec1/Munc18 (SM) family of proteins are essential for proper intracellular membrane trafficking through their interactions with both individual SNAREs and assembled SNARE complexes. Formation of the SNARE complex, between v-SNAREs associated with the vesicle and t-SNAREs associated with the target organelle, is required for fusion between a vesicle and its target membrane. Syntaxin-type t-SNAREs often form an autoinhibited structure, called the "closed conformation". The closed conformation precludes SNARE complex assembly, thus blocking membrane fusion. In mammalian cells, the SM protein associated with endosomal membrane fusion is VPS45, and the endosomal SNAREs, including the t-SNAREs Syntaxin16, Syntaxin6 and Vti1a, and the v-SNARE Vamp4. VPS45 interacts with Syntaxin16 and is proposed to regulate SNARE complex assembly of the endosomal SNAREs. VPS45 also interacts with the endosomal Rab5 effector, Rabenosyn-5, and the function of this complex is currently under investigation. Using recombinantly expressed, purified proteins, we demonstrate a direct interaction between VPS45 and Rabenosyn-5, and Rabenosyn-5 competes with VPS45 binding to Syntaxin16, which likely regulates SNARE complex assembly. Single particle cryoEM data was collected to characterize this interaction at higher resolution. Furthermore, mutational analysis of VPS45 suggests

that variants of VPS45 associated with severe congenital neutropenia type 5 (SCN5) disease may alter the affinity between VPS45 and Syntaxin16 and thus affect endosomal trafficking.

ABS#663

Poster session, July 14

Effects of zinc and carnosine on aggregation kinetics of Amyloid-β40 peptide

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The accumulation and amyloid formation of amyloid-β (Aβ) peptides is closely associated with the pathology of Alzheimer's disease. The physiological environment wherein Aβ aggregation happens is crowded with a large variety of metal ions including Zn2+. In this study, we investigated the role of Zn2+ in regulating the aggregation kinetics of Aβ40 peptide using ThT assay, AFM and CD. Our results show that Zn2+ can shift a typical single sigmoidal aggregation kinetics of Aβ40 to a biphasic aggregation process. Zn2+ aids in initiating the rapid self-assembly of monomers to form oligomeric intermediates, which further grow into amyloid fibrils in the first aggregation phase. The presence of Zn2+ also retards the appearance of the second aggregation phase in a concentration dependent manner. In addition, our results show that a natural dipeptide, carnosine, can greatly alleviate the effect of Zn2+ on Aβ aggregation kinetics, most likely by coordinating with the metal ion to form chelates. These results suggest a potential in vivo protective effect of carnosine against the cytotoxicity of Aβ by suppressing Zn2+-induced rapid formation of Aβ oligomers.

ABS#664

Poster session, July 14

A Dimer-Partner Tug of War Mechanism for Building Fluorescence Protein Biosensors for the Detection of SARS-CoV-2

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We introduce a novel strategy for developing protein fluorescence biosensors for viral detection, and apply it to SARS-CoV-2 as proof of concept. Our strategy employs: 1) A protein decoy that mimics the binding interface of the viral host receptor to attain highly specific binding to virions; 2) Conversion of protein decoy into a biosensor by placing a solvatochromic fluorophore onto the decoy as signal reporter; 3) A transducer based on an engineered tug of war between the dimerization of a protein biosensor and the high affinity binding to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. The decoy was developed from human ACE2: we engineered the 35 amino acid long a-helix ACE2 as minimalist scaffold (decoy) to recapitulate the binding interface while increasing the sequence's intrinsic propensity to form a-helical conformation. We then introduced the solvatochromic fluorophore 4DMN from the Imperiali lab in a key position expected to become buried when bound to RBD and produce an increase in the fluorophore's emission intensity thereafter. BLI experiments show the protein decoy binds to RBD with KD∼1.4 mM and shows ability to bind to Spike Trimers in the low nM range. Self-titration experiments using fluorescence, circular dichroism and size-exclusion chromatography indicate the biosensor forms a coiled-coil complex with KD∼2.3 mM, resulting in >14-fold increase in green fluorescence, thus enabling the high-sensitivity detection of the monomer-dimer interconversion. Once prepared near the dimerization midpoint (2.5 mM), the sensor can effectively detect the presence of 900 Spike-covered full virions in microwell plates by increasing its fluorescence. Therefore, we conclude that the dimer-partner tug of war mechanism can be used jointly with a host-decoy binding strategy to engineer fluorescence biosensors for the detection of viruses.

ABS#665

Poster session, July 14

Single-Molecule Imaging to Reveal How Chaperones Engage Condensates

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Heat shock and other cellular stresses trigger protein aggregation, which is resolved by the action of stress-induced molecular chaperones. These aggregates are often presumed to be the result of unregulated protein misfolding. However, emerging work shows that many stress-induced protein aggregates form by dynamic, regulated processes of biomolecular condensation. Our group recently showed that one such species, heat-induced condensates of poly-A binding protein (Pab1) from yeast, is a far more efficient substrate for yeast molecular chaperones than misfolded protein aggregates (Yoo et al. 2022). In this study, we sought to investigate the molecular-scale dynamics of chaperone recruitment to Pab1 condensates, and how these dynamics may differ between condensates and misfolded aggregates. A single-molecule imaging approach provides the most direct access to visualizing how protein condensates recruit and engage chaperones. We developed a custom two-color total internal reflection fluorescence microscopy (TIRFM) platform to track colocalization and interactions between purified, fluorescently labeled chaperones and labeled Pab1 condensates in vitro. Using our published fluorescence anisotropy-based assay to measure disaggregation (Yoo and Drummond 2022), we show that fluorescently labeled chaperones are competent in Pab1 condensate dispersal in vitro. Using our two-color imaging platform, we report the size and fluorescence characteristics of labeled Pab1 and labeled chaperones individually, and we observe the colocalization of Pab1 condensates with Hsp40, a class of chaperone required for Pab1 condensate dispersal. Building on these results, we will compare chaperone recruitment to condensates between Pab1 and misfolded protein aggregates as model substrates, and between different classes of chaperones such as Hsp40s, in order to reveal the molecular-scale dynamics of chaperone-condensate interactions.

ABS#666

Poster session, July 13

Engineering Larger Pore Proteins: A Study on Loop-to-Hairpin Evolution in Outer Membrane Beta-Barrel Proteins

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Bacterial outer membrane porins uniquely form transmembrane beta-barrels rather than transmembrane alpha-helical bundles. These beta-barrels consist of repeating beta-hairpins, with the number of beta-hairpins determining the diameter of the barrel. These robust proteins are ideal for nanopore applications like biosensing and filtration. Engineering these porins with larger pores mimics nature's gene duplication principle, where a beta-hairpin is copied and inserted. Here, we explore an alternate method of strand accretion observed in beta-barrel evolution, where a non-beta-hairpin structure converts into a new beta-hairpin fold.
To investigate this mode of strand accretion, we selected the 16-stranded PorB from Neisseria gonorrhoeae and the 18-stranded MOMP from Campylobacter jejuni, where the extra hairpin of MOMP sequentially aligns with the extracellular loop L3 of PorB. We engineered a chimeric protein named PorB18, where we replaced the loop L3 of PorB with the extra hairpin of MOMP. Despite a low sequence identity of 21.7% between PorB and MOMP and the replacement of a significant domain, the chimeric protein was stable enough to express and fold into the outer membrane of E. coli. Circular dichroism indicated an increased beta-sheet percentage, suggesting the inserted domain retained its secondary structure. Crosslinking confirmed a trimeric tertiary structure, akin to PorB and MOMP.
Predicted AlphaFold models suggested two possibilities, either an 18-stranded or a 16-stranded structure. Proteinase K digestion showed that the inserted hairpin is buried in the structure, as is the case in both models, where the inserted strand is either part of the barrel or forms a plug within the barrel. The channel conductance matched the theoretical value of the 18-strand model, validating the 18-strand model. Our study unveils an alternative method of beta-barrel engineering using the loop-to-hairpin evolutionary method, opening avenues to engineer proteins with larger barrels as well as domain swapping.

ABS#668

Poster session, July 15

Design Principles of Caveolins Across Evolution Revealed by Cryo-Electron Microscopy and Alphafold2

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Caveolins are an ancient family of proteins best known for their role as the building blocks of caveolae, flask-shaped nanoscale plasma membrane invaginations that serve as mechanosensors and scaffolds in vertebrates. For many years, the structural basis for the membrane remodeling activity of caveolins was unknown. Our groups recently showed using cryo-EM that human caveolin-1 (Cav1) assembles into a tightly packed, disc-shaped oligomer composed of 11 Cav1 protomers, with a flat membrane facing surface and prominent beta barrel emanating from the center of the cytoplasmic side of the complex [1]. Which features of the complex are optimized to build caveolae and which are required for broader functions of the protein family, especially in organisms which lack caveolae, remain unknown. To address this question, we used AlphaFold2 (AF2) to predict the structure of 72 caveolins from representative species of 13 different Metazoan phyla/superphyla. We show that AF2 predicts many features of human Cav1, including the fold of the protomer, and assembles it correctly into oligomeric complexes [2]. Furthermore, AF2 predicts that most caveolins pack in a spiral pattern, forming oligomeric discs with one hydrophobic and one hydrophilic surface, similar to human Cav1 [3]. In support of this possibility, single particle electron microscopy of negatively stained caveolin complexes from the choanoflagellate Salpingoeca rosetta, a sister of Metazoa, and the purple sea urchin Strongylocentrotus purpuratus form well-ordered disc-shaped complexes. These findings suggest that the organization of caveolins into oligomeric discs is an ancient and highly conserved feature of the protein family and provide a new structure-based framework to probe the functional roles of caveolins across Metazoa and beyond.

ABS#669

Poster session, July 15

Molecular mechanism of coenzyme specificity of Trypanosoma cruzi D-3-hydroxybutyrate dehydrogenase

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d-3-Hydroxybutyrate dehydrogenase (HBDH) catalyzes the conversion between d-3-hydroxybutyrate and acetoacetate. Most HBDHs use the coenzyme nicotinamide adenine dinucleotide (NAD), but not its phosphorylated analog NADP, presumably because of steric clashes and/or electrostatic repulsion between the 2'-phosphate of NADP and the "NAD/NADP-specificity loop". However, trypanosomal HBDHs differ, such as Trypanosoma brucei HBDH, which uses both NAD and NADP with similar affinity, and Trypanosoma cruzi HBDH, which only uses NADP, as shown here. Using enzymes kinetics and crystallographic analysis of T. cruzi HBDH in complex with the reduced coenzyme NADPH and a substrate mimic, we determined the molecular mechanism of its strict NADP specificity. The structure revealed a conformational change of the longer NAD/NADP-specificity loop with respect to bacterial HBDHs, which enables accommodation of the additional 2'-phosphate. We identified the T. cruzi-specific residues responsible for the strict NADP specificity of TcHBDH. Additionally, we found two TcHBDH mutant enzymes with a 10-fold increased turnover number with respect to wild type enzyme. These residues specifically diverged across Trypanosoma, demonstrating that they are not only involved in coenzyme recognition, but also catalysis. Collectively, our studies define the roles of unique residues in Trypanosoma HBDHs and provide a framework for future in vitro and in vivo studies on the enigmatic physiological role of trypanosomal HBDH.

ABS#670

Poster session, July 14

Proteomic analysis and comparison of Stage IIA T1N1 ER/PR negative Breast Cancer serum to Controls for identification of potential biomarkers for Breast Cancer

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Breast Cancer (BC) is among one of the leading causes of death in women. BC tumors are classified by the presence of Estrogen receptors (ER), Progesterone receptors (PR), and Human Epidermal Growth factor 2 (HER2) in

addition to anatomic features (size and involvement of axillary lymph nodes). The early stages of BC may cause dysregulation of proteins which can be characterized as biomarkers that can be used in early detection including in younger women. Serum analysis potentially aids in detection and analysis of secreted proteins from the tumor and whole body responses to the disease. Mass Spectrometry is important for the study of proteomics because of its high sensitivity and ability to detect low-abundant proteins. In this study serum samples from 5 women with ER PR-negative breast cancer were compared to 5 age-matched control counterparts. The 1N1 tumors were less than 20 mm in size with tumor cells found in up to 3 axillary lymph nodes. Samples were prepared using in-solution proteomic techniques followed by Nano-liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS) to identify proteins that are dysregulated between the matched pairs. This was performed in biological replicates with a total of 15 cancer samples and 15 control samples digested & analyzed. Raw data were then analyzed using ProteinLynx Global Server (v 2.4) Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. Any dysregulated proteins will be compared to those found in various cancer case studies in this project from our lab.

ABS#671

Poster session, July 15

Probing the effects of pathogenic mutations and small-molecule ligands on prion-like conversion of superoxide dismutase-1

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Multiple neurodegenerative diseases feature the accumulation of misfolded proteins in and around neurons. In some cases, the misfolding propagates through a prion-like mechanism whereby interactions between misfolded conformers and natively folded conformers induce the latter to convert into the misfolded conformer. Such prion-like propagation of misfolding is an attractive therapeutic target, but difficulties with assaying conversion directly have severely limited efforts to find drugs targeting conversion in most disease-related proteins. We developed a unique assay to monitor prion-like conversion of superoxide dismutase 1 (SOD1), whose misfolding is linked to amyotrophic lateral sclerosis (ALS). In this assay, a misfolded SOD1 mutant monomer that is associated with familial ALS is tethered to a wild-type (wt) SOD1 monomer, and the enzymatic activity of the wt SOD1 domain is monitored over time to detect its conversion into inactive misfolded conformers. Tethering the mutant vastly increases the effective local concentration of misfolded protein in the assay while keeping the global concentration low, decoupling conversion from aggregation. We used this assay to test if compounds that have been reported to inhibit aggregation of SOD1 are able to inhibit prion-like conversion. Examining a panel of small-molecule compounds, we found that some did indeed significantly delay conversion, but others had no effect. Some, like statins, even accelerated conversion, suggesting that they are contraindicated for treatment of ALS. The results underline the fact that conversion and aggregation are distinct biophysical processes, and point the way towards future drug discovery efforts targeting conversion specifically. We also explored how the choice of misfolded mutant affected the conversion kinetics, finding that some mutants converted wt SOD1 much more rapidly than others.

ABS#672

Poster session, July 14

A Proteomic Investigation of Human Serum from donors with triple-negative breast cancer and matched controls to identify Protein Biomarkers for Breast Cancer Detection

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In the United States, Breast Cancer (BC) is a leading cause of death in women, with a rate of 1 in 8 women developing breast cancer in their lifetime. An aggressive form is Triple Negative Breast cancer (TNBC) where the cancer cells score negative for estrogen receptor (ER), progesterone receptor (PR), and a human epidermal growth factor receptor 2 (HER2). These diagnoses make early detection vital. Early stages of BC can show dysregulation or proteins, which then can be referred to as biomarkers. Mass Spectrometry (MS) is used for protein analysis due to its specificity. This study uses MS-based

proteomics to identify differences between the proteins found in human serum from 8 women who have TNBC and their 8 control counterparts. Samples were analyzed via in-gel and in-solution digestion, subsequently by Nano liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS), using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. Raw data Are under analysis using ProteinLynx Global Server (v 2.4) Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. Dysregulated serum proteins will be compared to other dysregulated proteins found in numerous breast tumor case studies.

ABS#674

Poster session, July 14

Biochemical Characterization of the Yersinia Effector YopM and Interaction with Host Protein Pyrin

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Yersinia spp use a type III secretion system (T3SS) to inject effectors into the host cell to disrupt multiple cellular processes. Two effectors, Yersinia outer protein E (YopE) and YopT, disrupt phagocytosis by inactivating RhoA. Bacterial modifications to RhoA GTPases trigger assembly of the pyrin inflammasome as a compensatory host response. Another Yersinia effector, YopM counteracts the YopE- and YopT- triggered immune response by inactivating the pyrin inflammasome.YopM co-opts the host kinases protein kinase C-like kinase (PRK) and p90 ribosomal S6 kinase (RSK),to phosphorylate pyrin, locking it in an inactive form. YopM is comprised of an N terminal secretion signal, a central leucine rich repeat (LRR) region which binds PRK and a short C-terminal tail which binds RSK. YopM was shown to bind to two regions in the N terminus of pyrin. In-vitro kinase assays have demonstrated that the N terminal pyrin domain (PYD) of pyrin and specific LRRs are required for RSK-mediated phosphorylation of pyrin by YopM. However, the mechanism underlying this interaction is not fully understood. Using GST pulldowns and the bacterial two hybrid (BACTH) assay, we show that YopM directly binds to the PYD. Size exclusion chromatography (SEC) analysis confirms that purified YopM and PYD co-elute as a complex. Different Yersinia strains encode different YopM isoforms that vary in size due to variations in the number of LRRs. Comparisons of two YopM isoforms (YopMKIM, YopMPestA) for binding to the PYD showed no differences in affinity. We also determined the structure of the 13 LRR YopMPestA. YopM PestA is structurally similar to the previously published 15 LRR YopMKIM. In both crystals, YopM exists as a tetrameric superhelix made up of four monomers. Our studies give insights into YopM inhibition of the pyrin inflammasome through direct interactions with the PYD and provide additional biochemical insights on the YopM protein.

ABS#675

Poster session, July 14

Towards an Accurate Prediction of Protein (Un) Folding Rates and Stability from Structure and Sequence

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Proteins are biological nanomachines that self-assemble and change shape on cue to perform most functions in living systems and are thus key targets for myriad biomedical and biotechnological applications. These applications typically involve engineering the sequence of natural proteins to achieve desired properties (e.g., higher thermal stability), or designing entirely new ones. The ability to predict (un)folding rates, stability and/or disorder of proteins from the amino acid sequence is an essential tool towards fully enabling such protein engineering. At a coarse level, it has been shown that protein (un)folding rates obey simple rules that can be reproduced with basic statistical mechanical models and very limited protein-specific information (sequence length and structural class). However, increasing the sequence-structural detail and accuracy of these calculations to resolutions practical for protein engineers has proven tremendously challenging.

Our goal is to enhance such protein folding models to effectively produce accurate predictions of (un)folding rates, stability, and intrinsic disorder for use by protein engineers. Our approach starts with a basic physical model that defines the folding funnel as a one-dimensional projection of free energy where the (un)folding rates are defined as diffusion along such surface. With an expanded database of experimental protein (un) folding rates and mutational effects and an analytical

pipeline to modify the model and assess the changes in performance, we sequentially introduce parameters that account for specific sequence, topology, and structural properties, and examine significance/predictive power using rigorous statistical analysis. Our current results demonstrate large improvements in prediction accuracy (particularly for unfolding rates and stability) over existing methods using a relatively small number of structure-based physical parameters and a novel parameter defining protein topology on the energetic contribution of atomic interactions. We are now introducing sequence information with the goal of accurately predicting the effects of changing the sequence by a single amino acid.

ABS#676

Poster session, July 14

Thermodynamic Specificity Controls Pab1 Condensate Structure across Temperatures and Orthologs

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Stress-induced condensation occurs in response to a variety of environmental insults across eukarya. Yet, due to their heterogeneity and intractability towards typical structural approaches, little is known about the actual structures and formation mechanisms of condensates. Poly(A)-binding protein (Pab1 in budding yeast) is a canonical stress granule marker, whose condensation acts as a physiological stress sensor. Our recent work has found that Pab1 condenses by a sequential activation and local unfolding mechanism of its RNA-recognition motif (RRM) domains. We proposed the concept of thermodynamic specificity where the free energy surface of the RRM controls its activation and participation in the condensation process. Here, we show using hydrogen-deuterium exchange mass spectrometry (HDX-MS) that Pab1 condensates formed at different temperatures exhibit different degrees of RRM activation and participation in condensation. Further, Pab1 orthologs with different condensation temperatures have RRM stabilities tuned to their activation thresholds, leading to morphological differences in the condensate. Experimental demonstration that different stress temperatures generate different condensate structures and that RRMs from Pab1 orthologs with different condensation temperatures have different activation thresholds provides additional evidence supporting the role of thermodynamic specificity in Pab1 condensation.

ABS#679

Poster session, July 14

Probing the sequence determinants of FUS phase separation with synthetic repeats

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Fused in sarcoma (FUS) is a human RNA-binding protein whose aggregation into solid inclusions is linked to amyotrophic lateral sclerosis and frontotemporal dementia. FUS is also in an ever-growing class of proteins known to form liquid-like condensates via phase separation. The low complexity domain of FUS (FUS LC), is a key mediator in phase separation. FUS LC is enriched in serine, tyrosine, glycine, and glutamine, which each contribute to multivalent interactions that stabilize the liquid phase of the protein. The details underlying these weak interactions and the importance of specific amino acid sequence motifs are not well understood. Here we created a synthetic repetitive FUS LC-like sequence to specifically modify sequence motifs to probe the biomolecular interactions that contribute to phase separation of a disordered protein. The two engineered FUS sequences include a 14mer sequence and a 15 mer repeat sequence, with preliminary data suggesting synthetic FUS forms non-liquid aggregates while WT FUS remains liquid-like. Many differences between synthetic FUS and WT could be responsible for this aggregation, including sequence charge and amino acid order. We present our experimental and computational efforts to (1) modify engineered FUS to learn the impact of the two charged residues on the formation of liquid droplets in FUS LC and (2) alter the level of sequence disorder within synthetic FUS to learn how the organization of repeats, or lack thereof, determine the phase separation and material properties of FUS LC. Our data suggest that the addition of aspartic acids enhances liquidity of 14mer FUS, and that the sequence order of amino acids has a profound impact on 14mer phase separation. In improving our understanding of FUS phase separation thermodynamics, we can better understand the physiological functions of FUS and design new treatments for ALS and FTD.

ABS#680

Poster session, July 14

Engineering of a Broadband Genetically Encodable Ratiometric Fluorescent Calcium Biosensor with a 1 ms Folding Time in Response to Calcium

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Calcium is a ubiquitous secondary messenger in eukaryotic cells and failures in calcium level regulation can have severe physiological implications. To aid in testing the roles of calcium levels in these processes, accurate and responsive calcium sensors are required. Existing genetically encoded calcium sensors lack the time response and the dynamic range to resolve calcium signals and function in diverse cellular environments. To overcome these limitations, we developed a broadband molecular scale biosensor capable of detecting Ca+2 ion concentrations with 1 ms response times using the folding coupled to binding strategy. The Ca+2 ion binding domain of a human protein called calnuc was used as a template. The apo form of the protein folds in presence of calcium and the structural change in the protein is exploited as a transducer for a FRET based calcium biosensor. Using single molecule confocal microscopy, we estimated the ligand-induced changes in protein structure in intracellular Na+ and K+ conditions and found a response time of ~1 ms with a sensing range ranging from the nM to the mM scale. We also designed and tested genetically encodable versions for use in cells. Further engineering efforts resulted in encodable calcium sensors with a broadened dynamic range. The successful in-vitro detection of Ca+2 ions paired with the in-vivo sensor response in HEK293 cells confirms the viability of using calnuc with the "folding coupled to binding" strategy to overcome the limitations of existing encodable calcium biosensors.

ABS#681

Poster session, July 14

Prominin family proteins make biophysically similar extracellular vesicles

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Prominin-1 (Prom1) is a five-transmembrane pass integral membrane protein with characteristically large (200-300 amino acid) extracellular loops[1]. The Tweety homology protein family (Ttyh) has been identified as a distant homolog of Prom1 and shares the five transmembrane topology, but Ttyh has shorter (100-120 amino acid) extracellular loops[2]. Prom1 localizes to highly positively curved membranes, such as microvilli and cilia, and creates extracellular vesicles (EVs) that bleb off from these protrusions[3,4]. A point mutation implicated in inherited retinal dystrophy, located in one of the transmembrane regions of Prom1 (W795R), can also bend membranes and create EVs[5]. Given the topological similarities between Prom1 and Ttyh1 and the functional similarities between wild-type and W795R Prom1, our studies look to compare the EVs created by Prom1, W795R Prom1, and Ttyh1 to determine whether they share a mechanism of membrane bending. We reconstitute EV formation by Prom1 and Ttyh1 in cultured mammalian cells and purify them for mechanistic characterization. We then use sucrose density gradient ultracentrifugation and dynamic light scattering to determine if purified EVs produced by the different proteins are biophysically similar. We find that Ttyh1 induces EV formation in cultured cells to a similar extent as Prom1, and that the W795R Prom1 produces EVs at a lower level than wild type. We further show all three proteins produce EVs that co-purify over sucrose gradients, and so they have similar densities, and the EVs can be directly compared. These findings establish a foundation of biophysical similarities, and allow the morphological differences observed in EVs to be attributed to the differences in how each protein bends the plasma membrane.

ABS#683

Poster session, July 15

Association of the amyloidogenic C123 polypeptide with extracellular B- compared to Z-DNA within Streptococcus mutans biofilms

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The cariogenic pathogen Streptococcus mutans produces several amyloidogenic proteins, including the C123

truncation derivative of adhesin P1. In S. mutans, amyloid is associated with biofilm detachment of aging cultures rather than initial adherence. Specific monoclonal antibodies (MAbs) can distinguish C123 monomer and amyloid aggregates. Extracellular DNA (eDNA) is a well-known component of biofilm matrices. Recently, extracellular Z-DNA was reported to promote bacterial biofilm stability, while B-DNA enhanced disruption. Differential impacts of monomeric versus aggregated forms of the amyloidogenic protein A-beta on Z- to B-form DNA conversion has also been reported. We therefore evaluated co-localization of S. mutans C123 with B- and Z-DNA within S. mutans biofilms. Confocal microscopy and immunostaining were performed on adherent and non-adherent biofilm fractions of green fluorescent protein-labelled S. mutans. Commercial rabbit anti-Z DNA and murine anti-B DNA monoclonal antibodies, followed by Alexa fluor 647-labeled secondary antibodies, were used to visualize eDNA. Exogenous Alexa fluor 594-labeled C123 was added to biofilms to assess co-localization with eDNA. Co-localization was also assessed with MAb 2-8G, which loses reactivity with C123 when it aggregates into amyloid form. Specific staining with the anti-B-DNA reagent was observed in adherent and non-adherent biofilm fractions, while anti-Z-DNA staining was restricted to the adherent fraction. When labeled C123 was introduced during biofilm development, or used as a staining reagent, substantial co-localization with B-DNA staining was observed; however, C123 did not co-localize with immunostained Z-DNA. In sharp contrast, MAb 2-8G, which reacts primarily with monomeric C123, co-localized with Z-DNA but not with B-DNA. Thus, a known bacterial amyloid-forming protein associates with eDNA in biofilm matrices. In addition, the association varies depending on both the form of the DNA and the aggregation status of the protein.

ABS#685

Poster session, July 15

Probing Translational Control During Drosophila Oogenesis

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Translational control provides a key mechanism for the spatial and temporal regulation of eukaryotic gene expression. It plays a particularly important role in early embryonic development in organisms that rely heavily on maternally supplied mRNAs. The Drosophila developing oocyte serves as a valuable model for studying translational control. The Drosophila posterior determinant, nanos (nos), is translationally repressed throughout the oocyte cytoplasm except at the posterior pole. A multifunctional RNA-binding protein Glorund (Glo), the homolog of the mammalian hnRNP F/H family of proteins, represses translation of nos during oogenesis by targeting both translation initiation and translation elongation. To elucidate the molecular mechanism by which Glo regulates nos, I identified dFMRP as a Glo-interacting protein. By biochemically dissecting repression of nos translation in vitro, I demonstrated that dFMRP specifically inhibits translation elongation. Furthermore, I combined mutational analysis and in vivo and in vitro binding assays to show that Glo's qRRM2 domain specifically and directly interacts with dFMRP, suggesting that Glo's RNA-binding domains can also function as protein-protein interaction interfaces critical for its regulatory functions. Additionally, I applied ribosome footprint profiling to the Drosophila ovary at different developmental stages to identify maternal transcripts regulated during the translation elongation phase. A footprint peak-finding tool has been developed to detect transcriptome-wide ribosome stalling sites. My preliminary results suggest that developmentally regulated ribosome stalling sites may be widely present on Drosophila maternal transcripts.

ABS#686

Poster session, July 14

The Role of ATP in P1-P4 Domain Interaction in Bacterial Chemotaxis Kinase CheA

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Bacterial chemotaxis receptors allow for bacteria to sense their environment and bias their swimming towards nutrients. In this system, receptors associate with a kinase CheA and coupling protein CheW to form hexagonal arrays of receptors embedded in the membrane.

The rate of CheA autophosphorylation, and therefore phosphorylation of a response regulator, is controlled by this receptor signaling. For autophosphorylation, ATP must bind to the catalytic domain P4, and the P4 domain must interact with the P1 domain to transfer the phosphoryl group to its only substrate site H48 on P1. Currently, the mechanism of CheA activation by receptor is not known. Several have proposed that receptor signaling controls the state of CheA kinase activity by modulating ATP binding to P4, and binding of ATP to P4 causes structural changes that allow P1 to bind to P4 in a productive manner. However, the change in interaction between P1, P4, and ATP in the active vs. inactive state is not fully characterized.

We use NMR chemical shift perturbation experiments and STD-NMR experiments to characterize the binding of ATP, P1, and P4 in E. coli CheA. Our chemical shift perturbation studies on an isolated CheA P1 domain suggest the P1 domain has a previously unreported weak affinity for ATP. Mapping of the chemical shift perturbations on P1 shows that ATP binds to a region of P1 near the phosphorylatable substrate H48 residue. We measured a millimolar Kd binding affinity that may be relevant at physiological levels of ATP. We propose this weak affinity provides additional affinity for P1 to the P4 site when ATP is bound to P4. STD-NMR experiments are ongoing to measure binding affinity of ATP to the P4 domain. These experiments will elucidate the role of ATP in the interaction of P1 and P4 in receptor activated vs. inactivated CheA.

ABS#687

Poster session, July 15

A Hydrophobic Core Stabilizes the Residual Structure in the RRM2 intermediate State of the ALS-linked Protein TDP-43

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Folding intermediates mediate both protein folding and the misfolding and aggregation observed in human diseases, including amyotrophic lateral sclerosis (ALS), and they are thus prime targets for therapeutic intervention. We identified the core nucleus of structure for a folding intermediate in the second RNA recognition motif (RRM2) of the ALS-linked RNA-binding protein, TDP-43, using a combination of experimental and computational approaches. Urea equilibrium unfolding studies by NMR spectroscopy revealed that the RRM2 intermediate state consists of collapsed residual secondary structure localized in the N-terminal half of RRM2; the C-terminus, including the nuclear export sequence, is largely disordered. Alanine scanning mutagenensis combined with steered molecular dynamics simulations yielded key stabilizing hydrophobic contacts that, when mutated to alanine, severely disrupt the overall fold of RRM2. In combination, these results suggest a role for this RRM intermediate in normal TDP-43 function as well as a potential role in dysfunction by serving as a template for misfolding, abberant interactions, and aggregation through the low stability and non-native secondary structure.

ABS#688

Poster session, July 14

Structural Basis of Met Exon14 Skipping in Cancers

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The Met receptor tyrosine kinase is frequently selected for exon 14 (Juxtamembrane-N region) skipping mutations in the cancers of lung and brain origin. Our research establishes that a deletion in exon14 in cell lines enhances Met's responsiveness to lower HGF concentrations, leading to enhanced downstream signalling. Through phos-tag and IP-MS techniques, we found two sites within exon 14 that were phosphorylated in a manner dependent on upstream kinases. In vitro, exon14 structure within the protein demonstrated instability, only achieving stability through a complex geometric arrangement. mutations identified hindered the process of protein oligomerization and affected Met activation, while exon14 skipping bypassed negative regulation and amplified receptor signaling in a HGF dependent manner. Our study also revealed that a secondary structure within the Met Juxtamembrane-C region is crucial for Met activation. Our findings suggest that Met exon14 acts as a critical regulator in receptor assembly and activation

in response to HGF.Cumulatively, these results contribute to a comprehensive understanding of the role of exon14 skipping in Met activation and support the strategy of targeting Met in cancers.

ABS#689

Poster session, July 13

Exploring the Binding Interaction Between S100A1 and Titin's UN2A region

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Titin, the giant sarcomere protein, consists of multiple domains and regions of repeated structural elements that are essential for sarcomeric integrity of the myofibril. The N2A region within titin has been identified as a signaling hub in skeletal muscle that is responsible for various mechanics and interactions in the muscle. The N2A region is located at the junction between the proximal tandem Ig region and the PEVK region and is comprised of four Ig domains and a unique 117 amino acid insertion sequence (UN2A). The insertion sequence resides between the Ig80 and Ig81 domains, and exists in skeletal muscle, as well as the N2BA isoform of cardiac muscle. The N2A region has been shown to be regulated by calcium, therefore we investigated possible binding interactions with calcium-binding proteins. S100A1, a calcium-binding protein, is the most abundant S100 isoform in cardiac and skeletal muscle and is critical for mediating regulatory calcium signaling pathways through interactions with its target proteins. S100A1 is known to bind and activate the ryanodine receptor and PEVK region in titin, in the calcium-bound state. A sequence analysis of UN2A suggested that a S100A1 binding site might exist in the UN2A region. HPLC was used to confirm this interaction occurs and has an affinity of 6.8 μM, as measured by SPR. We have shown that binding affinity increases as a function of calcium concentration, indicating binding is calcium-dependent. Binding of S100A1 to UN2A induces a conformational change in UN2A as shown by FRET. The high glutamate clusters in the UN2A region led us to hypothesize that a change in pH could influence the UN2A/S100A1 binding affinity, and circular dichroism and SPR have shown that binding affinity is stronger at lower pH. These results provide insight on a previously unidentified interaction, and future work will determine how this interaction regulates muscle contraction.

ABS#691

Poster session, July 15

Novel Mechanism for Modulating Rapid Calcium Oscillation and Signaling by Calcium Sensing Receptor

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The spatial-temporal change of Ca2+ dynamics is essential for Ca2+ signaling and constitutes one of the most ubiquitous modes of signal transduction, involving muscle contraction, fertilization, and gene regulation. Calcium-sensing receptor (CaSR) is known to play an imperative and essential role in regulating Ca2+ dynamics by coupling between extra and intracellular Ca2+([Ca2+]o and [Ca2+]o). Intracellular calcium oscillation is largely altered due to abnormal expression or function of the CaSR in multiple human diseases, including cancer, cardiovascular and neurological diseases, as well asthma. However, the mechanisms of modulating rapid calcium oscillation and signaling by [Ca2+]o and CASR are largely unknown. Here we report the first CaSR protein-protein interactome with 94 novel putative and 8 published interactors using mass spectrometry and proteomics.[Ca2+]o promotes enrichment of 66% of the identified CaSR interactors, pertaining to Ca2+ dynamics, endocytosis, degradation, trafficking, and primarily to protein processing in the endoplasmic reticulum (ER).We also demonstrated that [Ca2+]o promoted direct interaction between CaSR and a trafficking protein: VAMP-Associated Protein A (VAPA) via Time-resolved försters resonance energy transfer (TR-FRET). Our studies suggest that the intracellular domain (ICD) of CaSR is responsible for interacting with VAPA. More importantly, ER-Plasma Membrane hotspots related to VAPA are mediated by this [Ca2+]o dependent association and are critical for regulating forward trafficking of CaSR to the cell surface. It further promotes sensitive intracellular calcium response and oscillation upon [Ca2+]o change. In addition, using our novel calcium sensors, we report ER Ca2+oscillations mediated by extracellular calcium and CaSR and there is ER Ca2+-based functional cooperativity for CaSR. The release of ER calcium and oscillation directly controls intracellular oscillation. The major finding of our studies significantly extends the repertoire of the CaSR interactome and reveals a novel

molecular mechanism for modulating rapid calcium oscillation and signaling.

ABS#692

Poster session, July 13

Titin Ig Domains Demonstrate Variable Stability Within the Proximal Ig Segment

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Titin is a giant filamentous protein contributing to the passive elasticity of muscle and is composed of approximately 300 serially linked immunoglobulin-like (Ig) domains. The tandem domains of titin's proximal Ig segment have been shown to separate at relatively low forces and thus contribute to the protein's elasticity. While titin's elastic behavior is attributed to extension of the proximal Ig and PEVK regions beyond slack length, the potential unfolding of individual Ig domains has been posited to contribute to even greater extensibility. Computational modeling and mechanical stability testing have demonstrated Ig domain unfolding under physiologically relevant force, with neighboring domains exhibiting variable degrees of stability. Thermal and chemical stability experiments were conducted on domains I65-I70 of the proximal tandem Ig segment to evaluate the stability of adjacent domains. Testing revealed variable free energies of unfolding, with I67 demonstrating the lowest thermal and chemical stability and I70 demonstrating the highest. This work confirms theoretical modeling of differential Ig domain stability and provides greater insight into the role of the proximal Ig segment in titin's elasticity. The observed variations in stability may allow for greater control over sequential unfolding events, with domains extending in an alternating fashion to prevent unwanted overextension.

ABS#693

Poster session, July 15

Robust Automated Backbone Triple Resonance NMR Assignments of Proteins Using Bayesian-Based Simulated Annealing

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We present a new approach called BARASA for the automated assignment of protein backbone triple resonance spectra, which is a challenging task in nuclear magnetic resonance (NMR) studies. Our algorithm combines Bayesian probabilities of predicted and observed chemical shifts with simulated annealing to find the optimal assignment solution. To drive the assignment, we calculate a pseudo-energy potential using predicted chemical shifts derived from experimental or predicted structures. Surprisingly, we find that the readily available predicted shifts can provide more useful information than the HNCO/HN(CA)CO spectra in some cases. BARASA, implemented in C++, is tested on eight different protein systems, ranging up to 450 amino acid residues, including folded and intrinsically disordered proteins (IDPs). We also evaluate the algorithm's performance under conditions of sparse data and artifacts by randomly sampling experimental peak lists to generate simulated conditions. The results demonstrate that BARASA is robust, capable of handling sparse and artifact-contaminated data, and fast enough for real-time data evaluation. Furthermore, when compared to commonly used algorithms (FLYA, AutoAssign, and I-PINE), BARASA outperforms them, particularly in cases of sparse data. Lastly, we show that using predicted chemical shifts from AlphaFold2 structural models greatly enhances the assignment process. This research is supported by a fellowship from the Cancer Therapeutics Training Program (CTTP) and CPRIT Grant No. RP210043.

ABS#694

Poster session, July 13

Combining protein language and structure models to redesign E. Coli proteome

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We're working on designing a strain of E. Coli using only 19 canonical amino acids, starting from redesigning essential genes one at a time with the goal of preserving fitness. Since there are many diverse genes to design, we're repurposing and extending machine learning-

driven protein design methods to accomplish this task. Each of the currently available methods captures specific properties of the design landscape due to its approach and training data provided to the models. Protein language models like ESM have access to vast number of sequences and can learn patterns favored by nature. Methods using AlphaFold as a loss function like AFDesign or MCMC hallucination are aware of the final structure of the protein, but are susceptible to adversarial examples. Finally, models which perform "inverse folding" like ProteinMPNN have both sequence and structure information and can output structure-aware designs. We're developing optimization methods that combine these models as part of the design process to find sequences scored highly by models coming from different approaches, and how this translates to their biological properties.The preliminary experimental results show that our methods can generate designs comparable with wild-type versions in fitness with a small number of attempts.

ABS#695

Poster session, July 15

Structural Characterization and Comparison of Temperature and Pressure Stress on a Protein Library Across pH and Concentration Using Microfluidic Modulation Spectroscopy

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Thermal stress is the most common stress condition utilized for gauging protein stability, however, melt curves from a protein under different conditions can appear very different but still produce the same Tm from a derivative plot. Microfluidic Modulation Spectroscopy (MMS) is an automated mid-IR technique capable of measuring protein secondary structure at very high resolution across a broad range of concentrations and buffer conditions. MMS can be used to measure and monitor the structural changes that are occurring, leading to protein unfolding, and then thermally induced aggregation. With MMS we can monitor the loss of native protein secondary structure elements as the protein is being subjected to thermal stress. We looked at thermal unfolding for lysozyme across a range of pHs and found the Tm to be pH dependent. We looked at an IgG across a range of concentrations and found the Tm to be modestly concentration independent. Additionally, we used MMS to compare temperature and pressure stress applied to ovalbumin and found that each stress unfolds ovalbumin differently.

ABS#696

Poster session, July 15

IRAK4 and its Ethnic Protein Variants

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Interleukin 1 receptor associated kinase 4 (IRAK4) serves as a central regulator in innate immune response pathways and has been identified as a therapeutic target to treat inflammation and cancer. In spite of intensive efforts, a clinically approved IRAK4 inhibitor does not exist and IRAK4 continues to "defy conventional signaling models" (Bennett 2022) and crystallization of the full-length protein. We hypothesize that understudied structural and functional features of this protein may provide critical insights. A secondary analysis of the IRAK4 gene using the aggregated genome database gnomAD identified two ethnic variants. Ethnic protein variants are characterized as: 1) changes in coding (amino acid) or non-coding (regulatory) sequences that have the potential to impact isoform selection (protein size and sequence); 2) being present in a "substantive" number of individuals (for example, with a minor allele frequency (MAF) of ≥10%) from at least one of the major ethnic groups; and 3) ranging "widely" in their frequency among major populations (for example, ≥20%). In the canonical IRAK4 sequence, a noncoding rs4251500 variant is located in the intron between Ser275 and Asp278 and occurs in 14% of Middle Easterners but not at all among East Asians; notably, the location of this variant is predicted to shift to other introns, depending on which of the eleven possible IRAK4 transcripts are made. By comparison, the missense Ala428Thr coding rs4251545 breast cancer risk variant we had previously reported occurs in 27% Africans/African Americans but only 9% of Europeans; the location of this variant shifts to Ala304Thr or to the noncoding transcription regulatory 3' untranslated region (UTR) depending on which of eight possible transcripts are made. Further studies may reveal how IRAK4 transcripts are generated/selected and whether unrecognized functional domains exist, e.g. the extreme carboxy terminus

(XCT) or the long disordered linker between MyD88 binding and kinase domains.

ABS#698

Poster session, July 13

Structural and Biochemical Studies of the Mechanism of Condensation in Nonribosomal Peptide Synthetases (NRPSs)

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Nonribosomal peptide synthetases (NRPSs) are a large family of megaenzymes whose pharmaceutically important products have saved millions of lives. NRPSs are configured as a series of modules, with each module responsible for incorporating a single amino acid to the growing peptide. A canonical module consists of an adenylation (A) domain that activates the module's cognate substrate, a peptidyl carrier protein (T) domain that transports covalently bound substrates between domains, and a condensation (C) domain that links amino acids and peptide moieties from adjacent modules. This modular organization makes these megaenzymes a very attractive target for bioengineering and the synthesis of new-to-nature molecules with novel and/or improved therapeutic properties. Although structural, biochemical and computational studies have contributed nicely to understanding of NRPSs, the C domain's dynamics, precise chemical mechanism and selectivity are keenly debated. To address this, we obtained high-resolution structures of the full NRPS condensation state. Forming the appropriate complexes was challenging because the two condensation substrates are each covalently linked to separate T domains within the same protein. To overcome this challenge, we made independent constructs of the two modules of the NRPS LgrA, covalently ligated different non-hydrolyzable substrate analogues to each T domain and stitched the modules together using protein ligation. This strategy allowed us to solve, for the first time, two sub-3Å crystal structures of the dimodular LgrA complex, in the substrate- and product-bound states. Through these structures we have hypothesized new functions for conserved residues in the chemical mechanism of condensation in NRPS C domains. We are currently using chemical biology strategies to develop novel biochemical assays to study the importance of these residues. Collectively the structural and biochemical data will help answer standing questions about the chemical mechanism of condensation, an important step in NRPS-mediated biosynthesis.

ABS#700

Poster session, July 13

The Key to Allosteric Inhibition in Human UDP-glucose 6-dehydrogenase is a Buried and Unsatisfied Polar Group

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Human UDP-glucose 6-dehydrogenase, hUGDH, is an essential enzyme in the synthesis of extracellular matrix components like hyaluronic acid. The overproduction of hyaluronic acid has been implicated in the development of aggressive prostate cancers and their metastasis. Understanding how hUGDH is regulated may lead to new strategies in cancer treatment. The activity of hUGDH is regulated through allosteric feedback inhibition by UDP-Xylose. The binding of UDP-Xylose alters the conformation of hUGDH from an active hexamer (E-state) to a horseshoe-shaped, inactive complex called the EΩ-state. The conformational change between these states is slow and can be observed as a lag in progress curves (hysteresis). Our structural studies on hUGDH allostery and hysteresis have identified a buried, unsatisfied polar group (Thr127) within the hydrophobic core of the E-state. In the EΩ-state, the hydroxyl group of Thr127 resides within a small cavity just large enough to accommodate a water molecule. We hypothesize that Thr127 will stabilize the EΩ-state and favor the allosteric transition. Consistent with our hypothesis, the residue corresponding to Thr127 in C .elegans UGDH is an isosteric valine, and the enzyme has a 16-fold lower affinity for UDP-Xylose. We confirmed this observation with a T127V substitution in hUGDH (hUGDHT127V). The resulting construct had similar KM and kcat values but a 10-fold lower affinity for UDP-Xylose. Additional support for the role of Thr127 can be seen in a heavily diminished hysteresis, suggesting that the equilibrium has shifted away from the EΩ-state. Our results show how a buried residue plays a key role in the allosteric mechanism of this essential enzyme.

ABS#702

Poster session, July 13

A Cryptic Allosteric Site Defines a Novel Feedback Inhibition Mechanism in hUGDH

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Human UDP-Glucose Dehydrogenase (hUGDH) catalyzes the NAD-dependent oxidation of UDP-glucose (UDP-Glc) to produce UDP-glucuronic acid. The activity of hUGDH is regulated by an atypical allosteric mechanism where the feedback inhibitor UDP-Xylose (UDP-Xyl) competes with UDP-Glc. Upon binding, UDP-Xyl induces the Substrate Binding Site (SBS) to change conformation to SBSΩ and convert hUGDH (E) to an inactive horseshoe-shaped complex (EΩ). This allosteric transition is remarkable in that the affinity for UDP-Xyl is 100-fold greater than that for UDP-Glc despite the fact that the only difference between the two molecules is the absence of a C-5 hydroxymethyl in the inhibitor. How does this relatively small structural change explain the large difference in affinity? Crystal structures show that both the SBS and SBSΩ sites fully satisfy the respective ligands, suggesting the difference in affinity is not simply from a bad contact or missing H-bond. Here we present evidence that the high UDP-Xyl affinity results from a cryptic allosteric site that works cooperatively with the SBSΩ site to inhibit the enzyme. The cryptic site is formed from the restructuring of the NAD binding site during the E to EΩ transition. Thus, UDP-Xyl can compete with both the substrate and the cofactor, which can be observed as either mixed or competitive inhibition depending on whether the NAD concentration is subsaturating or saturating, respectively. We have derived an equation that describes this 'bi-competitive' mechanism and successfully predicts the observed switching of inhibition mechanisms. The inherent cooperativity of the bi-competitive mechanism also explains how, under physiological conditions, a low abundance feedback inhibitor like UDP-Xyl (100 nM) can outcompete a more abundant ligand like UDP-Glc (10 mM) to inhibit an enzyme.
This study was supported by NIH grant-GMS number 2R01GM114298-06

ABS#705

Membrane Proteins: From Natural to Designed (July 14, PM)

Visualizing the Gymnastics of a Transition Metal Importer and Other LeuT-fold Transporters

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The LeuT-fold superfamily of membrane transporters contains 15 evolutionarily related families that transport diverse substrates like cations, polyamines, amino acids, and neurotransmitters. One of these families is the Nramps (Natural resistance associated macrophage proteins), which enable the cellular uptake of essential transition metals like iron and manganese in most organisms. We have determined high-resolution crystal structures of all steps in the manganese transport cycle from a model Nramp transporter, enabling apples-to-apples comparisons in unprecedented detail. The structures reveal how the global changes in the protein are associated with changes in the geometry of the divalent Mn(II) ion coordination sphere at the metal-binding site in the center of the protein. The manganese is nearly completely dehydrated, with only one bound water molecule, in the occluded state of the transporter cycle, and the manganese coordination sphere deviates from the ideal 6-coordinate octahedral geometry at all steps. Zooming back out to the LeuT-fold superfamily to investigate how the same fold can support such diverse transport functions, we developed analyses of distance difference matrices to compare conformational changes in distantly related homologs. Comparing dozens of LeuT-fold structures using these matrices, we find that the superfamily uses a combination of common transmembrane helix motions—all LeuT-fold transporters rock!—and specialized adaptations in their conformational cycles.

ABS#708

Poster session, July 15

Examining the effect of removing a conserved disulfide bond on the structural stability and activity of a lysozyme-specific nanobody

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Nanobodies have remarkable potential as immunotherapeutic agents, given their reduced size, greater tissue penetration, rapid clearance and enhanced manufacturability compared to conventional antibodies. Disulfide bonds are important to folding and stability of nanobodies; however, they complicate the production of these therapeutics in bacterial systems with reducing cytoplasm, due to aggregation of the target protein products, impairing their activity and manufacturability.

To address these problems, we designed a disulfide-free mutant of a 15 kDa lysozyme-binding nanobody, 1ZV5, useful in classifying lymphoproliferative disorders and monocytic leukemia and explored the effect on folding, stability and activity of the protein. The disulfide-free mutant was designed rationally based on the structural features of the N-terminal extracellular domain of CD2 molecule. The histidine-tagged wildtype (WT) and mutant proteins were expressed and purified using the pET-23a/BL21DE3 E. coli system and characterized thereafter. The CD spectrum of WT displayed characteristic β-sheet structures with contributions from beta strands, cross beta-sheet and beta turn, and a melting temperature of 77°C. However, the mutant was shown to be thermostable and had a slightly altered structure on CD measurements due to alterations in the hydrophobic core and the absence of the conserved disulfide. Both proteins displayed a comparable chemical denaturation profile with Cm in the range of 2.8-3.0 M of guanidium hydrochloride. Initial characterization by ELISA of 1ZV5 and its mutant verified a positive binding relationship with lysozyme, indicating a retained affinity thereof. The lysozyme-susceptible cell lysis assays showed reduced affinity of binding in mutant as compared to the WT. However, saturating the reaction with five times molar excess yielded absolute neutralization in both the proteins. Our work provides insight into designing a small yet effective, stable version of a nanobody, such that the expression-destabilizing disulfide bonds are removed, manufacturing yield is enhanced, and physically stable neutralization of target proteins is preserved.

ABS#709

Poster session, July 15

HeliX® and heliXcyto biosensors: dissect binding interactions from small molecules to cells in real-time

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heliX® and heliXcyto are fluorescence-based, 384 well plates compatible, modular biosensors that detects binding interactions between small molecules, proteins, nucleic acids, antibodies, and cell surface receptors. They utilize two core technologies: switchSENSE® and Real Time Interaction Cytometry (RT-IC). They use disposable, NFC-tagged chips with intrinsic microfluidics for a cost-effective and maintenance-free experimental pipeline.

switchSENSE® technology works by conjugating any ligand to a double stranded, fluorescently labelled DNA nanolever on the surface of a gold electrode. The label-free analyte binds in solution and modifies the fluorescent properties of the dye, yielding a real-time binding curve. switchSENSE® is particularly suited to unravel complex binding interactions such as:

- High performance binding kinetics.
- Ternary vs binary complex formation for PROTACs screening.
- Cooperative binding and sequence rearrangement for nucleic acid binding proteins.
- Affinity vs avidity of bispecific antibodies.
- Conformational changes.

RT-IC measures molecular interaction on the cell surface directly. This technology works by trapping live or fixed cells in a biopolymer cage on top of the same gold electrode. The fluorescent analyte interacts with the cell surface generating a real-time binding curve. RT-IC technology has been applied to measure affinity, avidity, and kinetic of antibodies, Bispecific T-cells engagers (BITEs) with cell surface antigens and to dissect G-Protein-Coupled Receptors (GPCR) density on the cell surface.

heliX® and heliXcyto are extremely versatile tools for molecular interaction studies. A wide range of biophysical parameters can be obtained with one single measurement and in real time. RT-IC is a revolutionary technology that measures real-time binding kinetics on the cell surface directly in an automated fashion.

ABS#710

Poster session, July 13

Comparing the Kinetic Stability of the 11S Globulin Storage Protein in Tree Nuts: Implications for Their Potential Allergenicity

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Kinetically stable proteins (KSPs) are proteins which display a high activation energy for unfolding that traps the protein in a folded/native conformation. This increase in activation energy of unfolding is what defines kinetic stability (KS), a characteristic of some proteins that plays important biological roles such as degradation and protease resistance. This novel study compares the KS of the 11S globulin seed storage protein (SSPs) from the nine most common tree nuts (TN) and explored how increased KS may correlate with potential allergenicity within this model system. This was achieved by first using polyacrylamide gel electrophoresis (PAGE) to assess a KSPs resistance to denaturation by the strong detergent sodium dodecyl sulfate (SDS) and weak detergent sodium lauroyl sarcosinate (SAR) as a proxy for different levels of KS. The resulting KS assessments were then used to categorize each TN 11S globulin into the high, medium, or low KS groups. Finally, the experimental data was correlated with allergy prevalence data within the clinical allergy field. Specifically, individuals allergic to each individual TN were assessed to determine how many of the allergic patients recognized the 11S globulin allergen. This way, the importance of the 11S globulin in eliciting allergic reactions across patients allergic to each TN, when compared to other SSPs is established. By correlating experimentally determined KS with allergy prevalence statistics for the model system of TN, a positive correlation between the two factors was observed, suggesting KS may have a significant role in developing allergy.

ABS#714

Poster session, July 13

Structural Insights into Prestin, the Auditory Amplifier Motor Protein

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Hearing is a process in which sound-induced mechanical vibrations are converted into electric signals within the inner ear and relayed to the brain. Outer hair cells (OHCs) electromotility, driven by prestin, is essential for mammalian auditory amplification 1, 2) (Fig. A). Prestin amplifies the auditory signal, increasing our hearing sensitivity up to 1000-fold, allowing humans to detect sound pressure as low as 20 μPa 3). The motor activity of prestin coincides with the voltage-induced movement of voltage sensor charges, which manifests as nonlinear electric capacitance (NLC). Here, we report the cryo-EM structures of thermostabilized prestin (PresTS) in complex with chloride ion, sulfate ion, and salicylate at the resolutions of 3.63, 3.52, and 3.57 Å, respectively 4) (Fig. B). PresTS forms a domain-swapped homodimer, with each protomer consisting of 14 transmembrane α-helices and the C-terminal cytosolic Sulfate Transporter and Anti-Sigma factor antagonist (STAS) domain crossing each other. The transmembrane domain is divided into two subdomains: the core and the gate domains. The central positively-charged cavity allows flexible binding of various anion species, which likely accounts for the reported distinct modulations of NLC by different anions. Comparisons of our PresTS structures with recently-reported ones suggest rigid-body movement between the core and gate domains, and provide insights into inhibition mechanism by salicylate 4-7). Mutations at the dimeric interface severely diminished NLC, suggesting that stabilization of the gate domain facilitates core domain movement, thereby contributing to the expression of NLC. These observations suggest that the bound anion and its surrounding environment work as the extrinsic voltage sensor in prestin, and their longitudinal migration due to the elevator-like movement might be detected as NLC, while other charged residues in the core domain may also contribute to the net charge movement. These findings advance our understanding of the molecular mechanism underlying mammalian cochlear amplification.

ABS#715

Poster session, July 15

Mass Spectrometry Analysis of Myoglobin Unfolding in Acetonitrile

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Apo-myoglogin's folding has been extensively studied and modeled1. However, while experiments have also looked at the unfolding of holo-myoglobin, this process is complicated by mixed protein populations due to the dissociation of heme2. Mass spectrometry can easily differentiate apo versus holo myoglobin but the technique is incompatible with traditional chemical denaturants like urea and guanidine. However, acetonitrile can be used to denature proteins and is compatible with mass spectrometry3. Experiments were conducted to measure changes in mass spectrometry charge state distributions as a function of acetonitrile concentrations for holo-myoglobin. Data revealed the changes in the populations of apo and holo myoglobin as the protein unfolds. Holo-apo dimers and higher order oligomers were also detected as the protein unfolds.

ABS#718

Poster session, July 13

Alternative Surfactant Probes to Investigate Kinetically Stable Proteins

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Kinetically Stable Proteins (KSPs) are characterized by a large energy barrier to unfolding. These proteins are hyperstable, unfolding very slowly with longer half-lives compared to non-Kinetically Stable Proteins (nonKSPs). Such hyperstability is vital in certain biological and industrial systems where persistence in harsh conditions such as extreme temperature or in the presence of proteases is necessary. Sodium Dodecyl Sulfate (SDS) is a harsh, anionic surfactant that has been shown to be an effective probe for high Kinetic Stability (KS). NonKSPs unfold in the presence of SDS, but KSPs are resistant SDS-induced denaturation and require further energy input such as heat. While SDS-resistance is a highly effective proxy for identifying high KSPs, it sets a very high threshold for defining KS and is unable to identify proteins that possess a significant level of stability but do not attain SDS-resistance. This study identifies a set of less denaturing surfactants to probe proteins that possess moderate Kinetic Stability (mKS) that cannot be identified by SDS alone. By identifying a new set of mKSPs, we can explore the characterization of kinetic stability in a spectrum, beyond a binary system of SDS-resistance or SDS-susceptibility. Further, with the range of moderate to high KSPs that we identify with these surfactants, we can establish a database of high, moderate, and nonKSPs and apply bioinformatic and computational methods to assess key structural characteristics that contribute to kinetic stability at each level.

ABS#719

Poster session, July 14

Novel Interleukin-6 Analyte-Responsive Biopolymers

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In response to the fourth industrial revolution and the next generation of regenerative medicine and synthetic organ production, a novel sensor is needed to detect biomolecular analytes specific to monitoring cellular health status. Presented herein is the design and synthesis of a novel interleukin-6 responsive protein polymer. Our analyte responsive protein polymer (ARP) design is a fusion protein consisting of a binding element and an elastin like polymer (ELP) body, with two main variations in the binding element, either an antibody short chain variable fragment (scFv) or a peptide sequence derived from hotspot residues in the human IL6 receptor.

ABS#724

Poster session, July 14

Toward Robust Bacterial Production of IL-6 for Biosensor Development

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Interleukin 6 (IL-6), a proinflammatory cytokine, is of rising interest regarding its many uses in scientific contexts. This protein is highly desirable for researchers whose aim is to study its functions. In particular, we are developing protein polymers for incorporation into IL-6 biosensors. Therefore, the generation of milligram quantities of pure IL-6 is of great importance as well. The primary objective of this project is to establish a robust system for bacterial expression and purification of IL-6, based on adaptions of existing published methods, thereby enabling further investigations into

its intricate biological functions and offering valuable opportunities for its application in scientific contexts.

ABS#725

Poster session, July 15

Evidence for a Skp1 Sequestration Mechanism for the Skp1 α-Galactosyltransferase Gat1 in Toxoplasma gondii

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The SCF (Skp1/Cullin-1/F-Box protein (FBP)) complex is involved in proteomic regulation within all eukaryotes. Protein targets are specifically recognized by a variety of FBPs which bind the Skp1 adaptor protein linking the substrate recognizing FBPs to the SCF complex. In the agent for human toxoplasmosis, Toxoplasma gondii, the oxygen-dependent prolyl hydroxylase PhyA hydroxylates a conserved proline on Skp1 priming it for addition of 5 sugars the last of which is mediated by the glycosyltransferase Gat1. Substantial evidence Indicates that glycosylation promotes both disassembly of the strong Skp1 homodimer and association with FBPs. Genetic deletion of Gat1 results in a modest lytic defect in fibroblast monolayers that is strongly exacerbated in a PhyA-KO background. This was surprising since Gat1's only known substrate is the Skp1 glycan, whose generation depends on the prior action of PhyA. Co-immunoprecipitation data indicate that, unlike the other GTs, Gat1 stably associates with Skp1 in vivo suggesting that Gat1 plays an additional role in associating with non-substrate glycoforms. Gel filtration and analytical ultracentrifugation (AUC) studies indicate that Gat1, a stable homodimer, directly and stably interacts with monomeric Skp1 with a submicromolar Kd. Glycosylation is suggested to affect how many Skp1 molecules associate with the highly stable Gat1 dimer. This may be related to the dissociative effect of glycosylation on Skp1 homodimerization as observed using gel filtration and AUC. Building on the known structure of each protein, AlphaFold modeling suggests that the Gat1 binding interface on Skp1 overlaps with its homodimerization and FBP interfaces. This model is supported by evidence that Gat1 inhibits Skp1 hydroxylation and competes with FBP binding to Skp1 in vitro. These findings offer a biochemical mechanism for the genetic data by suggesting a second role for Gat1 in titrating the availability of Skp1 for the SCF complex. A related effect was previously proposed for the unrelated terminal glycosyltransferase of Dictyostelium Skp1, suggesting that this mechanism was a driving force for convergent evolution.

ABS#726

Poster session, July 13

Characterization of the Insulin-Binding and Stimuli-Responsive Behavior of Candidate Insulin-Responsive Protein Polymers

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Elastin-like polymers (ELPs) possess a unique ability to reversibly self-assemble in response to various stimuli. This property makes ELPs promising for applications in biosensing. We hypothesize that ELP fusion proteins can be designed for incorporation into biosensors, such that specific ligand binding results in a change to the stimuli-responsive behavior of the ELP, reflected by a shift in the transition temperature (Tt) at which assembly occurs. In this research, we investigate the influence of two putative insulin-binding recognition elements, a peptide and a single chain variable fragment (scFv), on the Tt of ELPs. We performed dynamic light scattering and isothermal titration calorimetry to characterize ELP transition temperature behavior and the kinetics and thermodynamics of insulin binding, when present. The engineering principles discovered here will allow for more successful prediction and efficiency of analyte-responsive polymer design based on the physicochemical properties of the recognition element, analyte, and their association, as well as the desired functionality of the polymer and behaviors of the stimuli-response.

ABS#728

Poster session, July 15

Investigating caspase-6 and tau interactions for exosite identification

Andrew Smith (1); Jeanne Hardy (1)

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Caspases are proteases involved in apoptosis and inflammation which can cause debilitating diseases if their activity isn't properly regulated. These enzymes are classified as inflammatory, initiator, or executioner caspases and cleave protein substrates predominantly after aspartic acid residues. Caspase-6, one of three executioner caspases, is of particular interest due to its involvement in Alzheimer's Disease and its unique structural features relative to its closest relatives, caspase-3 and -7. In Alzheimer's Disease, microtubule-associated protein tau forms neurofibrillary tangles, aggregates which lead to progressive memory loss and deterioration of brain function. Caspase cleavage of tau appears to be an upstream event prior to aggregation. Both caspases-3 and -6 are known to cleave tau at several sites distal from amyloidogenic sequences commonly found in tau aggregates, however only blocking of caspase-6 cleavage influences disease outcomes. Thus, there is a need for the ability to block cleavage of tau by caspase-6. Our aim is to study protein:protein interactions between caspase-6 and tau to identify exosites critical for substrate recruitment and selection. An exosite is a site distal from the active site of a protein and can be utilized by an enzyme and may be used for recruiting a set of substrates more efficiently than others. Once characterized, the exosite on caspase-6 or tau may be ripe for discovery of compounds that block the caspase-6:tau interaction, ideally allowing caspase-6 to otherwise function properly in other pathways.
Caspase-

ABS#731

Poster session, July 13

Investigation of the effects of overexpression of Human Jumping Translocation Breakpoint protein (hJTB) in MCF7 cells using in-gel digestion based Proteomics

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Human JTB (hJTB) is a gene located on the human chromosome 1 at q21 which is involved in the unbalanced translocation in various types of cancer. JTB protein is ubiquitously present in normal cells and is found to be overexpressed in various types of cancer including prostate and breast cancer. Hence this protein could be a biomarker for tumor malignancies and a potential target for their treatment. However, the biological function and the pathway through which this protein causes increased cell growth and proliferation are not entirely clear. Investigation and comparison of the proteomes of cells with upregulated and downregulated JTB can be a good approach to understanding the protein's function and its contribution to tumorigenesis. In this study, MCF7 breast cancer cell lines were transfected with the sense orientation of the JTB cDNA in HA, His, and FLAG tagged CMV expression vector as well as with shRNA plasmids. Proteins extracted from transient and stable transfected cells were separated using 1D-SDS-PAGE. The expression of JTB was confirmed by the western blotting technique. In-gel digested peptides were analyzed by a Nano Acquity UPLC coupled with QTOF Xevo G2 Mass Spectrometer. Data processing was done using Mascot 2.4 server and Scaffold 4.1 software. We found several proteins such as HSP's, Actin, and/or tubulin proteins are closely associated with hJTB function. Furthermore, we performed a GSEA analysis to identify the biological processes and pathways associated with the JTB protein. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the JTB protein as a potential drug target for malignancies with overexpression of the protein.

ABS#732

Poster session, July 15

Structural Characterization of Selective and Potent Allosteric Inhibitors of Caspase-6 for Treatment of Neurodegeneration

Irina Sagarbarria (1); Jeanne Hardy (1)
(1) Chemistry, University of Massachusetts Amherst, Amherst, United States of America

Caspase-6 has been shown to be involved in several neurodegenerative processes such Alzheimer's (AD) and Huntington's Diseases (HD). Hyperactivity of caspase-6 in the context of neurodegeneration leads to the cleavage of neuronal proteins tau and tubulin, resulting in the formation of neurofibrillary tangles, a key pathological hallmark in AD, as well as axonal degeneration. Its involvement in the pathogenesis of these neurodegenerative diseases makes it an attractive target for drug development. Selective inhibition of caspase-6 and none of the

other 11 human caspases that share the same active site chemistry is crucial to achieve the desired therapeutic effect. A series of compounds has been developed in the Hardy Lab which selectively inhibits caspase-6 by covalently modifying a unique, non-catalytic cysteine residue, Cys 264. Structural characterization of the covalent complex formed between caspase-6 and one of our most potent analogs in this compound series, KT-60, is being performed using x-ray crystallography. X-ray data from co-crystals of caspase-6 and KT-60 do not show clear density for the inhibitor. Since the furoxan moiety of KT-60 is thiol-reactive, it is hypothesized that the presence of dithiothreitol prior to crystallization may cause the inhibitor to react instead with it. Crystallization trials in the absence of reductant are currently in progress. Mutational analyses of certain aromatic residues in the pocket where the extra density lies show that some of them may be involved in forming non-covalent interactions with KT-60, which may underlie its increased potency in comparison to the lead compound identified from the high throughput screen. We also aim to evaluate the in vivo efficacy of our inhibitor in a mouse model by comparing the levels of active caspase-6 and its cleaved substrates in brain and intestinal tissue with and without inhibitor treatment. The results of this study will enable us to understand the mechanism, specificity, and binding mode of this compound series to determine their suitability for AD-directed drug development.

ABS#734

Poster session, July 13

Vaccination with Stabilized Dengue Envelope Dimers Using Liposomal Adjuvant as a Display Method

Thanh Thanh Phan (1); Matthew Hvasta (1); Shaomin Tian (2); Gisselle Prida Ajo (1); Aravinda De Silva (2); Brian Kuhlman (1)
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The envelope (E) glycoprotein of Dengue viruses (DVs) is the main target of neutralizing antibodies. In humans, potently neutralizing antibodies target regions that span across two monomers in a homodimer. As a result, it is believed that an effective E subunit vaccine should remain dimeric under vaccination conditions. Previous immunization studies in mice using alhydrogel as an adjuvant have shown moderate protective response against the virus which targets mainly the E monomer. Recently another adjuvant platform using liposomes synthesized with or without cobalt porphyrin phospholipid (CPQ vs LPQ) has been demonstrated to be effective for vaccine formulation against different viruses. CPQ liposomes display proteins through interaction with a poly-histidine tag, providing multivalent display of antigen on the surface of the liposome. In this study, we employ these liposomes as adjuvants and display method to compare the immunogenicity of wildtype and stable E dimers from different DVs (DV1-4). Biochemical and structural characterization of the samples showed that conjugation of proteins to CPQ liposomes does not affect liposome integrity or protein oligomeric state. We then vaccinated mice with CPQ wild type E, LPQ stable E dimer or CPQ stable dimer in a monovalent DV2/3, bivalent DV2 and DV3 or tetravalent DV1-4 fashion. Our data show that, while both liposome formulations outperform alhydrogel, there is a difference in antibody profile between CPQ and LPQ adjuvants. We also observe that the wildtype protein had lower titer than stable dimer, which was significantly boosted by the second injection. More importantly, our data show that the CPQ platform is a viable option for multivalent, cocktail formulation of E proteins from different DVs. In conclusion, our study highlights a novel adjuvant platform for the display of E dimers as subunit vaccine antigen.

ABS#735

Poster session, July 14

Investigation of the effects of overexpression of Human Jumping Translocation Breakpoint (JTB) Protein using in-solution digestion-based Proteomics

Krishan Weraduwage (1); Madhuri Jayathirtha (2); Danielle Withad (3); Costel C Darie (2)
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Human JTB (hJTB) is a gene located on the human chromosome 1 at q21 which is involved in the unbalanced translocation in various types of cancer. JTB protein is found to be overexpressed in many types of cancer including prostate and breast cancer and is also present in normal cells. The biological function and the pathway

through which this protein causes increased cell growth and proliferation are not fully deciphered.Upregulated and downregulated JTB conditions can be a good approach to understanding the function of the protein and its interacting partners, as well as the biological pathways andmetabolic processes through which it functions. MCF7 breast cancer cell lines were transfected with the sense orientation of the JTB cDNA in HA, His, and FLAG-tagged CMV expression vector as well as with shRNA plasmids. Proteins extracted from transient and stable transfected cells were separated using in-solution digestion-based Proteomics. These samples were analyzed by a Nano Acquity UPLC coupled with QTOF XevoG2 Mass Spectrometer. Data processing was done using Mascot 2.4 server and Scaffold 4.1 software. We found several proteins such as HSP's, Actin, and tubulin proteins and pathways which are closely associated with hJTB function. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the JTB protein as a biomarker for early diagnosis and treatment of breast cancers.

ABS#736

Poster session, July 15

Direct observation of the evolution of oligomer populations during protein aggregation using single-molecule mass photometry

Simanta Paul (1); Aaron Lyons (1); Russell Kirchner (1); Michael T. Woodside (1)
(1) Department of Physics, University of Alberta, Edmonton, Canada

Many neurodegenerative diseases feature aggregation of misfolded proteins. Small oligomers are thought to be key neurotoxic species in these diseases, making the early stages of the aggregation cascade crucial to understand. However, early aggregation is difficult to study because it features heterogeneous mixtures of transient states. We use single-molecule mass photometry to track the populations of different oligomers as they evolve over time during aggregation by measuring individually the masses of the set of oligomers present in solution. Applying this approach to tau protein, which aggregates in Alzheimer's disease, we found that wild-type tau existed in an equilibrium between monomers, dimers, and trimers before aggregation was triggered. After triggering aggregation, oligomers up to 25-mers were detected. The number of monomers dropped monotonically, alongside first a rise in the population of the smallest oligomers followed by a steep drop as larger oligomers and fibrils began to grow rapidly. We fit the evolution of these populations to kinetic models to test different models of aggregation and identify the most likely mechanism, thereby quantifying the microscopic rates for each step in the aggregation. Applying the same approach to the pathogenic P301L mutant revealed that it could aggregate without any inducer, unlike wild-type tau, but it aggregated much more slowly, leading to larger populations of large oligomers. The approach we demonstrate here provides a powerful tool for characterizing previously inaccessible regimes in protein aggregation and building quantitative mechanistic models.

ABS#737

2023 Stein & Moore

Learning from nature's switches to make biotech tools and therapeutics

Kevin Gardner (1)
(1) Registrations Project Manager, AGORA OPUS3, Québec City, Canada

Environmental cues regulate many biological processes, coordinating cellular pathways to respond to changing conditions. Such regulation is often initiated by sensory protein domains which expand their chemical repertoire by using small molecule ligands to convert environmentally-triggered changes into altered protein/protein interactions. Combining biophysics, biochemistry and synthetic chemistry, we study the mechanistic controls of such domains to understand fundamentals of biological signaling and how this might be altered in disease or artificially controlled for therapeutic or biotech purposes. Here I will discuss several examples of this principle, showing how our work into light- and oxygen-regulated signaling proteins has led to novel optogenetic tools and a first in-class anti-cancer therapeutic (Merck's belzutifan HIF-2 inhibitor). Future directions stemming from this work will also be discussed.

ABS#738

Poster session, July 15

Structure of the Wnt-Frizzled-LRP6 Initiation Complex Reveals the Basis for Coreceptor Discrimination

Sunhee Hwang (1)
(1) Genentech

Wnt signaling is an important pathway that regulates numerous cellular and development processes. Canonical Wnts form ternary receptor complexes composed of tissue-specific Frizzled (Fzd) receptors together with the shared LRP5/6 coreceptors to initiate β-catenin signaling. A structural view of this ternary initiation complex has not been elaborated, primarily due to the low affinity of the Wnt-LRP6 interaction. We used in vitro protein evolution to engineer a high-affinity Wnt with enhanced Wnt-LRP6 interactions and signaling potency. The engineered Wnt enabled biochemical reconstitution and structural visualization of the soluble Wnt-Frizzled (Fzd)-LRP6 ternary complex. In the structure, Wnt binds to LRP6 in a tenuous interaction mediated by two flexible segments on Wnt. The Wnt N-terminus forms a relatively minor interaction, while the major interaction is formed by the "NC-loop" emanating from the globular core of Wnt, that simultaneously insert into the central funnels of the E1 and E2 domains of LRP6, respectively. The 40Å spacing separating the two Wnt binding determinants is matched by the distance between the E1E2 funnels, and is maintained between the E3E4 funnels to enable a similar binding mode for LRP6 E3E4-binding Wnts. We further constructed chimeric Wnts bearing NC-loop 'grafts' that were able to transfer LRP6 domain specificity between different Wnts. Synthetic peptides comprising the NC-loops of multiple Wnts were able to inhibit Wnt binding to LRP6 and Wnt signaling. The structure of the ternary complex provides a topological blueprint for the orientation and proximity of Fzd and LRP6 within the Wnt cell surface signalosome.

ABS#739

Poster session, July 15

Temperature Dependent Catalytic Activities of the Intra-Melanosomal Domain of Tyrosinase-Related Protein 1 (TYRP1) and Oculocutaneous Albinism Type 3 (OCA3) Related Mutants

Isabella Osuna (1)
(1) NIH

Oculocutaneous Albinism Type 3 (OCA3), an autosomal recessive disorder, is caused by mutations in Tyrosinase-Related Protein 1 (TYRP1). OCA3 leads to vision abnormalities and photophobia and presents with rufous or brown OCA. TYRP1 catalyzes the oxidation of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) to indole-5,6-quinone-2-carboxylic acid (IQCA). To determine the TYRP1 enzymatic activity, a subsequent reaction using 3-methyl-2-benzothiazolinone hydrazine hydrochloride (MBTH) is required to enhance the reaction and allows the product to be determined spectrophotometrically. However, reports on TYRP1 activity in human and mice remain controversial. Here, we focus on the effects of temperature on the enzymatic activity of TYRP1 and OCA3 mutant variants, R326H and D308N. The proteins were expressed using the baculovirus in Trichoplusia Ni larvae and then purified using IMAC and SEC. The product of the enzymatic reaction was confirmed using MBTH which forms a visible dye complex with IQCA at 505 nm at 25, 32, 37, and 43 ºC for 4 hours. We then tested the enzymatic activity of the proteins using the same experimental parameters but without MBTH and for 24 hours. When comparing the two reactions, the one using MBTH shows increasing activity with increasing temperature for 4 hours of incubation for TYRP1 and D308N. Although, R326H reaches maximum activity at 37ºC, indicated with the same absorption level. However, TYRP1 and R326H show maximum activity at 37 ºC and decrease at 43 ºC (heat shock conditions) for a 24-hour incubation whereas D308N increases. In the absence of MBTH, TYRP1 and R326H are more susceptible to heat shock conditions whereas D308N not only retains but also increases activity. In the future, we aim to use these results to determine the temperature dependent kinetic parameters and Van't Hoff analyses of the oxidation of DHICA to IQCA by TYRP1 and mutant variants.

ABS#740

Poster session, July 15

Evaluating the Versatility of Gator® Next Generation BLI Platform for Biotherapeutic Development and Gene Therapy

Harsha Agarwal (1); Johnny Zhang (1); Katie Trieu (1); Wai Choi (1); Imndrani Chakraborty (1); Vivienne Lee (1); Rich Wang (1); Pu Li (1); Robert Zuk (1)
(1) GatorBio

The Gator® next generation BLI platform is a label-free, real-time monitoring system that enables characterization and optimization of therapeutic antibodies, proteins, small molecules, LNPs, and viruses. The Gator® instruments and biosensors offer a wide range of assays for protein-protein interactions and quantitation including epitope binning, kinetics, affinity, and specificity. Here, we present data on these applications that demonstrates versatility, throughput and accuracy for accelerating the

development of biotherapeutic and gene therapy candidates.

ABS#741

Poster session, July 13

Developing a model to predict whether single nucleotide variants are likely benign or pathogenic

Ramit Goyal (1)
(1) School of Engineering & Applied Science, Yale University

Many human diseases like cystic fibrosis are caused by single amino acid mutations. These mutations can change protein structure, influence their ability to interact with biological macromolecules, and hinder their function. Previous studies have assembled a large data set of single amino acid mutations and categorized them as likely pathogenic (LP) or benign (LB). We seek to identify features that distinguish mutations that are classified as LP versus LB. Protein three-dimensional (3D) structure determines amino acid solvent accessibility, and it is well-known that the driver of protein folding is sequestering hydrophobic amino acids into a solvent inaccessible core. The relative solvent-accessible surface area (rSASA) is a scale between 0 and 1 that describes whether an amino acid is in the core (0) or on the surface (1). The deep learning model NetSurfP-3.0 can predict rSASA for each amino acid from sequence alone. We ran NetSurfP-3.0 to generate rSASA for each amino acid for proteins in a database of 81,000 mutant sequences and their wildtype counterparts since most of the sequences do not possess experimentally determined structures. We show that the change in rSASA is largest at the mutation site, decaying rapidly after a few sites from the mutation. Further, we show that core mutations have a higher probability of being LP. We also show that the average magnitude of the change in rSASA is similar for both LP and LB mutations. Our novel studies will contribute to the development of computational methods to design therapeutics to ameliorate the effects of pathogenic mutations.

ABS#742

2023 Marie Maynard Daly Award Plenary

Large-scale analysis of proteins to solve equity issues in Alzheimer's disease research

Renã A.S. Robinson (1)
(1) Department of Chemistry, Vanderbilt University

Alzheimer's disease greatly impacts the global population and especially minoritized populations in the United States, such as African American/Black adults. Disparities in disease incidence and burden are amplified by the lack of inclusion of African American/Black adults in basic science research and clinical trials to advance Alzheimer's research. In order to facilitate better understanding of disease and the search for effective biomarkers, proteomics lends as an effective subfield of protein science which provides high-throughput analysis of proteins in biological fluids. The RASR Laboratory has developed mass spectrometry-based high-throughput and quantitative workflows that enable enhanced sample multiplexing and analysis of thousands of proteins in a single experiment. These workflows have been applied to study post-mortem brain and plasma tissues that come from diverse participants in Alzheimer's disease research studies. This presentation will provide an overview of the proteomics strategies and highlight major findings in the pathogenesis of Alzheimer's disease in studies inclusive of African American/Black adults. Additionally, preliminary plasma protein biomarker candidate data and discussion on achieving equity in biomarker research efforts for Alzheimer's will also be provided.

ABS#743

Poster session, July 15

Characterizing Protein Binding with OpenSPR™ and Alto™ Surface Plasmon Resonance

Sajni Shah (1)
(1) Nicoya

Surface plasmon resonance (SPR) is a gold standard in biologics research and the development of safe and effective treatments against diseases. The data derived from SPR can help better understand molecular mechanisms and provide key insights into signaling pathways that are critical in advancing drug development. We demonstrate how Nicoya's OpenSPR™ and Alto™ platforms are ideal for therapeutics development by accurately and precisely providing binding kinetics characterization of a range of protein and other biomolecular applications, including transmembrane protein, oligonucleotide, virus-like particles and antibody-antigen binding systems.

ABS#744

Peptide Modalities: Size Doesn't Matter (July 14, AM)

Tales of macrocycle medchem: Balancing cell permeability and target binding

Josh Schwochert (1)
(1) Unnatural Products Inc.

Nearly all cyclic peptides found from ribosomally synthesized library techniques; e.g. phage display and mRNA display, are too large and far too polar for cell permeability. To overcome this, we have utilized a non-traditional medicinal chemistry approach focused on large leaps in scaffold and pharmacophore space. A model medicinal chemistry campaign will be discussed utilizing our chemistry and computational platform. Through the use of iterative focused libraries and direct-to-biology screening we rapidly optimized a series of MDM2 binding cyclic peptides from unobservable intrinsic permeability to small-molecule-like permeability while improving potency 100-fold, yielding compounds with nM cell activity. Efforts to improve the drug-likeness of de novo peptide ligands through macrocycle DNA-encoded libraries, will also be discussed.

ABS#745

Poster session, July 14

Development of a Selective CD16a-Based NK Cell Engager Utilizing Antibodies Targeting a Single Amino Acid Variation

Wuxiang Liao (1); Christine Tumanut (1); Lin Li (1); Adam Corper (1); Dillip Challa (1); Alex Chang (1); Hydari Begum (1); Elinaz Farokhi (1); Rich Wang (1); Catherine Woods (1); Xiaomin Fan (1)
(1) Gator Bio

Natural killer (NK) cells play a vital role in the human innate immune system and are being explored as a promising approach for cancer immunotherapy. Of particular interest are NK cell engagers that can target and activate NK cells to attack cancer cells. In this study, we developed novel NK cell engagers by targeting the NK cell activating receptor CD16a using antibodies that selectively distinguish between CD16a on NK cells and CD16b on granulocytes, which are highly homologous to each other. To generate antibodies with high developability, we used AvantGen's Germliner™ Library Collection and AvantSabre Fab antibody yeast display platform, and discovered two classes of antibody clones that selectively recognize CD16a without cross-reactivity to CD16b. Epitope mapping revealed that a single amino acid difference confers over 10,000-fold selectivity for one class of antibody clones, while for the other class a second unique epitope on CD16a was identified. In bispecific engager format, these antibodies exhibited potent tumor cell-dependent activation of NK cells and effective killing of tumor cells, even in the presence of 10 mg/mL of human IgGs and outperforming a CD16a antibody currently in the clinic. Our findings indicate that anti-CD16a antibody-based NK cell engagers have significant potential for cancer immunotherapies.

ABS#746

Poster session, July 15

Transmembrane Allostery: Identifying the Network and Energetics of Ion Channel Inactivation by NMR

Yunyao Xu (1); Eric Keeler (1); Zhiyu Sun (1); Dinghy Zhang (1); Manasi Bhate (1); Ann McDermott (1)
(1) Columbia University

Potassium channels, which conduct potassium ions selectively through lipid membranes, are ubiquitous and critical for various physiological functions, such as hearing, heart beating and signal transduction. Fulfilling these functions involves accurate control of two gates in the potassium channel to transit the protein into different states. Using a bacteria potassium channel named KcsA as a model system, we applied SSNMR to characterize various states and demonstrate that the two gates are allosterically coupled to achieve a functional phenomenon called C-type inactivation, where potassium channel spontaneously shut down the conduction of potassium ions. Here we quantitatively characterized this allosteric coupling network, identified key residues participating in this coupling.

ABS#747

Poster session, July 13

Ubiquitinated H2B is a gatekeeper to the nucleosomal acidic patch

Chad Hicks
(1) Johns Hopkins University

Ubiquitination of H2BK120 (H2Bub) and H2AK119 (H2Aub) on histone proteins of nucleosomes regulates fundamental biological processes. A handful of previous experiments have observed that H2Bub interferes with protein binding to nucleosomes, but the mechanism by which this occurs has remained unclear. Here, we determined the cryoEM structures of H2Bub and H2Aub-modified nucleosome to reveal the mechanism by which H2Bub excludes RCC1 from the nucleosome. H2Bub adopts multiple positions on the surface of the nucleosome occluding portions of the nucleosome acidic patch, a hotspot binding surface commonly recognized by nucleosome binding proteins like RCC1. H2Aub also adopts multiple positions on the surface of the nucleosome but does not occlude access to the acidic patch. Furthermore, we performed binding studies showing that H2Bub but not H2Aub excludes RCC1 from the nucleosome acidic patch in vitro. These results suggest that H2Bub acts as a gatekeeper to the nucleosome acidic patch.

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