PS36

Abstracts Special Issue

THE PROTEIN SOCIETY

JULY 7 - 10, 2022

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THE 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 36 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.

#PS36

1986 - 2022

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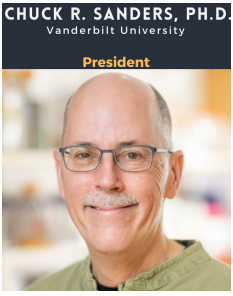
Welcome



Thank you for joining us at the 36rd Annual Symposium of the Protein Society! We are grateful and proud to have had one of our best meetings yet, after waiting eagerly to reconvene.

We are also proud of the amazing line-up of poster presentations (350+), stimulating talks (110+ speakers), and much-awaited networking across the 3.5 days in San Francisco. This year we awarded a record number of travel grants, including our Diversity, Equity and Inclusion Travel Awards – part of our unwavering commitment to supporting young scientists of all backgrounds. We also did not increase our already-reduced registration rates – a commitment we will continue to make for years to come.

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 in the United States and 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.

Kind Regards,

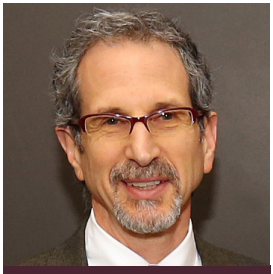
Charles R. Sanders

Charles (Chuck) Sanders, Ph.D.
President

PS37 THE PROTEIN SOCIETY
37th Annual Symposium
Boston, Massachusetts
July 13 - 16, 2023

Program Planning Committee

San Francisco | July 7 - 10, 2022



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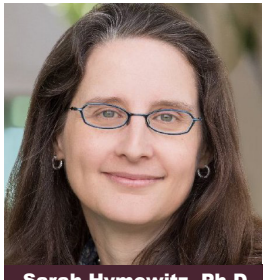

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Thank you for helping us celebrate 36 years of impact.

2022 Award Winners

Squire Booker, Ph.D., Penn State University

2022 Hans Neurath Award Winner - Sponsored by the Hans Neurath Foundation

Throughout Squire Booker's scientific career, he has studied the most challenging and interesting enzyme systems. Having had the prescience as a postdoctoral scholar to investigate the founding member of the iron-sulfur/radical-S-adenosyl-L-methionine (radical-SAM) enzyme family, which is approaching a million assigned members and has unquestionably become the foremost frontier area in enzyme research, Booker then taught the community how to produce and handle with these fragile enzymes in his early work at Penn State. This past decade, his investigations into reactions that append sulfur, methyl(ene), and methylated sulfur moieties upon unactivated aliphatic, olefinic, and aromatic carbons have provided deep insight into the molecular logic of biosynthetic pathways to enzyme cofactors and drugs and the mechanisms of antibiotic resistance. Most recently, he overcame a longstanding barrier to the study of radical-SAM enzymes with cobalamin cofactors, allowing dissection of pathways to fosfomycin and carbapenems, clinically important antibiotics. This work elucidated fundamentally novel chemistry, including reactions that grow fully-saturated alkyl chains – one carbon at a time – using SAM as methyl donor and cobalamin as intermediary. For his elucidation of the structures and mechanisms of radical-SAM enzymes in antibiotic biosynthesis and resistance, Squire Booker is most deserving of the 2022 Protein Society Hans Neurath Award.

Previous Hans Neurath Award Winners

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

Daniel Herschlag, Ph.D., Stanford University

2022 Stein & Moore Award Winner

Dan Herschlag is renowned for his extraordinary rigor, intellectual breadth, creativity, and accomplishment in diverse areas of protein science. His unique style of scientific inquiry, applying fundamental chemical, biophysical and enzymological principles to long-standing and emerging questions in protein science and biology, has borne fruit in numerous ways. In protein enzymology, Dr. Herschlag identified Catalytic Promiscuity, a critical missing link in evolution and a foundation for the fertile area of directed evolution. His group has provided the deepest and most extensive dissection of hydrogen bonds and their contributions to enzyme catalysis. In Protein-RNA interactions, he developed the RNA Chaperone hypothesis, presaging the discovery of cellular RNA chaperones and recognition of their widespread importance. He demonstrated the ubiquitous role of RNA binding proteins in coordinating gene expression. Most recently, his has group transformed our understanding of Protein-RNA recognition 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.

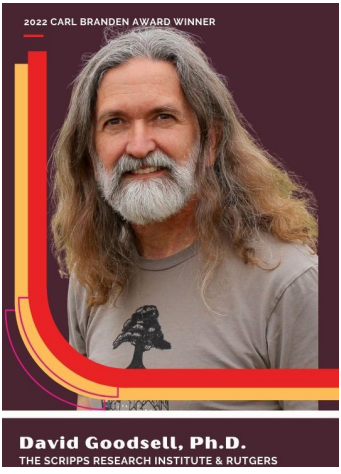
Previous Stein & Moore Award Winners

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
1990 - Kurt Wuthrich
1989 - Hans Neurath
1988 - Fred Richards
1987 - Emil Smith

2022 Award Winners

David Goodsell, Ph.D.; The Scripps Research Institute

2022 Carl Brändén Award Winner - Sponsored by Rigaku Corp.



David S. Goodsell's iconic educational materials and paintings, made freely available for use and reuse, that have introduced students around the globe to the science of structural biology using crystallographic and 3DEM structures, from basic biology to emerging public health crises such as Ebola, measles, and now COVID-19. David combines his training in structural biology and experience in scientific research and software development with active practice of the visual arts. He pioneered extraordinary visual methods for exploring molecular and cellular structure that are the foundation of his public outreach/education work. His distinctive, non-photorealistic technique creates easily-interpretable illustrations of molecules and the structure of living cells, both as watercolor paintings and computer-generated images. His passion for and remarkable ability to illustrate and describe molecular landscapes to expert researchers and beginners alike has led to collaborations with science museums, filmmakers, educators, and popular authors on the creation of educational and outreach materials. David has also written several general-interest books on molecular biology, cell biology, and bionanotechnology. Throughout his unique and impressive career, David has promoted and enabled effective teaching and learning of biochemistry and molecular biology and has inspired the public with his vision, his creativity, and his artistic genius.

Previous Carl Brändén Award Winners

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

Jin Zhang, Ph. D., University of California, San Diego

2022 Christian B. Anfinsen Award Winner

The 2022 recipient of this award is Professor Jin Zhang. Dr. Zhang pioneered a native biochemistry approach and developed innovative photophysical tools to enable the precise interrogation of biochemical networks within living cells. The timing and location of biochemical processes encode specific signals for life processes. To extract the key spatiotemporal information, Dr. Zhang developed general strategies and specific fluorescent biosensors to study signaling molecules in their native biological context, from living cells to live animals. These strategies have proven generalizable for the study of many signaling molecules, sparking the development of an ever-expanding repertoire of fluorescent biosensors. Dr. Zhang also developed first-in-class technologies for imaging protein-protein interactions and enzymatic activities in living cells at a spatial resolution below the diffraction limit. Enabled by these technologies, pioneering work in the Zhang lab has led to a breakthrough discovery of fundamental mechanisms underlying spatial compartmentation of a ubiquitous second messenger, cAMP, as well as revelation of the oncogenic mechanism of fibrolamellar carcinoma, an atypical liver cancer. This and other discoveries in Zhang's laboratory have begun to establish a new conceptual framework — cellular biochemical activities are spatially organized into an activity architecture to encode essential information that profoundly impacts cell physiology and disease.

Previous Christian B. Anfinsen Award Winners

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

2022 Award Winners

Sun Hur, Ph.D., Boston Children's Hospital

2022 Dorothy Crowfoot Hodgkin Award Winner:

Supported by a grant from Genentech, a member of the Roche Group

Sun Hur's structural and biochemical work on a family of vertebrate innate immune receptors, RIG-I-like receptors (RLRs), led to the discovery of receptor polymerization and clustering during foreign RNA detection and immune activation. Her laboratory elucidated how RLR filament formation enables detection of various types of viral and host RNA signatures, such as secondary structure and modification, and integration of such disparate information for nucleic acid discrimination. By reconstituting the signaling complex with purified components for the first time, they determined long sought-after structures of an activated RLR in complex with its co-factor (K63-linked polyubiquitin) and the signaling adaptor MAVS. These studies revealed precisely how receptor oligomerization activates the downstream signaling pathway. Her group also showed that certain mutations in the receptor and regulators can shift the immunological "threshold" for self-tolerance, leading to constitutive activation of RLRs by self-RNAs in lupus-like inflammatory disorders. Finally, her laboratory's findings that RLRs remodel protein-RNA complexes demonstrated an unanticipated signaling-independent, effector-like function of RLRs, challenging the conventional view of immune receptors as simple signaling molecules. In summary, her investigations have provided a molecular framework for understanding the RLR pathway, which now serves as a model for other nucleic acid sensors in innate immunity.

Previous Dorothy Crowfoot Hodgkin Award Winners

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

Philipp Kukura, Ph. D., University of Oxford

2022 Emil Thomas Kaiser Award Winner

Dr. Philipp Kukura is a physical chemist who has devoted his career to developing spectroscopic techniques to study biomolecules. Consistently pushing the boundaries of what can be detected with light, he has developed techniques sensitive to ever-smaller signals – in the form of magnetic field effects, ultrafast changes in vibrational signatures, and light scattered by nano-objects such as proteins. Most importantly, he developed mass photometry, a technology that measures the mass of single biomolecules using light scattering and which is proving ground-breaking for the study of proteins.

Dr. Kukura pursued the research that led to mass photometry despite his goal — to detect single proteins by light scattering alone — was considered 'impossible'. His perseverance resulted in the first single-molecule optical technique that is both universal (requiring no labelling) and specific (providing direct information on protein identity and structure). Mass photometry has diverse applications, from assessing sample purity for structural biology to in vitro science in general.

Dr. Kukura founded a company to commercialize the technology. Both the company and uptake of mass photometry have grown rapidly; they are a testament to the technology's value and impact for protein research, and to Dr. Kukura's commitment to ensuring it is widely accessible.

Previous Emil Thomas Kaiser Award Winners

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

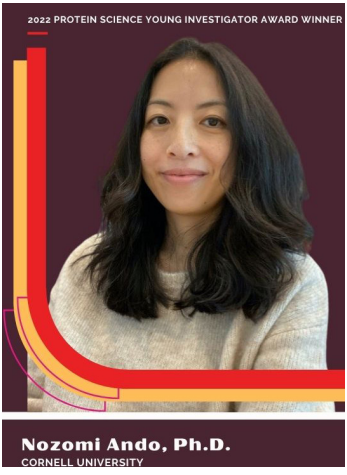
Previous recipients, sponsored by SynPep Corporation, include:

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

2022 Award Winners

Nozomi Ando, Ph. D., Cornell University

2022 Protein Science Young Investigator Award Winner - Sponsored by Wiley



Nozomi Ando has innovated new experimental and computational methods to understand the molecular mechanism of protein allostery. An outstanding example is her work on diffuse scattering, a faint and smeary signal in the background of X-ray diffraction images from protein crystals. For many decades, this signal was ignored by the structural biology field although it was known that it contains information on how atoms in a molecule move relative to each other. Although diffuse scattering from simple molecules can be interpreted, it was thought that diffuse scattering from protein crystals is intractable. Nozomi's group was the first to develop methods enable rigorous validation of data statistics and data-model agreement. In doing so, they were able to show for the first time, compelling evidence that two types of correlated motions exist in a protein crystal: atoms in different unit cells, as well as atoms within a protein molecule. These correlated protein motions are the key to allostery, and this breakthrough result has the potential to bring to life high-resolution structures from x-ray crystallography. Nozomi is known as a leader not only for her science but also for her work in advancing structural biology education and advocating for diversity in STEM.

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

Nicolas Fawzi, Ph.D., Brown University

2022 Protein Science Young Investigator Award Winner - Sponsored by Wiley

The 2022 recipient is Professor Nicolas Fawzi. Dr. Fawzi is an internationally recognized leader in a field that his technological advances have helped to open. Dr. Fawzi uses solution NMR spectroscopy, a unique and powerful tool, to investigate the structural biology of biomolecular condensates and the biophysical chemistry of the disordered regions of RNA-binding proteins. He is able to examine the structural details of these proteins and their complexes with atomistic resolution, and his efforts were among the first to bring biomolecular clarity to phase separation of this class of proteins. For example, his research described, for the first time, how three of the most important proteins in ALS remain structurally disordered in normal function and how mutations change transient structure and lead to protein aggregation in the disease. His current efforts to visualize protein structures and contacts within condensates in cells represent the next frontier. Dr. Fawzi's work at Brown has been published in top journals, including last author on high impact independent publications.

Previous Protein Science Young Investigator Award Winners (cont.)

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

Protein Science Best Paper *Award Winners*

Chelsea Vickers, Ph.D., Victoria University of Wellington

2021 Best Paper Award Winner - Sponsored by Wiley

Chelsea Vickers is a postdoctoral fellow with Wayne Patrick at Victoria University in Wellington, and she received her PhD from the University of Waikato, in New Zealand. Her paper in Protein Science concerns what she and her colleagues refer to as the "enzymatic dark matter" within the tree of life (Vickers CJ, Fraga D, Patrick WM. Quantifying thetaxonomic bias in enzymology. Protein Sci. 2021 Apr;30(4):914-921. doi: 10.1002/pro.4041). Using bioinformatic tools, Vickers and her coauthors analyze the extent to which enzymes in different branches of life have been characterized. They use the BRaunschweig ENzyme DAtabase (BRENDA), a compendium of enzymatic data (specifically, Michaelis-Menten constants), to map the limits of biochemical exploration along the different branches of the tree of life, and they find that many branches are completely unexplored (the "enzymatic dark matter,"). They find that the enzymes that have been characterized biochemically are from a limited number of species and phyla, reflecting the historical development of biochemistry and the use of model organisms, as well as a focus on human biology.

The most important conclusion of this paper is that there is an enormous untapped opportunity to discover new enzymes in life, as the results of deep-sequencing efforts keep pouring in. As one of the reviewers of the paper puts it, "the kinetic data in the BRENDA database present enzymologists with a survey of which enzymes occur on earth, and what kinetic parameters are typical. However, as the authors argue, if the database is heavily biased, then any take-home messages we try to infer from this database will not be representative of what is possible for enzymatic activity in the biosphere." Another reviewer complimented the authors by saying that this "is a very interesting and thought- provoking paper. I enjoyed reading it. It's an important point, we work on a few model enzymes and the (non-metabolomic) genomic databases are the tip of the iceberg, so what are we studying?"

Her mentor, Wayne Patrick has this to say about Chelsea Vickers: "It has been an absolute privilege hosting Chelsea in my research group. She has pushed me to think differently about my own research and she has been a superb role model for my postgraduate students. I am excited to see what future contributions she makes to protein science!"

Ryan Woloschuk,
University of Toronto
2021 Best Paper Award Winner
Sponsored by Wiley

Max Reed,
University of Toronto
2021 Best Paper Award Winner
Sponsored by Wiley

Ryan Woloschuk and Max Reed are both graduate students, working under the mentorship of Prof. Andrew Woolley at the University of Toronto. Their paper in Protein Science concerns the design of a photoswitchable binding protein (Woloschuk RM, Reed PMM, Jaikaran ASI, Demmans KZ, Youn J, Kanelis V, Uppalapati M., Woolley, GA. Structure-based design of a photoswitchable affibody scaffold. Protein Sci 2021 doi: 10.1002/pro.4196). The design is based on the Z domain, a small three-helix bundle that is a versatile scaffold for the design of variants that bind with high affinity and specificity to desired targets. To make the Z domain photoswitchW_able, Woloschuk, Reed, and their colleagues adapted a strategy first exploited by Stewart Loh and his group, which is to create a fusion protein in which the two proteins that are joined together exhibit positive or negative cooperativity in folding. If one of the proteins is easily converted between folded and unfolded states by the action of an external agent, then the folding, and therefore the function, of the second protein becomes responsive to that agent. Woloschuk, Reed, and colleagues describe the fusion of a Z domain to photoactive yellow protein, which is well folded in the dark, but adopts a molten globule state when exposed to blue light. In their designed fusion protein, the Z domain can fold into a stable structure only when the photoactive yellow protein is destabilized. As a result, exposure to blue light activates the binding capacity of the Z domain to an IgG molecule.

The paper by Wolloschuk, Reed, et al., provides a thorough biophysical and functional characterization of the designed photoswitchable binding protein. As one of the reviewers of the paper notes, “The function of the switches is thoroughly characterized through a range of approaches from yeast two-hybrid to protein NMR. In a field were many papers lack a clear design framework and the constructed switches are minimally characterized, this work stands out.” Another reviewer states that “… the NMR structural experiments and yeast assays are compelling, and they support the proposed switching mechanism in convincing fashion.”

Travel Awards

Congratulations to the following outstanding students and early-career investigators for receiving travel assistance to attend The 36th 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.

2022 DEI Awards

Ankan Bhadra, Washington University, St. Louis
Noah Wake, Brown University
Emily Saccuzzo, Georgia Institute of Technology
Lydia Kenney, Georgia Institute of Technology
Akshaya Arva, Texas Women's University
Behnoush Seifinoferest, University of California, Merced
Claire Stewart, University of North Carolina at Chapel Hill
Taylor Witter, Mayo Clinic
Davina Adderley, Georgetown University
Kevin Ramirez, University of California, Merced
Valentina Dimitrova, Northwestern University
Fiona Aguilar, Massachusetts Institute of Technology
Karen Acosta, University of Pennsylvania
Connor Parker, Rutgers University
Velia Garcia, Vanderbilt University
Gwendell Thomas, Georgia Institute of Technology
Mona Minkara, Northeastern University
Henry Pan, University of Texas at Austin
Zara Bukhari, University of the Pacific
Zirui Wang, Michigan State University
Mubark Mebrat, Arizona State University
Carmen Giovana Granados-Ramirez, Universidad Distrital Francisco Jose de Caldas
Shankar Devkota, Monash University
I-Te Chu, University of North Carolina at Chapel Hill
Jimin Yoon, Massachusetts Institute of Technology
Karen Ramirez Quintero, The University of Georgia
Rachana Rao Battaje, Indian Institute of Technology Bombay
Karen Noguera, The University of Georgia

2022 DEI Awards (cont.)

Andre Phan, San Jose State University
Cynthia Okoye, University of Cambridge
Glorisé Torres-Montalvo, Texas A&M University
Sai Pooja Mahajan, Johns Hopkins University
Amber Byrd, Loyola University New Orleans
Allyson Li, Columbia University
Ram Bhusal, Monash University
Tehya Littleton, Loyola University New Orleans
Mehrsa Mardikoraem, Michigan State University
Benedikt Dolgikh, Michigan State University
Minh Thu Ma, Georgia Institute of Technology
Sachith Roch De Silva, Georgetown University
Sebastian Swanson, Massachusetts Institute of Technology
Taariq Woods, National Eye Institute
Jennifer Ramirez, University of Pennsylvania
Silvana De Souza, University of Costa Rica, San Pedro
Uthayasuriya Sundaramoorthy, Australian National University
Rajesh Jaiprashad, Georgetown University
Phillip To, Johns Hopkins University
Yuran Zhang, University of British Columbia
Jonathan Besna, Université de Montréal
Jonathan Eicher, University of North Carolina at Chapel Hill
Blessing Oyiogu, University of Witwatersrand
Arjan Bains, University of California, Merced
Iliana Levesque, University of Michigan
Vy Dang, University of Texas at Austin

Travel Awards

2022 Protein Science Young Investigator and Finn Wold Travel Award Recipients

Areetha D'Souza, Vanderbilt University
Karishma Bhasne, University of Massachusetts, Amherst
Saacnicteh Toledo Patino, Okinawa Institute of Science and Technology
Gianluca Veggiani, University of Toronto
Sandor Babik, University of Massachusetts Amherst
Rohit Jain, Case Western Reserve University
Ilona Christy Unarta, University of Wisconsin Madison
Prabhat Tripathi, Northeastern University
Christopher T. Schafer, University of California San Diego
Asif Ali, University of Chicago
Andrew Morris, Miami University
Ethan Goulart, University of Massachusetts Amherst
John Mintken, Loyola University New Orleans
Saba Shahzadi, Nationwide Children's Hospital
Atharva Bhagwat, Johns Hopkins University
Ryan Judy, University of California Santa Barbara
Lily DeBell, California Institute of Technology
Alexandra Van Hall-Beauvais, EPFL
Nicholas Schneider, Marquette University
Emily Lewkowicz, Boston University School of Medicine
Shilpa Sharma, IIT Delhi
Patrick Carmody, Indiana University
Jeremiah Gaiser, University of Montana, NIH/NIAID
Lila Halbers, UCI
Karla Castro, École Polytechnique Fédérale de Lausanne
Weimin Tan, Texas A&M University
Dustin Luu, Arizona State University
Fangying Huang, University of Massachusetts Amherst
Can Ozden, University of Massachusetts Amherst
Daniel Olson, University of Montana
Julisia Chau, Stanford University

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2022 Protein Science Young Investigator and Finn Wold Travel Award Recipients

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Alexandra Barlow, California Institute of Technology
Kasun Pathirage, Northeastern University
Caitlyn McCafferty, University of Texas
Patrick Buckley, Yale University
Xizi Zhang, University of California, Berkeley
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Yu-Hsiu Lin, University of Texas Health San Antonio
Yichi Su, Stanford, School of Medicine, Neurobiology
Vikas Chonira, Texas A&M
Sergio Romero-Romero, University of Bayreuth
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Gabriella Gerlach, University of Pittsburgh
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Darya Stepanenko
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Rebecca Stowe, Miami University
Sanjay Kumar, Jawaharlal Nehru University, New Delhi
Adem Hadjabdelhafid-Parisien, Université de Montréal
Ngoc Thu Hang Pham
Bao Nguyen Viet
Amanda Laseke
Tracy Yu, Penn State University
Sam Schmidt, Michigan State University

At A Glance

	July 7		July 8	
7 a.m.	Registration (7 a.m.)			
7:30 a.m.			Registration (7:30 - 10 a.m.)	
			TPS Business Meeting/Breakfast	
8:30 a.m.	**Plenary Award: David Agard, UCSF 2021 Stein & Moore Winner**		**Plenary Award: Daniel Herschlag, Stanford University 2022 Stein & Moore Winner**	
9:10 a.m.	**Parallel Session 1:**	**Parallel Session 2:**	**Parallel Session 1:**	**Parallel Session 2:**
11:20 a.m.	**Synthetic Biology & Biosensing - Engineering Protein Components for Cellular Tasks**	**Seeing 3D Structures in Cells: Cryo-electron Tomography Blazes the Trail**	**Protein Science Addressing Health Disparities**	**Machine Learning in Protein Science**
Noon	**Networking Tables Event w/DEI, Careers & More (RSVP required)**		***Protein Science* Workshop/ Educator's Panel**	
1:45 p.m.	**Parallel Session 1:**	**Parallel Session 2:**	**Parallel Session 1:**	**Parallel Session 2:**
	Integrating Techniques to Address Challenges in Protein Structural Biology	**Imaging & Tracking of Proteins in Space and Time**	**Miniproteins: Are There Really That Many Additional Genes That We Have Been Missing?**	**Celebrating 100 Antibody Drugs**
4 p.m.	**Posters & Exhibits Networking Reception New: Guided Posters**		**Posters & Exhibits Networking Reception New: Guided Posters**	
4:45 p.m.				
5:30 p.m.				
7 p.m.				
	*Abstracts accepted for posters through **May 30**. Discounted, refundable registration through*			

July 9		July 10	
Registration (7:30 - 10 a.m.)		Registration (7:30 - 10 a.m.)	
Plenary Award: **Petra Fromme, University of Arizona** **2021 Christian B. Anfinsen Winner**		**Parallel Session 1:** **High Throughput Protein Science**	**Parallel Session 2:** **Protein & Ligand - A New Marriage Between an Old Couple**
Parallel Session 1: **Protein Phase Separation in Biomolecular Condensates**	**Parallel Session 2:** **Structure & Dynamics; Perspectives on Enzyme Function**	**Plenary Award:** **Squire Booker, Penn State University** **2022 Hans Neurath Winner** **David Goodsell, Scripps Research Institute & Rutgers University** **2022 Carl Brändén Winner** **Closing Remarks**	
Undergrad Research Session/ NSF Workshop			
Plenary Awards **Nicolas Lux Fawzi, Brown University,** **2022 *Protein Science* Young Investigator** **Sun Hur, Harvard University,** **2022 Dorothy Crowfoot Hodgkin Winner** **Philipp Kukura, Oxford University** **2022 Emil Thomas Kaiser Winner** **Jin Zhang, UCSD** **2022 Christian B. Anfinsen Winner** **Nozomi Ando, Cornell University** **2022 *Protein Science* Young Investigator**			
Posters & Exhibits Networking Reception **New: Guided Posters**			
Members' Reception (8:30 - 10 p.m.)			

***June 1**. All information subject to change.*

Protein Science

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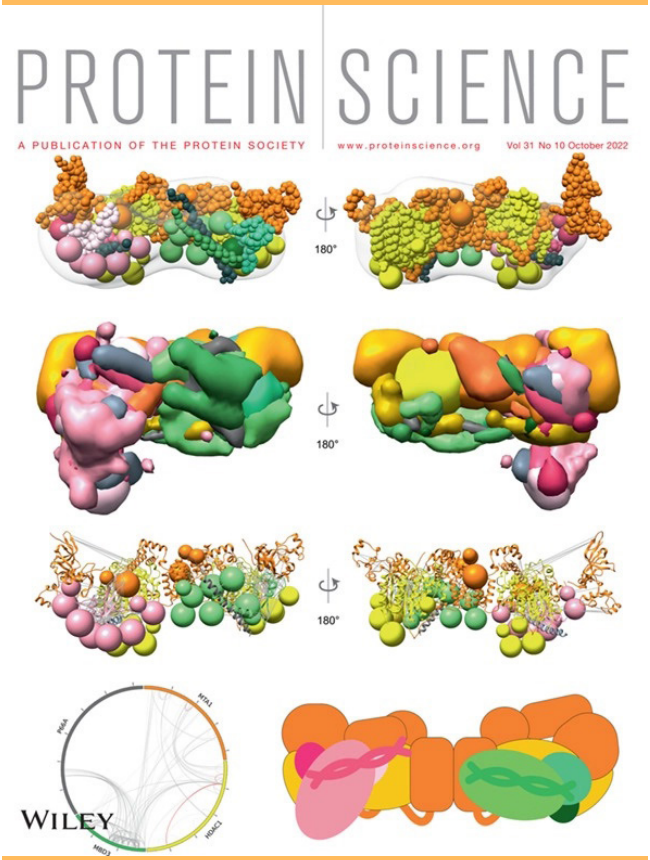

Abstracts

The following abstracts were presented at The Protein Society's 36th Annual Symposium in San Francisco, California.

DOI: 10.1002/pro.4494

THE PROTEIN SOCIETY WILEY

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS007 | Oscillating Machinery of a Circadian Clock

Andy LiWang
University of California, Merced (United States)

Circadian clocks provide a biochemical representation of local time inside cells and control the timing of gene expression in anticipation of sunrise and sunset. In cyanobacteria, a circadian oscillator comprised of three Kai proteins — KaiA, KaiB, and KaiC — relay temporal information downstream through two kinases, SasA and CikA, to regulate the transcription factor RpaA. Identifying the mechanisms by which circadian clocks exert temporal control over gene expression has been challenging in the complex milieu of cells. Thus, we reassembled an intact clock including oscillator and signal transduction components under defined conditions in vitro. Together with structural studies and biochemical analyses of partial clock reactions, we acquired new insights into mechanisms by which the cyanobacterial circadian clock functions to control gene expression. The in vitro oscillator is known to function under a relatively narrow set of Kai protein concentrations; we show here that a KaiABC-only mixture that fails to oscillate in a sustained manner due to limiting levels of KaiB can be rescued by SasA, which acts to recruit KaiB to the KaiC hexamer through heterotropic cooperativity. Cooperativity is based on structural mimicry between SasA and KaiB, and mutations that eliminate heterocooperativity in vitro profoundly affect circadian rhythms in vivo. CikA also rescues period defects under low levels of KaiA; together, our data help explain how the clock compensates in vivo for changes in concentrations of oscillator components that occur as part of the transcription-translation feedback loop and protein turnover. The coupling between oscillator and input-output components blurs their distinction. We developed the in vitro clock to establish causal links between clock biochemistry and in vivo phenotypes, and it provides a platform to explore how changes in factors such as temperature or ATP levels are propagated to regulate transcription.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS021 | A comprehensive structural view of target recognition in the Streptococcus Class A sortase catalytic mechanism

Jeanine Amacher, Alex Johnson, Isabel Piper, Brandon Vogel, Sophie Jackson, Justin Svendsen, Hanna Kodama, Jay McCarty, John Antos
Western Washington University (United States)

The cell wall is a critical extracellular barrier for bacteria and many other organisms. In bacteria, this structural layer is comprised of peptidoglycan, which maintains cell shape and integrity and provides a scaffold for displaying various protein factors. To attach proteins to the cell wall, gram-positive bacteria utilize sortase enzymes, which are cysteine transpeptidases that recognize and cleave a specific sorting signal, followed by ligation of the sorting signal-containing protein to the peptidoglycan precursor lipid II. This mechanism is the subject of considerable interest as a target for therapeutic intervention and as a tool for protein engineering, where sortases have enabled sortase mediated ligation or sortagging strategies. Despite these uses, there remains an incomplete understanding of the stereochemistry of substrate recognition and ligation product formation. Here, we solved the first structures of sortase A from Streptococcus pyogenes (spySrtA) bound to two substrate sequences, LPATA and LPATS. In addition, we synthesized a mimetic of the product of sortase-mediated ligation involving lipid II (LPAT-lipid II), and solved the complex structure in two ligand conformations. These structures were further used as the basis for molecular dynamics simulations to probe SrtA ligand dynamics and to construct a model of the acyl enzyme intermediate, thus providing a complete structural view of key states in the catalytic mechanism. Overall, this structural information provides new insights into the recognition of the sortase substrate motif and lipid II ligation partner and will support the continued development of sortases for protein engineering applications.

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Track: Structure and Dynamics Perspectives on Enzyme Function

ABS022 | Filamentation/Polymerization as a Novel Wide-Spread and Evolutionarily Conserved Enzyme Regulatory Mechanism

Nancy Horton, Niloofar Ghadirian, Dmitry Lyumkis, Zelin Shan
University of Arizona (United States)

We use a model system, the sequence dependent endonuclease SgrAI, to investigate important mechanistic and biological questions regarding enzyme filamentation and enzyme regulation. SgrAI forms structured assemblies of heterogeneous stoichiometries under conditions where its DNA cleavage activity is 200-1000 fold accelerated, and surprisingly, its DNA sequence specificity is altered (Biochem. 49, 8818). We have shown that these assemblies are helical filaments composed of SgrAI (Biochem. 52, 4373, Structure 21, 1848), and have proposed hypotheses for 1) how filamentation activates the DNA cleavage activity of SgrAI, as well as 2) how filamentation modulates the enzyme's DNA sequence specificity using the sequence specific energy of DNA distortions (Structure 27, 1). We have also carried out a complete kinetic investigation to create a full computational model of the entire DNA cleavage pathway including filamentation and all forward and reverse rate constants for each step (JBC 293, 14585 & 14599). This model has allowed us to predict the behavior of SgrAI within a cell, at biologically relevant concentrations, and showed that the filamentation mechanism, and in particular the slow filament assembly step, allows SgrAI to target invading DNA while minimizing damage to its host genome (J. Virol. 93, e01647). We now present new Cryo-EM structural data supporting the hypothesis that filamentation stabilizes a conformation of SgrAI where a second divalent cation binds in the active site, thereby enhancing the rate of DNA cleavage (JBC, in press). We also propose a model for control of SgrAI filamentation and DNA sequence specificity involving a disorder-to-order transition in SgrAI. Finally, new kinetic modeling also show how the secondary recognition sequences alter the thermodynamic states of SgrAI to affect filamentation and DNA cleavage preferences.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS023 | Biochemical and Biophysical Characterization of Small Molecule Inhibition of Gankyrin as a Therapeutic Strategy for Cancers

Emma Kane, Dipti Kanabar, Abbas Kabir, Taylor Laflamme, Aaron Muth, Donald Spratt
Clark University (United States)

Gankyrin is a seven-ankyrin repeat containing oncoprotein associated with the 26S proteasome assembly that regulates numerous oncogenic and inflammatory pathways through facilitating protein-protein interactions. As a chaperone protein, gankyrin binds to the S6 ATPase subunit of the 19S regulatory cap of the 26S proteasome that enhances both mouse double minute 2 homolog (MDM2) and cyclin-dependent kinase 4 (CDK4) mediated proteolysis of tumor suppressor proteins (TSP) p53 and retinoblastoma protein (Rb), respectively. Previous studies have shown that gankyrin overexpression increases the degradation of tumor suppressor proteins resulting in uncontrolled cell proliferation and the onset of various cancers. The first small molecule identified for gankyrin inhibition, cjoc42, has been rudimentarily characterized to disrupt the interaction with S6 ATPase as a therapeutic approach for both liver and breast cancer. Here we show using a structure-based drug design approach that novel small molecule cjoc42 derivatives show marked improvement in inhibition of tumorigenesis with concurrent increases in TSP expression levels. Further biophysical evaluation revealed that these cjoc42 derivations mode of action induce global gankyrin unfolding. These findings also provide insight into clarifying essential residues within gankyrin that are required for small molecule interaction. Taken together, this work aims to establish optimal targeting of tandem repeat proteins to increase selectivity, mitigate off-target activity, and conceptualize a biochemical approach coupled with drug discovery.

Track: Protein Science Addressing Health Disparities

ABS024 | Mass spectrometry based protein assays for diagnosis and prognosis of COVID-19 infection

Sanjeeva Srivastava
Indian Institute of Technology Bombay (India)

During the past year, the understanding of COVID-19 severity has strengthened substantially. Using mass-spectrometry based proteomics, metabolomics & ML approaches, we discovered classifiers of COVID-19 severity such as AGT, FGG, APOB and SERPINA3 and also developed targeted protein assays based on Selected Reaction Monitoring for clinical translation. The altered plasma proteome of COVID-19 severe patients revealed dysregulation of peptidase activity, regulated exocytosis and myeloid leukocyte activation pathways. This study revealed that mass spectrometry-based peptide tests can be used by the clinicians for diagnosis as well as identified pathways/ markers as the predictors of the disease progression. Further, we also investigated the potential of attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy as a rapid blood test for classification of COVID-19 disease severity using a cohort of 160 COVID-19 patients. In summary, this study demonstrates the potential of ATR-FTIR spectroscopy as a rapid, low-cost COVID-19 severity triage tool to facilitate COVID-19 patient management during an outbreak.

Track: High Throughput Protein Science

ABS025 | Proteomic investigation of COVID-19 severity during the tsunamic second wave in India

Sanjeeva Srivastava
Indian Institute of Technology Bombay (India)

A mass spectrometry based proteomic analysis has been done on severe and non-severe group of COVID 19 patients using nasopharyngeal swab samples. We have concluded from our study that antimicrobial peptide pathway might have a key role in the severity of the disease during the second wave of COVID-19. Further exploration of this pathway can shed light to improve our understanding for SARS-CoV-2 pathogenesis and severity during the second wave. We have also identified severity markers and did their validation using MRM, but further validation is required before taking them forward for clinical translation. To the best of our knowledge, this is the first proteomic study depicting the role of antimicrobial peptide pathway in the severity of second wave of COVID-19.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS026 | Expressed Protein Ligation without Intein

Wenshe Liu, Yuchen Qiao
Texas A&M University (United States)

Proteins with a functionalized C-terminus such as a C-terminal thioester are key to the synthesis of larger proteins via expressed protein ligation. They are usually made by recombinant fusion to intein. Although powerful, the intein fusion approach suffers from premature hydrolysis and low compatibility with denatured conditions. To totally bypass the involvement of an enzyme for expressed protein ligation, here we showed that a cysteine in a recombinant protein was chemically activated by a small molecule cyanylating reagent at its N-side amide for undergoing nucleophilic acyl substitution with amines including a number of L- and D-amino acids and hydrazine. The afforded protein hydrazides could be used further for expressed protein ligation. We demonstrated the versatility of this activated cysteine-directed protein ligation (ACPL) approach with the successful synthesis of ubiquitin conjugates, ubiquitin-like protein conjugates, histone H2A with a C-terminal posttranslational modification, RNAse H that actively hydrolyzed RNA, and exenatide that is a commercial therapeutic peptide. The technique, which is exceedingly simple but highly useful, expands to a great extent the synthetic capacity of protein chemistry and will therefore make a large avenue of new research possible.1-2

1. Qiao, Y.; Yu, G.; Kratch, K. C.; Wang, X. A.; Wang, W. W.; Leeuwon, S. Z.; Xu, S.; Morse, J. S.; Liu, W. R., Expressed Protein Ligation without Intein. J. Am. Chem. Soc. 2020, 142 (15), 7047-7054.
2. Qiao, Y.; Yu, G.; Leeuwon, S. Z.; Liu, W. R., Site-Specific Conversion of Cysteine in a Protein to Dehydroalanine Using 2-Nitro-5-thiocyanatobenzoic Acid. Molecules 2021, 26 (9), 2619.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS028 | Practical Course in Macromolecular Crystallography

Kay Perry, David Neau, Frank Murphy
NE-CAT/Cornell University (United States)

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.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS029 | Allosteric network analysis in galectin-7 uncovers key residues controlling positive cooperativity between two opposite glycan binding sites

Ngoc Thu Hang Pham, Alex Paré, Myriam Létourneau, Marlène Fortier, Gabriel Bégin, David Chatenet, Yves St-Pierre, Patrick Lagüe, Charles Calmettes, Nicolas Doucet
Centre Armand-Frappier Santé Biotechnologie, Institut National de la Recherche Scientifique (INRS), Université du Québec, Laval, QC, Canada. (Canada)

Human galectin-7 (GAL-7) plays dual roles as a pro- and anti-tumorigenic protein. It is a prototypical galectin characterized by a beta-galactoside binding site (GBS) and a homodimeric molecular architecture. Due to high GBS similarity among several galectin homologs in the cell, the development of GAL-7 glycan-based inhibitors aimed at perturbing glycoreceptor interactions remains a high-risk strategy plagued by unwanted off-target effects. Functional allosteric modulation thus represents an alternative strategy to improve GAL-7 selectivity. In the present work, we used network analysis to predict residue positions that allosterically modulate the pro-apoptotic activity mediated by GAL-7. We uncovered how crucial residue contacts at the dimer interface can be altered to control its biological function by allosterically modulating communication between protomers. We show that introducing a covalent disulfide bridge at position G16 strengthens protomer interactions to improve positive cooperativity by favoring interprotomer communication between residue pair Arg20-Asp103 at the dimer interface. Accordingly, we describe a new approach to investigate the global communication flow between the two GBSs, revealing that altered electrostatic interactions between residue pairs Arg20-Asp103 and Arg22-Asp103 reduces interprotomer communication. This is consistent with decreased pro-apoptotic activity in GAL-7 variants R20A, R22A, and D103A, further suggesting that Arg20, Arg22, and Asp103 are potentially controlling positive cooperativity in GAL-7. Our study illustrates how the shortest pathway analysis can illuminate global flow communication to reveal key residues controlling allosteric communication between long-range functional sites within a protein.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS030 | Zinc as a modulator of PSD-95 palmitoyl modification

Yonghong Zhang
The University of Texas Rio Grande Valley (United States)

PSD-95 is an important postsynaptic membrane-associated protein. It is a central component of postsynaptic densities and plays a primary role in synaptic development and maturation. PSD-95 mediates postsynaptic location of AMPA receptors through its N-terminal C3/C5 palmitoylation/depalmitoylation switch. The palmitoylation-site N-terminus contains a zinc finger motif with unknown function. In this study, the interaction between Zn2+ and PSD-95NT was investigated for the first time. The NMR titration of 15N-labeled PSD-95NT by ZnCl2 was performed and demonstrated Zn2+ binds to PSD-95NT with a binding affinity (Kd) in the micromolar range. The zinc binding was confirmed by fluorescence and mutagenesis assays, indicating two cysteines and two histidines (H24, H28) are critical residues for the binding. These results suggested the concentration-dependent zinc binding is likely to influence PSD-95 palmitoylation since the binding site overlaps the palmitoylation sites, which was verified by the mimic PSD-95 palmitoyl modification and intact cell palmitoylation assays. This study reveals zinc as a novel modulator for PSD-95 postsynaptic membrane association by chelating its N-terminal region, indicative of its importance in postsynaptic signaling.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS031 | Investigating the structure of TolA and its relationship with the proton motive force

Cara Press, Melissa Webby, Christina Redfield, Simon Newstead, Colin Kleanthous
University of Oxford (United Kingdom)

In Gram-negative bacteria the outer membrane (OM) provides a barrier to the outside world and confers intrinsic antibiotic resistance. It is a non-energised membrane, so energy requiring processes such as nutrient import must use energy sources across the inner membrane (IM) or in the cytoplasm. The Tol-Pal system utilises the proton motive force (PMF) across the IM for the purpose of maintaining the integrity of the OM.

One of the five core components of the Tol-Pal system, Pal, is a lipoprotein anchored into the OM that binds to peptidoglycan (PG) forming a tether between the two layers (Parsons, Lin and Orban, 2006). The other four components TolA, TolB, TolQ and TolR, work to modulate the binding state of Pal. When bound to TolB, Pal can diffuse freely, allowing it to be recruited to the septum during cell division (Petiti et al., 2019; Szczepaniak et al., 2020). The inner membrane complex of TolQ-TolR transduces the PMF through the effector protein TolA and pulls TolB from Pal, thereby allowing Pal to bind to PG. The periplasmic second domain of TolA is essential for the function of the system and has been shown to undergo a PMF dependent conformational change (Germon et al., 2001). In this study we characterise the nature of TolA domain II using a range of biophysical techniques and examine the structure of the domain using both NMR and X-ray crystallography. It is hoped that elucidating the structure of TolA domain II will shed some light on the mechanism of action of the Tol-Pal system and how PMF-driven conformational changes in TolA translate to force transduction.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS032 | A multipronged footprinting map for interaction sites of stress biomarker Neurotransmitter Y (NPY) and novel peptide biorecognition elements

Rohit Jain, Erik Farquhar, Janna Kiselar, David Lodowski, Mark Chance
Case Western Reserve University (United States)

Neuropeptide Y (NPY) signals important stress and mood phenotypes in the brain. Novel peptide biological recognition elements (BREs) have been developed as a first step towards developing hormone biosensors for stress and weight management. We employed protein footprinting mass spectrometry (PF-MS) to provide readout of NPY residues responsive to BRE binding. Three complimentary PF-MS approaches – Hydroxyl radical footprinting, Trifluoromethyl radical footprinting and Carboxyl group footprinting were applied independently.

This multipronged approach mapped changes in NPY solvent accessibility with 42% labeling coverage. The location and mechanism of binding of two BREs (N2, N3) with NPY was evaluated, revealing changes in NPY structure. PF-MS derived solvent accessibility for the NPY-N2 and NPY-N3 complexes shows distinct structural conformations. Deprotection of residues in NPY due to N2 binding suggest an extended NPY conformation, whereas the absence of deprotection in the NPY-N3 complex points to a hairpin conformation. Protection of sites in the NPY-N3 complex agree with reported MD results, confirming a C-terminal binding site.

NMR structures of NPY shows multiple distinct conformations, including an extended structure with unfolded N-terminus and a poly-proline α-helix that is back folded on the C-terminus of NPY. Knowing the correct NPY conformation is essential to better design sensors based upon the NPY-N2 and NPY-N3 complexes. To do so, we reversed the order of PF-MS experiments via a "digest and label" strategy. We found the baseline structure of NPY has a higher order structure and by comparison to intact NPY BRE complexes FP, structural determinants impacting NPY solvent accessibility are being elucidated.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS033 | InDel Mix & Match. Reshaping coenzyme binding pockets through insertions and deletions

Saacnicteh Toledo Patino
Okinawa Institute of Science and Technology (Japan)

Enzymes that employ cofactors to perform catalysis are ubiquitous across the tree of life and are necessary to assist half of the enzymatic reactions in nature. Among cofactors, nucleotide derivatives are believed to be the catalytic fossils of a hypothetical RNA-based world. They may have become associated with early peptides, which evolved towards the enormous catalysis we observe today. Given their importance, it is fundamental to understand how their binding modes emerged within protein scaffolds. This understanding will allow us to switch cofactor specificities and therefore functions across protein families and superfamilies. To date is widely accepted that protein scaffolds may have evolved through amplification and combination of ancient peptides. It has been shown that these ancestral units usually bind to nucleotide-moieties and how their combination in the laboratory leads to solubly expressed chimeras. Similarly, random mutagenesis has proven to alter protein functions. However, the role of insertions and deletions (InDels) in the emergence of protein functions remains underexplored. Not only are InDels highly deleterious, but also difficult to implement in randomized libraries, compared to amino acid substitutions.

We systematically searched for InDels within coenzyme binding pockets to study the hypothesis that insertions

and deletions were involved in the divergence of coenzyme-binding in early evolution, and as a proof of principle, we engineered InDels to remodel the nicotinamide dinucleotide (NAD) binding pocket of oxidoreductases into the S-adenosylmethionine (SAM) binding pocket of Rossmann methyltransferases. The elucidation of the mutants' crystal structures and binding studies corroborated the orthogonal binding switch from NAD to SAM, providing the first example where the implementation of InDels in rational design leads to a coenzyme-specificity switch that links two distinct enzymatic chemistries (redox and methylation) in Rossmann proteins. We believe this strategy can be generalized and will constitute a handy asset to the current protein engineering methods.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS034 | RCSB PROTEIN DATA BANK: Enabling Next Generation Exploration of PDB Data

Connor Parker, Zukang Feng, Dennis Piehl, Stephen Burley
Rutgers (United States)

The Protein Data Bank (PDB) was established in 1971 as the first open-access digital data resource in biology. Beginning with seven protein structures, the PDB archive now hosts >185,000 structures of proteins, DNA, and RNA (totaling >1 billion atoms). Today, PDB is universally regarded as a core data science resource of fundamental importance to the life-science community and long-term preservation of biological data. PDB data impacts basic and applied research in fundamental biology, biomedicine, biotechnology, and energy.

The archive is managed by partners in the Worldwide Protein Data Bank (wwPDB): RCSB PDB, PDBe, and PDBj. This collaboration requires regular coordination and management of data across wwPDB data centers. The public data archive is 923 GB, with internal holdings significantly larger. A recently-developed application (File Access Application Programming Interface) will provide an updated means of synchronization between members. Using this new application, the process of sending and receiving data internally across wwPDB data centers will become less time consuming and more efficient.

Each wwPDB partner maintains a public website for data access and exploration. The RCSB PDB website (RCSB.org) is visited by millions of users annually. Features include a structural motif search that finds residue patterns that recur throughout the PDB; a 3D Protein Feature View that maps 1D sequence features and annotations onto 3D structures, and new annotations for membrane proteins, antibodies, and domain classifications.

These new tools are intended to enable the next generation of PDB data exploration.

RCSB PDB is a member of the Worldwide Protein Data Bank (wwpdb.org). RCSB PDB is funded by the National Science Foundation (DBI-1832184), the US Department of Energy (DE-SC0019749), and the National Cancer Institute, National Institute of Allergy and Infectious Diseases, and National Institute of General Medical Sciences of the National Institutes of Health under grant R01GM133198.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS035 | Dose-dependent nuclear delivery and transcriptional repression with a cell-penetrant MeCP2

Xizi Zhang, Alanna Schepartz
University of California, Berkeley (United States)

Methyl-CpG-binding-protein 2 (MeCP2) is a nuclear protein abundantly expressed in neurons and is essential for postnatal brain development. Mutations in the MECP2 gene cause Rett syndrome (RTT), a severe and incurable neurological disorder that disproportionately affects young girls. Despite the recent development in drugs capable of treating RTT-related symptoms, no disease modifying treatments exist for patients with RTT. Here we seek to develop a cell-penetrant MeCP2 that restores functional protein levels in cells as a potential therapy for RTT. We utilized the mini-protein known as ZF5.3 that is shown to be capable of efficiently delivering multiple proteins and enzymes to the cytosol and nuclei of multiple cells lines. Using confocal microscopy, flow cytometry and fluorescence correlation spectroscopy, we find that the fusion protein ZF5.3-MeCP2 reaches the nucleus 2-fold higher than unconjugated MeCP2 protein, achieving nuclear concentrations greater than 600 nM. Moreover, western blot analysis of isolated nuclear fractions shows ZF5.3-MeCP2 remains intact and retains the ability to associate with known MeCP2-interacting protein partners once delivered to the nucleus. Finally, using an in cellulo transcriptional repression assay, we determine

that nuclear-delivered ZF5.3-MeCP2 selectively binds and represses methylated gene expression.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS036 | Role of cystathionine--lyase from Pseudomonas aeruginosa in cysteine biosynthesis and H2S generation

Marco Pedretti, Carolina Conter, Luis Alfonso Martínez-Cruz, Paola Dominici, Alessandra Astegno
University of Verona (Italy)

Pseudomonas aeruginosa (Pa) is one of the most life-threatening multidrug-resistant (MDR) "superbug", declared a priority 1 pathogen by WHO. Due to its emerging nonsusceptibility to conventional antibiotics, the identification of new pharmacological interventions is urgently needed. In this scenario, a novel approach to tackle antibiotic resistance relies on targeting the primary enzymatic sources of the bacterial hydrogen sulfide (H2S)–mediated defense system. H2S has been shown to promote resistance to clinically relevant antibiotics in Pa and other bacterial pathogens [1], and an effective antimicrobial strategy targeting the cystathionine-γ-lyase (CGL) enzyme as the primary generator of H2S in two major human pathogens, Staphylococcus aureus and Pa, has been recently purposed [2]. To extend the knowledge about this targetable system, herein, we report a comprehensive kinetic and structural characterization of recombinant CGL from Pa (PaCGL). We established that native PaCGL is a homotetramer in solution, in which the PLP cofactor is covalently bound to each monomer through Lys208 via a Schiff base linkage. The kinetic analysis, in combination with the identification of reaction products by RP-HPLC, revealed that PaCGL specifically catalyzes the canonical α, γ-hydrolysis of L-cystathionine to form L-cysteine. Importantly, PaCGL can also efficiently produce H2S using L-cysteine or L-homocysteine as substrates. This study, together with the obtainment of the crystal structure of the enzyme (PDB: 7BA4), demonstrates a crucial role of PaGCL not only in cysteine biosynthesis but also in H2S generation and opens the avenue to innovative anti-Pa interventions based on pharmacological targeting of the CGL enzyme.

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Track: High Throughput Protein Science

ABS038 | Not refoldable, aggregation-prone, and toxic: A story about production of a high-value drug target

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The majority of targets pursued by the pharmaceutical industry are membrane proteins.1 However, designing a drug effective against such targets are highly challenging. High Throughput Screening (HTS) is a common but often inefficient approach. Our goal is to use structure-based and fragment-based discovery approaches, which can provide a more directed and efficient path to lead inhibitors. However, production of recombinant target protein in high quantity, high purity, and soluble represents a major barrier to pursuing structure-based drug discovery for membrane proteins. The goal of our team is to develop inhibitors of a human type II transmembrane protein (T), targeting the extracellular domain. However, several intrinsic characteristics pose major obstacles to production in E. coli as T: (i) contains four disulfide bonds, (ii) has two free cysteines are involved in the receptor oligomerization, (iii) has an extensive hydrophobic core as well as a significant surface hydrophobic cleft, and (iv) is toxic, functioning in a cell death pathway in mammalian cells and to a lesser extent in E.coli. In addition, T forms homo- and hetro-oligomers and binds carbohydrates. This presentation focuses on our efforts to produce T for our drug discovery campaign. We have expressed T in E.coli, which uniformly produced protein in inclusion bodies. Attempts to obtain soluble in bacteria included changing expression conditions and testing different adjuvants. In parallel, an extensive array of refolding approaches were assayed, along with screening for additives, detergent, and reducing agents. In-silico modeling was also used to predict less aggregation-prone mutants that might facilitate refolding and these are being produced and tested. We are also expressing T in genetically modified yeast that produce glycosylated protein closely mimicking those found in humans.

Track: Miniproteins: Are There Really That Many Additional Genes That We Have Been Missing?

ABS039 | Emergence of a catalytic activity in a SH3-like fold provides a powerful resistance mechanism

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Type B dihydrofolate reductases (DfrB), first identified in the 1970s, procure strong resistance to the synthetic antimicrobial trimethoprim. DfrB enzymes are constituted of a single SH3-like fold of 78 residues that assembles as an obligate homotetramer to form a central, highly symmetrical active site. Intriguingly, DfrB enzymes have no evolutionary homology to any characterized protein, such that their evolutionary origin is unknown. Here, I present our investigation of distant DfrB homologues that begins to fill gaps in our knowledge of the evolution of this SH3-like fold into a powerful antimicrobial resistance mechanism. Through metagenomics database searches of uncharacterized and putative proteins, we identified distant homologues to the DfrB, which we characterized for their capacity to provide trimethoprim resistance when expressed in E. coli. By means of kinetic characterization, circular dichroism, negative-stain electron microscopy and more, we demonstrate that a 365-residue distant homologue, having similarity with DfrB only in its small SH3-like fold, recreates the DfrB active site environment through multimerization, yielding undistinguishable dihydrofolate reductase activity and trimethoprim resistance. These results contribute to our goal of establishing the evolutionary path that has led the SH3-like fold of the DfrB enzymes to the modern resistome. This research will deepen our knowledge of evolution of antimicrobial resistance mechanisms, a major issue in modern medicine.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS040 | Multiscale modeling approaches for the protein-solid interface

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The adsorption of proteins on solid surfaces remains a complex but central issue in the biomaterials field, which requires us to understand this process at the atomic level. In particular, designing efficient devices using immobilized proteins requires us to grasp details of the protein-solid interface, such as the adsorbed enzyme orientation, the stability of the adsorption and its consequences on the protein function [1]. Such information can be obtained using molecular modeling approaches on different scales, either with classical all-atom Molecular Dynamics simulations, or with coarse-grain calculations based on Elastic Network Models [2]. Applications on [NiFe]-hydrogenases (which catalyze hydrogen oxidation) [3-4] and copper-billirubin oxidase (which catalyzes oxygen reduction) [5] show how simulations give us insight on factors determining enzymes orientation on the electrode surfaces, and how the adsorption on a solid surface can impact proteins structure, dynamics and mechanical properties, and therefore their
catalytic activity. Furthermore, simulations on cellulose degradation) adsorbed on bare and SAM-functionalized gold surfaces highlight how one must find an acceptable stability-activity trade-off when working with immobilized enzymes [6].

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Track: Structure and Dynamics Perspectives on Enzyme Function

ABS041 | Q-Repex: A python pipeline to increase sampling of EVB simulations

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The complex potential energy surfaces (PES) builds the basis for the exploration of chemical systems. These PES are formed by the multitude of different accessible molecular conformations. Various methods exist to analyze this chemical space. Among these methods is Molecular Dynamics (MD). MD simulations are commonly used to calculate the time evolution of various chemical systems, ranging in size from dimers in vacuo to solvated enzymes.

A common problem when applying MD simulations on large biochemical systems is a lack of sampling, caused by, e.g. large barrier heights separating stable basins or a rough surface with many local minima. This lack of sampling traps the dynamic system, preventing an efficient exploration of the PES.

Replica exchange molecular dynamics (REMD) allows for an extensive exploration of these less accessible PES, by performing simulations at differing conditions and exchanging these conditions at predefined intervals.

The Q software package, which was developed to perform empirical valence bond (EVB) and free energy calculations, is frequently used for the study of reaction mechanisms in biomolecules using reactive MD. The tool presented here, Q-Repex, increases the sampling efficiency of the robust EVB-FEP/US approach by means of REMD. Tests were performed on various, prior studied, biochemical reactions, including the TIM catalyzed isomerisation reaction of GAP. Q-Repex decreases the computational costs, and opens the possibility of new in depth studies of biochemical reactions.

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Track: Protein Science Addressing Health Disparities

ABS042 | Mechanism of protein protection by desiccation-tolerance molecules

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Protein-based drugs are amazingly effective, but solution instability of proteins means these therapeutics require costly refrigeration, impeding their accessibility [1,2]. Drying can increase stability, but most proteins do not withstand dehydration [3]. Protective molecules, excipients, are often added to safeguard proteins during drying [4,5]. Formulation, however, is empirical and of varying efficacy due to a paucity of high-resolution information about dry proteins; we do not fully understand the mechanism(s) of protection [4,6].

To solve this problem, we developed Liquid-Observed Vapor Exchange (LOVE) NMR, a technique that reports on dry protein structure at the residue level [7]. Using LOVE NMR, we showed that dry proteins comprise an immobile but diverse conformational ensemble [7]. We also showed that electrostatic interactions and disorder are important for protection by desiccation-tolerance proteins from a tardigrade and a midge [8].

We will describe our recent efforts on protection by sugars, including trehalose, a well-known excipient [9,10]. We will also present data on new client proteins that allow us to apply both LOVE NMR and activity assays, enabling investigation of both structure and function. Such efforts elucidate mechanisms of dehydration protection, allowing rational design of excipient formulations and making protein products more affordable and accessible [11].

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Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS043 | Ligand-specific mechanisms of allosteric regulation in FXR

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Farnesoid X receptor (FXR) is a member of the nuclear receptor family of ligand-regulated transcription factors that regulates bile acid, lipid and glucose metabolism. Bile acids are the endogenous FXR ligands. Bile acid binding to FXR induces association with FXR response element sequences (FXREs), and promotes selective recruitment of coregulator proteins via induced structural changes in the activation function AF-2 surface, i.e. the site of coregulator binding. However, there is a poor understanding of how FXR is allosterically regulated by bile acid, which acts as a molecular switch to initiate critical long-range of transcriptional events.

The goal of this work is to understand how minor structural modifications in bile acids give rise to differential FXR transcriptional activity. We used dual luciferase assays to determine how bile acids modulate FXR DNA binding preferences for a wide range of FXREs. We also used molecular dynamics (MD) simulations to study the effect of the bile acids on AF-2 signaling. We have identified key residues that mediate specific allosteric communication pathways in FXR, and the mutations of the key residues in FXR-LBD affect bile acid-specific FXR transcriptional activities, confirming their roles in ligand-AF-2 allostery. These findings can further our understanding of how bile acids impact FXR promoter selectivity and coregulator recruitment.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS044 | A CRYPTIC POCKET IN EBOLA VP35 ALLOSTERICALLY CONTROLS RNA BINDING

Matthew Cruz, Thomas Frederick, Upasana Mallimadugula, Rishi Samarth, Gaya Amarasinghe, Gregory Bowman
Washington University in St. Louis (United States)

Many proteins are difficult to target with drugs because their primary function is to participate in protein-protein interactions (PPIs) and protein-nucleic acid interactions (PNIs) through flat interfaces that are less conducive to small molecule binding. These interactions are found in many viral proteins that interact with host factors and viral nucleic acids in order to evade the host immune system. We focused our study on viral protein 35 (VP35), from the highly lethal Ebola virus. VP35 plays essential roles in Ebola's replication cycle, including binding the viral RNA genome to block a host's innate immunity. However, there are no FDA approved drugs targeting VP35. One promising opportunity for discovering and designing new drugs is cryptic pockets, or pockets that are absent in available protein structures but form due to protein dynamics. Identifying and exploiting cryptic pockets remains challenging as most known pockets were discovered alongside the identification of a small molecule effector. Here, we applied adaptive sampling simulations to sample states in VP35's conformational landscape with large pocket volumes which revealed a potentially druggable cryptic pocket. Then, using allosteric network detection algorithms, we predicted that VP35 harbors a cryptic pocket that is allosterically coupled to its RNA-binding interface. Thiol labeling experiments along with dsRNA binding experiments suggest the VP35 cryptic pocket is present and that stabilizing this pocket in its open form allosterically disrupts RNA binding. We then conducted an experimental high throughput screen for dsRNA binding inhibitors targeting VP35 that yielded a number of hits. These results demonstrate the potential of cryptic pockets to allosterically affect PPI and PNIs presenting new therapeutic opportunities for targeting these prevalent interactions.

Track: High Throughput Protein Science

ABS045 | The endoplasmic reticulum proteostasis network profoundly shapes the protein sequence space accessible to HIV envelope

Jimin Yoon, Emmanuel Nekongo, Jessica Patrick, Taffani Hui, Angela Phillips, Anna Ponomarenko, Rebecca Sebastian, Yu Meng Zhang, Vincent Butty, C. Ogbunugafor, Yu-Shan Lin, Matthew Shoulders
Massachusetts Institute of Technology (United States)

The sequence space accessible to evolving proteins can be enhanced by cellular chaperones that assist biophysically defective clients in navigating complex folding landscapes. It is also possible for proteostasis mechanisms that promote strict quality control to greatly constrain accessible protein sequence space. Unfortunately, most efforts to understand how proteostasis mechanisms influence evolution rely on artificial inhibition or altered expression of specific chaperones or quality control factors. Here, we use chemical genetic strategies to tune proteostasis networks via natural stress response pathways that regulate the levels of entire suites of proteostasis factors. Specifically, we upregulate the unfolded protein response (UPR) to test the hypothesis that the host endoplasmic reticulum (ER) proteostasis network shapes the sequence space accessible to human immunodeficiency virus-1 (HIV-1) envelope (Env) protein. Elucidating factors that enhance or constrain Env sequence space is critical because Env evolves extremely rapidly, yielding HIV strains with antibody- and drug-escape mutations. We find that UPR-mediated upregulation of ER proteostasis factors, particularly those controlled by the IRE1-XBP1s UPR arm, globally reduces Env mutational tolerance. Conserved, functionally important Env regions exhibit the largest decreases in mutational tolerance upon XBP1s induction. Our data indicate that this phenomenon likely reflects strict quality control endowed by XBP1s-mediated remodeling of the ER proteostasis environment. Intriguingly, and in contrast, specific regions of Env, including regions targeted by broadly neutralizing antibodies, display enhanced mutational tolerance when XBP1s is induced, hinting at a role for host proteostasis network hijacking in potentiating antibody escape. These observations reveal a key function for proteostasis networks in decreasing instead of expanding the sequence space accessible to client proteins, while also demonstrating that the host ER proteostasis network profoundly shapes the mutational tolerance of Env in ways that could have important consequences for HIV adaptation.

Track: High Throughput Protein Science

ABS046 | Deep mutational scanning of a predicted Syk-family kinase ancestor using a bacterial assay

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University of California, Irvine (United States)

Many tyrosine kinases cannot be expressed readily in E. coli, limiting facile access to these proteins for biochemical experiments. We used ancestral sequence reconstruction to generate a spleen tyrosine kinase (Syk) variant that, unlike the present-day members of this family (Syk and ZAP-70), can be expressed in bacteria. This bacterially-expressed protein has catalytic activity, substrate specificity, and allosteric regulation by phosphorylation that reflect those of human Syk and ZAP-70. Taking advantage of the ability to express this novel Syk-family kinase in bacteria, we developed a two-hybrid assay that couples the growth of E.coli in the presence of an antibiotic to successful phosphorylation of a bait peptide by the kinase. Using this assay, we screened a saturation mutagenesis library of the reconstructed Syk-family kinase. Loss-of-function mutations identified in the screen correlate well with residues established as critical to function and/or structure in protein kinases. We also identify activating mutations in the regulatory hydrophobic spine, catalytic hydrophobic spine, and activation loop, all of which are within key motifs involved in kinase regulation. Strikingly, one of the mutations to the bacterially-expressed Syk-family kinase identified in our screen resulted in a dramatic increase in expression. Thus, through ancestral sequence reconstruction followed by deep mutational scanning we have generated a high yield Syk-family kinase that can be expressed in bacteria.

Track: Machine Learning in Protein Science

ABS047 | Computational Development for Automatic Enzyme Design

Qianzhen Shao
Vanderbilt University (United States)

The a priori computational design of function-enhancing enzyme mutants is significant for expanding the biocatalytic toolbox for late-stage functionalization of drug-like molecules, environmental degradation of plastics and other pollutants, and medical treatment of food allergies. In this talk, I will introduce our ongoing efforts to develop computational database, software, and workflows that facilitate the discovery of beneficial enzyme mutants for catalyzing new-to-nature reactions. This includes an integrated enzyme structure-function database, IntEnzyDB; a high-throughput enzyme modeling software, EnzyHTP; a deep learning framework for predicting the outcome of enzyme-catalyzed hydrolytic kinetic resolution, EnzyKR. We will also talk about the use of these tools to advance the understanding of fundamental enzyme catalysis processes.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS048 | Insights into the function of an essential membrane protease Rv2903c from Mycobacterium tuberculosis

Indu Bhatia
National Institute of Immunology (India)

Cell envelope of pathogenic Mycobacterium tuberculosis (Mtb) is intriguingly a complex structure representing a significant barrier to drug delivery and provides substantial survival advantages to the pathogen within the granulomas. Furthermore, the functional knowledge on proteins harboured within the cell membrane is scarce owing to the substantial experimental challenges of working with the membrane proteins. The extracytosolic secretion of proteins is a key cellular process for cell survival and inarguably play a vital role in virulence of Mtb; promoting host cell invasion because of their direct involvement in the molecular interactions made between the pathogen and their target cells, therefore representing important molecules to study the host-pathogen study of Mtb. In the present study, we report the in vitro functional characterization of an essential full length membrane protein LepB (Rv2903c) which encodes the sole homolog of the type I signal peptidase in Mtb. LepB full length membrane protein was purified to homogeneity using various chromatographic techniques and its native folded state was studied using circular dichroism (CD) and intrinsic tryptophan fluorescence spectrometry. Its subcellular localization studies infer that LepB proteins localizes to the cell membrane. Since, lepB is a type I signal peptidase which are critical for the release of translocated preproteins from the cell membrane. In light of its function, an essential mycP3 protein (Rv0291) and immunogenic implicated protein fbpA (Rv3804) has been studied as lepB substrates via surface plasmon resonance (SPR) and in vitro activity assays. Thus, this study on in vitro functional characterization of LepB which will aid in better understanding of enigmatic area of protein export of Mtb proteins.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS049 | Small Molecules Targeting the Disordered Transactivation Domain of the Androgen Receptor Induce the Formation of Collapsed Helical States

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Castration-resistant prostate cancer (CRPC) is a lethal condition suffered by ~35% of prostate cancer patients who become resistant to existing FDA-approved drugs. Small molecules that target the intrinsically disordered N-terminal domain of the androgen receptor (AR-NTD) have shown promise in circumventing CPRC drug-resistance. A prodrug of one such compound, EPI-002, entered human trials in 2015 but was discontinued after phase I due to poor potency. The compound EPI-7170 was subsequently found to have improved potency, and a related compound entered human trials in 2020. NMR measurements have localized the strongest effects of these compounds to a transiently helical region of the disordered AR-NTD but no detailed structural or mechanistic rationale exists to explain their affinity to this region or the comparative potency of EPI-7170. Here, we utilize all-atom molecular dynamics simulations to elucidate the binding mechanisms of the small molecules EPI-002 and EPI-7170 to the disordered AR-NTD. We observe that both compounds induce the formation of collapsed helical states in the Tau-5 transactivation domain and that these bound states consist of heterogenous ensembles of interconverting binding modes. We find that EPI-7170 has a higher affinity to Tau-5 than EPI-002 and that the EPI-7170 bound ensemble contains a substantially higher population of collapsed helical states than the bound ensemble of EPI-002. We identify a network of interactions in the EPI-7170 bound ensemble that stabilize collapsed helical conformations. Our results provide atomically detailed binding mechanisms for EPI compounds consistent with NMR experiments that will prove useful for drug discovery for CRPC.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS050 | Thioesterase enzymes in the updated ThYme database

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Thioesterases (TEs) hydrolyze thioester bonds. They catalyze important reactions in pathways such as fatty acid synthesis and polyketide synthesis, essential for the biological production of components in foods, cosmetics, detergents, insecticides, fungicides, antibiotics, and other medicinal compounds. Thioesterases are classified into thirty-five TE families,1 which appear in the publicly available thioester-active enzyme (ThYme) database.2 Enzymes in a family are classified according to sequence similarity, which allows inferring the tertiary structure and catalytic residues of all family members based on those that have been experimentally characterized. The TE families have been recently updated in a new version of the ThYme database, including the TE enzyme families, sequences, and structures. The new version of ThYme currently presents >100,000 sequences in a modern online interface that allows users to interactively search sequences, blast sequences in a short time, customize their preferences, and download content. The new ThYme also includes other enzyme groups that act on thioester bonds such as ketoacyl synthases. In this presentation, we will describe: i) the functions, structures, and mechanisms of the thirty-five TE families, ii) the catalytic residues in most TE families with known tertiary structures, iii) the phylogenetic analysis of experimentally-confirmed TEs that have a HotDog or an alpha-beta hydrolase fold, and iv) the new functionalities in the updated ThYme database. Convergent and divergent evolution is observed in TEs, which show that thioester hydrolysis can proceed in different structures and by other mechanisms, leading to multiple substrate specificities.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS051 | Thermostability and DNA-Binding Function of Engineered, Chimeric Proteins

Jessica DeSanctis, Nicole Boardman, Amber Persson, Michelle McCully
Santa Clara University (United States)

Functional thermostable proteins are beneficial for many industries including pharmaceutical, manufacturing, and transportation. Protein stability is imperative for safely handling and storing biopharmaceuticals as well as the efficiency of their transportation by eliminating the need for industrial freezers. Here, we attempt to rationally design a protein that is both thermostable and functional. The Engrailed homeodomain (EnHD), a protein isolated from Drosophila melanogaster, is a good target for assessing functionality and thermostability due to its moderate melting temperature (Tm = 52 °C) and its ability to bind to DNA. UVF, an engineered homologue of EnHD, is non-functional but very thermostable (Tm >99 °C). UVF shares a 22% sequence identity to EnHD. We created chimeras of EnHD and UVF by combining the functional components of EnHD and the thermostable elements of UVF using MODELLER. We conducted all-atom explicit solvent molecular dynamics simulations of the proteins

alone and with DNA at 25 and 100 °C. We compared their Cα RMSD between temperatures to assess thermostability and analyzed contact time with DNA to assess function. Proteins were expressed using recombinant protein methods in E. coli. After expression and purification, we used Circular Dichroism (CD) and Electrophoretic Mobility Shift Assays (EMSAs) in order to analyze the thermostability and functionality of the chimeric proteins.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS053 | Metabolic Engineering of Live Yeast for the Production of Novel Tetracyclines for In Situ Treatment of Antibiotic Resistance

Arden Lee, Ehud Herbst, Scott Snyder, Yi Tang, Virginia Cornish
Columbia University (United States)

Developing treatments for antibiotic resistant bacterial infections is among the highest priority public health challenges worldwide. Tetracyclines, one of the most important classes of antibiotics, have fallen prey to antibiotic resistance, necessitating the generation of new analogs. Many tetracycline analogs have been accessed through both total synthesis and semisynthesis, but key C-ring tetracycline analogs remain inaccessible. New methods are needed to unlock access to these analogs, and heterologous biosynthesis in a tractable host such as Saccharomyces cerevisiae is a candidate method. C-ring analog biosynthesis can mimic nature's biosynthesis of tetracyclines from anhydrotetracyclines, but challenges exist, including the absence of the unique cofactor F420 in common heterologous hosts. Here, we describe the biosynthesis of tetracycline from anhydrotetracycline in S. cerevisiae heterologously expressing three enzymes from three bacterial hosts. Additionally, our collaborators in the Tang laboratory reported the heterologous biosynthesis of a non-antibiotic anhydrotetracycline derivative, TAN-1612, in Saccharomyces cerevisiae from Aspergillus niger; we propose to convert TAN-1612 into a high titer tetracycline- and analog-producer by modifying the 2-, 4-, and 6-positions, proven critical for antibiotic activity. By hijacking biosynthetic hydroxylating and reducing enzymes, we will modify the 6-alpha-position, dearomatizing the C-ring. By taking advantage of yeast's natural biosynthetic pathways, we will create inexpensive, single-dose antibiotics, setting the stage to pursue yeast as a novel therapeutic. These state-of-the-art synthetic biology technologies will create entirely new paradigms, leading the charge against infections and diseases.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS054 | Detergent Alternatives: Membrane Protein Purification Using Synthetic Nanodisc Polymers

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The development of styrene maleic acid (SMA) and diisobutylene maleic acid (DIBMA) co-polymers provides an alternative to traditional detergent extraction of integral membrane proteins. By inserting into the lipid bilayer, these polymers can extract membrane proteins with lipids in the form of native nanodiscs. Unlike detergent solubilization, where membrane proteins may lose annular lipids necessary for proper folding and stability, native nanodiscs allow for the extracted targets to remain in the natural lipid environment. In addition, polymer-based nanodiscs can be purified using common chromatography methods, similar to protocols established for detergent-solubilized membrane proteins. Here we describe the solubilization screening and purification of an integral membrane protein using several commercial co-polymers. We focus our efforts on SapC, a transmembrane domain in the Type I ABC importer SapABCDF, and we demonstrate that SMA-bound SapC nanodiscs can assemble with the separately purified nucleotide binding domain SapF to build the SapCF complex. Our pursuits have since expanded to include the second nucleotide binding domain of the Sap transporter, SapD, as we seek to understand the mechanism of transporter regulation by the SapD/SapF domain pair.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS056 | Discovery of a reversible covalent inhibitor against Papain-like protease (PLpro) from SARS-CoV-2 virus

Teena Bajaj, Eddie Wehri, Rahul Suryawanshi, Kundan Pardeshi, Elizabeth King, Kamyar Behrouzi, Zahra Khodabakshi, G Renuka Kumar, Mohammad R.K. Mofrad, Daniel K. Nomura, Melanie Ott, Julia Schaltesky, Niren Murthy
University of California, Berkeley (United States)

Reversible covalent inhibitors have generated a tremendous interest because of their unique combination of

high efficacy and low toxicity. However, developing reversible covalent inhibitors is a very challenging task because the only strategy available for generating reversible covalent inhibitors are based upon either boronic acids or a retro-michael addition reaction, which are effective on only a small set of proteins. In this report, we demonstrate that a thiouridine fragment can serve as a scaffold for developing thiol triggered reversible covalent inhibitors. The thiouridine fragment was identified from a 115,000 molecule screen performed against the papain-like protease (PLpro) from SARS CoV-2 virus. This fragment inhibited PLpro with a IC50 of 3.8 μM and also inhibited SARS CoV-2 replication in cells. In addition, the thiouridine fragment covalently reacted with cysteines in PLpro, and its inhibition of PLpro was reversed via the addition of thiols. The thiouridine fragment undergoes an additional-elimination reaction with the active site cysteine of PLpro, and thiols that can enter the active site are able to displace the thiouridine fragment. These experiments demonstrate that the addition elimination reaction has the potential to generate reversible covalent inhibitors and dramatically expands the classes of warheads that can generate reversible covalent inhibitors.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS057 | Insight Into the Structural Evolution of Polyamine Acetyltransferases Across Prokaryotes, Eukaryotes, and Archaea

Huy Do, Nicola Bordin, Clemens Rauer, Christine Orengo, Misty Kuhn
San Francisco State University (United States)

Polyamines are critical small molecules that are found in a variety of organisms and regulate numerous processes such as bacterial biofilm formation, siderophore production, and transcription. One way polyamine concentrations are regulated is through their acetylation via polyamine acetyltransferases. The most widely studied polyamine acetyltransferases are the spermidine/spermine N-acetyltransferases (SSATs), which acetylate spermidine and spermine using the donor molecule acetyl-coenzyme A. These enzymes belong to the Gcn5-related N-acetyltransferase (GNAT) superfamily, which is comprised of a variety of enzymes that adopt a characteristic GNAT structural fold. While SSATs all adopt this fold, their oligomeric states and presence and absence of domain swapping vary across organisms. The two most prominent and structurally characterized SSATs in bacteria and humans are SpeG and hSSAT, respectively. However, SpeG is a dodecamer without domain swapping and has allosteric sites, whereas hSSAT is a dimer with domain swapping and to our knowledge has no allosteric site. In this study, we sought to determine the evolutionary structural changes that potentially contributed to the two distinct structural forms of these proteins. Therefore, we grouped GNAT protein sequences into functional clusters and identified sub-clusters of proteins that were located between the SpeG and hSSAT clusters. Since none of the proteins within these sub-clusters had been structurally determined, we generated Alphafold models to perform structural comparisons. Our analysis of these data revealed distinct groups of proteins that likely share similar structural characteristics.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS058 | Investigating the Importance of a Series of Active Site Residues on PA3945 Enzyme Activity

Cillian Variot, Misty Kuhn
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The diverse and widespread superfamily of Gcn5-related N-acetyltransferases (GNATs) are characterized by structural motifs and varying acceptor pockets that allow them to bind and acetylate a breadth of substrates. One example of a GNAT that exhibits substrate promiscuity is the PA3944 enzyme from Pseudomonas aeruginosa. We have previously shown this enzyme acetylates a variety of substrates, including the macrocyclic antibiotic polymyxin B, and examined the importance of numerous active site residues for substrate specificity. A homologous enzyme PA3945 shares a 71% sequence identity with PA3944, yet it is inactive toward polymyxin B. A comparison of a homology model of PA3945 and the 3D crystal structure of PA3944 indicates the proteins may adopt a similar overall structure but some active site residues do not appear to be conserved. To determine whether the lack of activity toward polymyxin B is due to the non-conserved proline residues or alteration of other active site residues, we generated point mutations of the PA3945 enzyme to mimic the active site residues in PA3944 and screened the mutants for activity. Our research details the importance of acceptor site residues on the activity of PA3945 and expands on our understanding of the evolution of these two enzymes.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS059 | Assessing the Importance of an Active Site Serine Residue on Binding and Enzymatic Activity of AAC(6)-Ig and -Ih Aminoglycoside Acetyltransferase Enzymes

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Aminoglycosides are a potent class of antibiotics that can be used against a wide variety of microbes. These drugs typically work by binding to a ribosomal subunit and causing errors in translation, leading to bacterial death. However, these antibiotics can become inactivated via acetylation by aminoglycoside acetyltransferases. These enzymes belong to the Gcn5-related N-acetyltransferase superfamily, which utilize a variety of chemical mechanisms to acetylate an assortment of substrates. The most common general acid/base chemical mechanism for GNATs tends to use a catalytic tyrosine residue as a general acid involving a direct transfer kinetic mechanism. However, other chemical and kinetic mechanisms are possible. For example, we recently showed that some GNATs can use a catalytic serine residue, which is involved in a hybrid ping-pong kinetic mechanism. Our comparative examination of AAC(6')-Ig and -Ih aminoglycoside acetyltransferases from Acinetobacter shows these two enzymes have a serine residue in a similar location in the active site. To determine whether this serine residue is indeed critical for activity, we generated point mutations of these serine residues and screened the enzymes for activity against a panel of aminoglycoside antibiotics. We also performed docking studies with this serine residue acetylated and non-acetylated to probe the binding modes of the aminoglycosides in these two conditions. These results provide an understanding of how these two enzyme acceptor site residues may bind diverse aminoglycosides and contribute to antibiotic resistance in these organisms.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS060 | Examination of the Structural Motifs that Underpin the GNAT Fold Across Diverse 3D Protein Structures

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Gcn5-related N-acetyltransferases (GNATs) are important enzymes that catalyze the acetylation of small and large biomolecules. The impact of acetylation ranges from enabling antibiotic resistance to post-translational modifications of proteins. While GNATs catalyze diverse reactions, they all tend to adopt a common structural fold that enables the acetyl donor and acetyl acceptor molecules to bind. This fold is defined by four motifs A-D, which were documented over twenty years ago when the number of GNAT 3D protein structures in the Protein Data Bank (PDB) were more limited. Today, there are numerous GNAT protein structures that have been determined, which has expanded our understanding of how diverse proteins from this superfamily fold. To determine whether the boundaries of these motifs remain applicable across this larger structural dataset or should be refined, we examined their locations on 56 representative structures from the PDB. Based on these data, we found some secondary structure extensions or deviations from the core motifs on a subset of proteins. We then grouped these structures based on these deviations and examined whether there were patterns to their functional or liganded state. Our results provide new insight into whether the originally defined GNAT motifs are retained or deviate in an expanded GNAT structural dataset.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS061 | Decarboxylase Enzymes in the Polyamine Biosynthetic Pathway are Post-translationally Modified by Lysine Acetyltransferase Proteins

Patricia Uychoco, Pamela Caro De Silva, Tiffany Chambers, Misty Kuhn
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Polyamines are small polycationic organic molecules that are biosynthesized from a variety of amino acids and are utilized by living organisms for many cellular processes. Polyamine biosynthesis and degradation are highly regulated processes, and in some bacteria polyamines act as regulatory molecules for biofilm formation and dispersion. Prior studies have shown that several lysine acetyltransferase (KAT) enzymes in E. coli acetylate a multitude of different proteins. While the corresponding substrate proteins and sites of acetylation have been identified, their locations on 3D protein structures have not been thoroughly investigated across all substrates. Moreover, not much is known about

whether lysine acetylation is an important post-translational modification for polyamine metabolic enzymes. Our analysis identified three regulatory polyamine biosynthetic decarboxylase enzymes that are enzymatically acetylated by KAT proteins. We performed a structural analysis to compare the location of these acetylation sites in 3D on the different enzymes. Our structural investigation revealed that while the enzymes catalyze similar reactions, they do not share the same structural folds and the sites of acetylation are scattered across protein domains. These results provide a framework for further elucidating the substrate specificity of KAT proteins and how polyamine metabolism may be further regulated by lysine acetylation.

Track: Cellular Tasks

ABS062 | Exploring the Regulation of Polyamine Biosynthesis and Degradation via Lysine Acetylation in E. coli

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Polyamines are aliphatic small molecules that are important for cellular homeostasis and bacterial survival under stressful conditions. High concentrations of polyamines in cells can be toxic and their acetylation by polyamine acetyltransferases neutralizes this toxicity. One way polyamine acetylation is regulated in E. coli is via the spermidine N-acetyltransferase SpeG enzyme. It also has an allosteric site that is thought to be important for regulating SpeG kinetic activity by binding polyamine at the allosteric site. Polyamine metabolism can also be regulated at the transcriptional, translational, and post-translational levels. At the post-translational level, many E. coli proteins involved in polyamine metabolism are modified via lysine acetyltransferases (KATs). To learn more about the potential effects of acetylation of these proteins on the regulation of polyamine metabolism in E. coli, we examined the location of acetylated and non-acetylated enzymes within polyamine biosynthetic and degradative pathways. We found the proteins that were acetylated are located at key regulatory points of these pathways or are global transcriptional regulator proteins that are important for transitioning between anerobic and aerobic metabolism. Our results highlight possible scenarios for the effects of acetylation on the flux of these pathways and uncover unexplored avenues for further studies on the regulation of polyamine metabolism in bacteria.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS065 | Structures of the Pro-Oncogenic Singly-Liganded HER2/HER3 Heterodimer Reveal Novel Asymmetry

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The Human Epidermal Growth Factor Receptor 2 (HER2) and Human Epidermal Growth Factor Receptor 3 (HER3) form a potent pro-oncogenic signaling unit upon binding to the extracellular ligand neuregulin-1ß (NRG1ß). The mechanism of HER2 heteromeric association with HER3 remains unknown in the absence of any structure of the complex. Through novel ligand-based purification and single particle cryo-electron microscopy (cryo-EM) on graphene oxide supported grids, we solved three novel structures of the HER2/HER3 heterodimer in the context of near-full length receptors. These structures represent the first depictions of a singly-liganded HER receptor dimer, HER2 actively engaged with a co-receptor, and HER3 in a ligand-bound configuration. Our structure of the HER2/HER3/NRG1ß heterocomplex in which only HER3 is bound to a ligand reveals unexpected dynamics at the HER2/HER3 dimerization interface and supports a model in which ligand binding allosterically coordinates a receptive pocket for the dimerization arm of the co-receptor. This novel allosteric mechanism of ligand occupancy sensing also explains an intrinsic mode of HER2 autoinhibition in which the extracellular domains of HER2 do not homo-associate via the canonical active dimer interfaces despite adopting an active-like conformation without a ligand. In a second heterocomplex structure featuring the most prevalent oncogenic extracellular domain HER2 mutant, S310F, we observe a compensatory interaction with the HER3 dimerization arm that stabilizes the dimerization interface. We show that both HER2/HER3 and HER2-S310F/HER3 retain the capacity to bind to the HER2-targeting therapeutic antibody, trastuzumab, but the mutant complex does not bind to pertuzumab. Our third structure of the HER2-S310F/HER3/NRG1ß/trastuzumab Fragment antigen binding (Fab) complex, the first structure of this therapeutic in the context of a HER receptor dimer, demonstrates how the receptor dimer subtly rearranges to accommodate trastuzumab. These findings have broad implications for understanding HER receptor dimerization and unveil previously unknown structural details for rational bispecific drug design.

Track: High Throughput Protein Science

ABS066 | Progress report on characterizing the stability of 500 repacked variants of a four-helix bundle protein

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the ohio state university (United States)

As a group of essential bio-macromolecules, most proteins become functional when folding into unique 3-dimensional structure. The limited understanding of the sequence basis of the protein folding and stability is a major obstacle for engineering therapeutic protein molecules. Many lines of evidence suggest that the residues packed in the hydrophobic core are important for a protein to maintain native-like properties. Here we analyze how the stability and other biophysical properties vary by repacking the hydrophobic core of the four-helix-bundle protein, Rop. We used combinatorial mutagenesis in selected positions within the core region of Rop to generate a large library of variants. Via a robust cell-based screening method, we can screen for the active variants based on the fluorescent phenotype. By employing a growth selection based on the function of Rop, we can enrich the active variants in the population and confirm the sequences in large-scale. A high-throughput thermal scanning technique has been developed to obtain insight into the stability effects of the hydrophobic core repacking. Combined with other biophysical characterization and MD simulation, we will further define the roles of the hydrophobic core in protein folding and functioning. Here we present a preliminary analysis of over 300 variants, which has revealed unexpected sequence patterns and also produced some of the most stable variants of Rop that have been engineered to date.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS067 | Developing Small-Molecule Probes for Activity-Based Profiling of Tyrosine Phosphatases

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Columbia University (United States)

Tyrosine phosphatases, enzymes that catalyze the dephosphorylation of tyrosine-phosphorylated proteins, make up one of the largest families of signaling proteins in humans. As protein tyrosine phosphorylation is an important post-translational modification that can modulate protein-protein interactions, protein localization, stability, and enzyme activity, tyrosine phosphatases possess significant roles in signaling pathways of both normal cellular processes and a wide variety of diseases, including cancer. However, we know relatively little about how and when tyrosine phosphatases are regulated, calling for development of more tools to probe their activities in various signaling contexts. Here, we describe our efforts to design chemical probes that covalently label the active sites of tyrosine phosphatases and report on their activity across the proteome. First, we designed a small library of candidate molecules, by combining a series of thiol-reactive functional groups that could label the catalytic cysteine residue, along with a variety of scaffold structures. The library was then screened for reactivity against thiols and specificity towards target active sites. Then, several candidates were augmented with alkyne groups to enable click chemistry with biotin tags for enrichment, and subjected to further biochemical assays. Finally, lysates and intact cells were treated with these candidates and analyzed in mass spectrometry proteomics experiments to detect labeled proteins. We showed that these molecules can label certain tyrosine phosphatases with specificity. With this ability, the probes offer a way to investigate changes on abundance and activity levels across the tyrosine phosphatase family upon differing signaling states or cell types. Furthermore, we anticipate that our design efforts will also yield new insights into the general development processes of activity-based probes.

Track: Cellular Tasks

ABS068 | X-ray crystallographic structure of BrxA, an enzyme involved in redox homeostasis in gram-positive organisms

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Bacillithiol is a low-molecular-weight thiol produced by many gram-positive bacteria, such as the pathogenic organisms Staphylococcus aureus and Bacillus anthracis. It is involved in detoxification of xenobiotic agents as well as redox homeostasis. During oxidative stress, bacillithiol is added to protein thiols by forming mixed disulfides with cysteine side chains to protect them from oxidative damage. Bacilliredoxins are small enzymes that remove these mixed bacillithiol disulfides from protected proteins through a disulfide-exchange reaction, which restores the thiol group on the target protein. BrxA is a bacilliredoxin from S. aureus that has been used in biosensor

applications for bacillithiol detection and antibiotic efficacy assessment. The broad thioredoxin family of proteins typically contains a CXXC motif, whereas bacilliredoxins like BrxA contain an unusual conserved CGC motif. Furthermore, their exact catalytic mechanism remains unclear. Here, we describe a 1.6 Å resolution X-ray crystallographic structure of the bacilliredoxin BrxA from S. aureus. The structure adopts the typical thioredoxin fold and contains bacillithiol in a mixed disulfide with Cys54. The crystal structure, coupled with a site-directed mutagenic study, demonstrates that an intersubunit disulfide linkage at Cys56 forms under oxidizing conditions and may play a role in dimer stabilization. This structure represents the first-ever crystallographic observation of bacillithiol, and the findings presented here will provide insight into the function of BrxA and other bacilliredoxins.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS070 | Use of Deepmind Alphafold to Recreate Prion Protein in silico Predicted Structures

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While prion proteins are known to be soluble in their PrPC state, in pathologies they aggregate to form fibrils. Although these rich in beta-sheet, the tertiary structure of fibril-forming prions remains elusive. Questions remain about how these structures rich in beta-sheet form.

In addition to several NMR structures of recombinant human prion proteins containing these mutations, several in silico models predicting the effects of these mutations have been published. Research suggests that destabilization of the beta2/alpha2 loop allows the prion to protein to sample conformations that can lead to protein misfolding.

Here, the ten point-mutations evaluated in silico by Meli, et al. [PLoS One. 2011 Apr 28;6(4)] were re-predicted using Deepmind Alphafold. Each structure was then compared against the NMR ensemble structure 1qlz using the 'super' command in Pymol, and RMSD values were recorded. These Alphafold predictions were also compared to the in silico predictions of Meli, et al.

In terms of overall tertiary structure and most elements of secondary structure, Deepmind Alphafold predictions successfully recapitulated the overall NMR solution structure.

However, Deepmind Alphafold predictions for the D178N, T188K, and E196K mutants revealed substantial deviations from the NMR structure 1qlz, within the beta2/alpha2 loop; these Alphafold predicted structures did not reveal a 3/10 helix. Interestingly, the alpha3 helix in the Alphafold prediction for the F198S mutant seemed to deviate from the 1qlz NMR structure more than any of the other predictions.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS071 | Engineering Protein-based Materials via Synthetic biology

Fuzhong Zhang
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Nature has evolved a wide range of protein-based, high-performance materials that can outperform the best available synthetic materials. Producing these protein materials can provide a renewable and environmentally-friendly route to replace current petroleum-derived materials. However, the natural biosynthetic process of these materials are often impossible to harness for scalable production as they are produced in limited quantity by slow growing organisms. Engineering microbes to produce these high-performance materials could provide an attractive solution, but face multiple technical challenges. In this talk, I will introduce our recently developed strategies using synthetic biology and protein engineering to microbially produce megadalton protein based materials, including titin polymers and amyloid-silk hybrid proteins. Our engineered hybrid proteins include structural and function domains from multiple proteins to deliver desirable and tunable properties. We then engineer bacteria to overexpress these highly repetitive, high molecular weight hybrid proteins that incorporate non-canonical amino acids. Additional, we developed a strategy to perform protein polymerization reactions in living cells using folded proteins as monomers. These synthetic strategies have resulted ultra-high molecular weight (UHMW) titin polymers and amyloid-silk proteins. To explore their applications, these proteins were spun to fibers that not only recapture highly desirable properties of their natural counterparts (i.e., high damping capacity and mechanical recovery for titin, stable β-sheet structures for amyloid) but also exhibit exceptionally high strength, toughness, and damping energy — outperforming many synthetic and natural polymers. Our strategies in protein engineering and synthetic biology may have broad applications, leading to various novel protein-based materials.

Track: High Throughput Protein Science

ABS072 | Complete mutational mapping of a GTPase switch in vivo reveals novel allosteric regulation

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GTPases are highly regulated molecular switches that control signaling in cells. Switching occurs via catalyzed GTP hydrolysis and nucleotide exchange, yet the importance of additional regulation for biological function - suggested by high GTPase conservation and centrality in protein networks - is unclear. Here we map the function of each residue in the central GTPase Gsp1/Ran in its native biological network by deep mutagenesis. Quantitative fitness measurements for 4333 point mutations revealed an unexpected toxic/gain-of-function response resulting from 29% of mutations, located throughout the Gsp1/Ran structure including at several novel allosteric sites. Using biochemical measurements and computational modeling in Rosetta, we show that these sites are coupled to the active site, and can modulate the kinetics of GTPase switching. We identify distinct mechanisms by which perturbations at these sites alter Gsp1/Ran cellular function. Our systematic discovery and functional annotation of new regulatory sites provides a residue-level map which we show generalizes to other GTPases such as Ras. This "functional anatomy" of a GTPase molecular switch opens avenues to interrogate and target GTPases controlling many essential biological processes. Finally, our study adds to the emerging linkage between allosteric regulation of switch balance, the ultrasensitivity of switches, and functional consequences for cellular regulation.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS073 | Allosteric Regulation of E3 Ligase Parkin Studied by HydrogenDeuterium Exchange Measured by Mass Spectrometry (nsHX-MS) and Single Molecule FRET (smFRET)

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Parkin is a multi-domain ubiquitin E3 ligase and is central to the controlled destruction of damaged mitochondria by autophagy (mitophagy). To function normally, Parkin needs to maintain a delicate balance between an auto-inhibited resting state and an activated state competent in ubiquitinating mitochondria outer membrane proteins. Its function is tightly regulated by several allosteric regulators and post-translational modifications. A number of Parkin genetic variants across the protein sequence are found to be correlated with human diseases such as early onset Parkinson's disease. Despite several crystal structures being available, the mechanism(s) by which these mutations affect Parkin function remains elusive.

To better understand the biophysical basis of allosteric regulation of Parkin, we build a state-ensemble based model derived from rigorous statistical thermodynamic principles. The population distribution of high-energy partially unfolded state ensemble and its shift in response to allosteric signals are crucial to understanding allosteric regulations. We perform nsHX-MS to detect and map the high energy states populated by Parkin. HX due to large scale sub-global and global unfolding events is promoted by titrating in low concentrations of chaotrope, which in turn, enables mapping Parkin folding stability in a structure-resolved manner. Here, we focus on interaction between the inhibitory Ubiquitin-like domain (Ubl) and the rest of Parkin. We've found the interaction is only marginally stabilizing which explains why many seemingly mild amino-acid substitutions could affect Parkin activation.

Our smFRET measurements further corroborate and complement the nsHX-MS results. In these measurements, we show Parkin partition into a major population corresponding to the auto-inhibited state and a minor one where the distance between the Ubl and the Parkin core is much larger than in the auto-inhibited state. The latter may represent an activated state. Supported by NIH (GM129076).

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS075 | Similar but different catalytic mechanism of N-hydroxy-L-arginine hydrolase: arginase framework used for hydrolyzing its inhibitor

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DcsB, an enzyme produced from D-cycloserine biosynthetic gene cluster, displays moderate similarity to

arginase in the sequence and three-dimensional structure. Arginase is a ubiquitous enzyme hydrolyzing L-arginine to generate L-ornithine and urea, whereas DcsB hydrolyzes Nω-hydroxy-L-arginine (L-NOHA), an arginase inhibitor, to generate L-ornithine and hydroxyurea. We determined the crystal structure of DcsB associated with L-ornithine and that with the tetrahedral derivative of 2(S)-amino-6-boronohexanoic acid, whose boron atom forms a covalent bond with an oxygen atom bridging two manganese ions at the active center. The substrate-binding pocket of DcsB is narrower than that of arginase, suggesting that DcsB is unsuitable for the binding of L-NOHA in an inhibitory manner. The transition state-like structure demonstrated that Asp210 and Glu241 have a role to trap a positively charged ion near the dimanganese cluster. Kinetic analysis using the mutated DcsB showed that the enzyme employs different catalytic mechanisms under the neutral and alkaline pH conditions. Glu241 in DcsB is likely involved in the recognition of the hydroxyguanidino group of L-NOHA, whereas Asp210, in cooperation with Glu241, seems to contribute to the reactivity toward the protonated L-NOHA, which is a preferable species under the neutral pH conditions. After entering of the protonated L-NOHA to the substrate-binding pocket of DcsB, a hydronium ion may be trapped at the positive ion-binding site. Then, the ion serves as a specific acid catalyst to facilitate the collapse of the tetrahedral intermediate of L-NOHA.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS076 | Static and Dynamic Characterization of Protein Structures on Ramachandran Plots

Soon Woo Park, Byung Ho Lee, Seung Hun Song, Moon Ki Kim
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Protein structures are directly related to their biological functions or processes. Therefore, various techniques have been developed to describe their shapes and among them, Ramachandran plot which expresses two torsion angles (phi, psi) of the backbone on a two-dimensional plane has played an important role in structural biology for decades. Although many studies have been carried out in terms of Ramachandran plot to analyze the protein structure, Ramachandran plot is still constructed by only small number of dataset and further it cannot specifically reflect a steric structure of proteins.

In this study, we analyzed both static and dynamic characteristics of the secondary structures of proteins. In the static part, Ramachandran plot was revisited for high-resolution proteins (<2.0 Å) released in the protein data bank (PDB) until March 2022. And then, amino acid propensities according to residue depth were expressed on a Ramachandran plot using the DEPTH program. In the dynamic part, normal mode analysis (NMA) based on elastic network model (ENM) was performed for dataset by the Korea SKKU morph server (KOSMOS). All NMA results were built as a database in KOSMOS web server so that all researchers could use them in various ways. In addition, by statistically analyzing the NMA results, the degree of conformational change according to the secondary structure of all proteins belonging to the dataset was expressed in the phi-psi space. Through this analysis, we quantitatively surveyed the contribution of the protein conformational change on Ramachandran plot. Provided that biological functions of proteins are closely related to their structures and dynamics, this bioinformatics approach will play an important role in functional biomaterial design as well as protein folding prediction.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS077 | InDels in the loop region shapes new protein function in the evolution of GH19 chitinase

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In addition to substitutions, insertions and deletions (InDels) play a key role in the emergence of enzyme functions. However, our understanding of the role of InDels is limited compared to the one of substitution. Most protein engineering methods focus on exploring the sequence space of the fixed length while sequence space including InDels has been overlooked due to the ambiguity in evolutionary information and the difficulties in creating libraries. Herein we choose glycoside hydrolase family 19 (GH19) chitinase as a candidate to investigate how InDels in the loop regions contribute to the emergence of new functions in proteins. The function of GH19 varies from chitinase activity to antifungal activity, depending on the presence/absence of loop regions. Phylogenetic analysis revealed the ancestral nodes where the InDels in loop regions occurred. Characterizations of eight protein variants based on two reconstructed ancestral proteins with different loop combinations revealed the critical loop for new function acquisition. This study suggests that non-catalytic loop additions/removals are one of the strategies that Nature applies, to acquire new protein functions.

Track: High Throughput Protein Science

ABS078 | Modulation of Ribosomal Frameshifting by Cotranslational Folding and Misfolding of the Nascent Polypeptide Chain

Patrick Carmody, Matthew Zimmer, Charles Kuntz, Haley Harrington, Kate Duckworth, Wesley Penn, Suchetana Mukhopadhyay, Thomas Miller, Jonathan Schlebach
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Membrane protein folding is an error-prone process that is subject to numerous forms of biochemical regulation. The ribosome and its ensemble of accessory proteins employ a variety of mechanisms to monitor membrane protein folding and misfolding during translation that are essential for the maintenance of protein homeostasis. Nevertheless, it's unclear how this biosynthetic machinery detects and responds to folding and misfolding in real time. We recently found that the mechanical forces generated by the translocon-mediated membrane integration of transmembrane domains can stimulate a translational recoding event known as -1 programmed ribosomal frameshifting (-1PRF). Alphaviruses modulate the efficiency of -1PRF through crosstalk between an mRNA structure that pauses the ribosome on a slippery-sequence of the transcript during the translocon-mediated cotranslational folding. Based on the features of this motif, we searched the human transcriptome for other potential sites that may promote this cotranslational feedback and identified hundred potential candidates. Preliminary efforts to validate certain hits have identified an active -1PRF motif within the cystic fibrosis transmembrane conductance regulator (CFTR), the misfolding of which causes cystic fibrosis. Ribosomal frameshifting at this site results in the premature termination of translation, and knocking out this motif partially restores the expression of a common CFTR variant (ΔF508) that undergoes cotranslational misfolding. These findings suggest a novel role for ribosomal frameshifting in eukaryotic protein homeostasis.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS079 | Developing An Ex Vivo Rubber Biosynthesis Platform From Prenyl Transferase Enzyme Complexes

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Natural rubber (NR) is a cis-polyisoprene biopolymer essential for the manufacture of thousands of industrial products. Synthetic rubber can be used to manufacture some of those products, but its physicochemical properties are suboptimal for many critical applications such as tires and medical products, and its carbon footprint is environmentally damaging. A single plant species, Hevea brasiliensis (Hevea), has been the sole producer of NR in the world for over a century. Biological and geopolitical concerns currently threatening Hevea rubber production is driving efforts to develop other rubber producing plants as well as ex vivo rubber biosynthesis platforms.
NR is synthesized in vivo within the lipidaceous monolayer membrane of round structures known as rubber particles (RP) where the hydrophobic rubber molecule is effectively sequestered from the aqueous environment of the plant cell cytosol. Plant functional genomics studies have identified several RP-associated proteins, and two of these, cis-prenyl transferase (CPT) and CPT-binding protein (CBP), appear to be essential for NR biosynthesis. These two proteins form either a heterodimer, or another yet to be characterized quaternary structure leading to an active enzymatic complex. Here we demonstrate that recombinant CPT and CBP proteins, from two rubber-producing plant species, are enzymatically active and synthesize polyisoprenes but not natural rubber. Hence, reconstitution of prenyl transferase enzyme activity is necessary but not sufficient for ex vivo rubber synthesis. However, we demonstrate changes in the reaction constituents, such as organic solvents, provide improved conditions for higher enzyme activity.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS081 | Guanidine degradation in bacteria reveals a new, broadly distributed carboxyguanidine deiminase enzyme family

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The recent discovery of operons controlled by guanidinium-binding riboswitches has generated renewed interest in bacterial guanidine degradation and export pathways. The genes encoding the enzymes urea carboxylase and allophanate hydrolase are well

represented in operons controlled by the guanidine-I riboswitch. These enzymes function in tandem to facilitate the ATP-dependent hydrolysis of urea to ammonia and carbon dioxide, yielding a similar outcome to the action of urease, but through a mechanistically distinct process. We and others have demonstrated that urea carboxylase has a strong substrate preference for guanidine over urea, suggesting that the primary enzymatic function is to act as a guanidine carboxylase (GC) to activate guanidine for subsequent degradation. We demonstrate that a heteromeric enzyme, previously annotated as a "urea carboxylase-associated protein", is required for the decomposition of guanidine to ammonia and carbon dioxide. This enzyme (CgdAB) acts as a carboxyguanidine deiminase to hydrolyze carboxyguanidine to allophanate for subsequent degradation by allophanate hydrolase. Closer inspection of CgdAB reveals that it belongs to a large, functionally uncharacterized family of metalloenzymes that may utilize a unique iminohydrolase mechanism. Here, we report on the structure and catalytic activity of GC and CgdAB from Pseudomonas syringae. This work delineates the molecular basis for catalysis and substrate selectivity in a set of bacterial enzymes with newly ascribed function.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS082 | Living Yeast Biosensor for Aspergillus Fumigatus

Davida Rios
Columbia University (United States)

Over 150 million people are estimated to be impacted by severe fungal diseases with high mortality rates. However, fungal infections remain a neglected public health issue despite early diagnosis and therapeutic intervention potentially preventing death or chronic illness. One of the most prominent causative agents of invasive fungal infections are from the Aspergillus species, with Aspergillus fumigatus responsible for over 200,000 yearly cases of invasive aspergillosis globally. We have developed a low-cost, easy-to-use, and accurate live yeast-based colorimetric assay for the detection of A. fumigatus in culture supernatants. This assay is based on detecting the A. fumigatus mating pheromone through a heterologously expressed G-Protein Coupled Receptor (GPCR) in the engineered yeast, Saccharomyces cerevisiae. The GPCR transcriptionally activates the biosynthesis of lycopene, a red pigment visible to the naked eye, upon binding with the mating pheromone peptide. We built an initial biosensor with the A. fumigatus GprA mating receptor (AfuSte2) into our parent biosensor strain with fluorescent readout and achieved activation of the GPCR with a 44-fold increase at 1 uM synthetic A. fumigatus pheromone peptide (AfuPep) concentration. We tested our biosensor with A. fumigatus clinical isolate culture supernatants, resulting in at least 8-fold increase in fluorescence. These results established a foundational A. fumigatus biosensor that is responsive to synthetic peptide and fungal culture supernatants. To develop our biosensor for real world use, we transferred our system to an optimized biosensing strain which yielded a 2000-fold increase in fluorescence with AfuPep and at least 30-fold increase with culture supernatants. Our technology offers a rapid, accurate, and simple result interpretation that could provide early diagnosis and thus early therapeutic intervention, leading to positive patient outcomes compared to the lengthy and complex diagnosis methods presently used.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS083 | Defining the topology of the XPA-RPA complex in Nucleotide Excision Repair

Areetha D'Souza, Walter Chazin
Vanderbilt University (United States)

Nucleotide excision repair (NER) is a primary DNA repair pathway that functions to remove bulky DNA lesions arising from endogenous and environmental toxins as well as UV irradiation from sunlight. Mutations in NER genes lead to xeroderma pigmentosum (XP), which is characterized by an extreme hypersensitivity to sunlight and a greater than 2000-fold increase in skin cancer. NER is a complex, multi-step pathway requiring >20 proteins that work together as a dynamic multiprotein machine leading to the excision of the damaged strand containing the lesion. XPA and RPA proteins are NER scaffolds recruited to guide the accurate positioning of proteins in the pre-incision complex. We have previously characterized the two interaction sites of XPA-RPA:- a primary contact between the XPA N-terminal (XPA-N) disordered domain and the RPA32 C-terminal domain (RPA32C), and a weaker secondary contact between the XPA DNA binding domain (DBD) and the RPA70AB subunit. Here, we report integrative structural models of full-length XPA, RPA and substrates mimicking the two ss-ds DNA junctions using a combination of previously determined structures from our lab, SAXS data and computational modeling. High quality SAXS data required optimization of the XPA and RPA constructs, and screening of DNA substrates using size

exclusion chromatography to identify stable complexes. The complexes bound to the 3' and 5' ss-ds junctions differed in the orientation of the RPA domains as it engages the ssDNA substrate, as well as the positioning of the contact between XPA-N and RPA32C. Our findings that the XPA-RPA scaffold can bind to either ss-dsDNA junction, consistent with our proposal that XPA may bind first to one and then the other junction over the course of the NER trajectory.

Track: Celebrating 100 Antibody Drugs

ABS084 | Engineering Bispecific Antibodies as Therapeutics

Paul Carter
Genentech (United States)

Bispecifics are emerging as an important new subclass of antibody therapeutics with 3 bispecific currently marketed and >270 bispecifics and multispecifics in active clinical development. Bispecific IgG – a common format - are complex heterotetrameric proteins that historically have been very challenging to produce in sufficient quantity and quality for clinical trials. This presentation will describe ways to facilitate the development of bispecific IgG. Firstly, intrinsic antibody heavy/light chain pairing preference were investigated and utilized to enable the efficient production of bispecific IgG in single mammalian host cells. Secondly, a mutational analysis was employed to probe and then mitigate high viscosity of some bispecific antibodies. This may facilitate applications requiring high concentrations of bispecific antibodies such as subcutaneous delivery.

Track: Protein Phase Separation in Biomolecular Condensates

ABS085 | Building the cilium with ATP-driven molecular motors

Anthony Roberts
University College London (United Kingdom)

Cilia/flagella are present on virtually all eukaryotic cells and have essential roles in signalling, sensing and cellular propulsion. The motor protein complex dynein-2 works with polymeric intraflagellar transport (IFT) trains to form a transport machinery crucial for cilia biogenesis and function. We have used cryo-electron microscopy (cryo-EM) and machine learning methods to visualise dynein-2 motor domains undergoing ATP-driven conformational changes used to power retrograde IFT. We have also recombinantly expressed and purified the full human dynein-2 complex and investigated its structure using cryo-EM, revealing a 1.4 MDa assembly. The structure, along with in vitro motility assays and live-cell imaging, provides insights into how dynein-2's motor activity is regulated, and how the complex recognizes the IFT train. These data give mechanistic insight into intraflagellar transport and the origin of diverse functions in the dynein family.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS086 | Elucidating the molecular landscape of bacterial Gcn5-related N-acetyltransferase (GNAT) enzymes

Misty L. Kuhn
San Francisco State University (United States)

Gcn5-related N-acetyltransferases are an exquisite superfamily of enzymes that exhibit diverse structures and functions and are widespread across domains of life. These enzymes adopt a unique structural fold that creates acyl donor and acyl acceptor sites that are located on opposite sides of a V-like splay of a beta sheet. While the donor site is relatively conserved, the acceptor site architecture is quite variable and creates unique environments for different substrates to bind. Thus, the composition of amino acids in the acceptor site contribute to substrate specificity or promiscuity, as well as enable variability in kinetic and chemical mechanisms for acylation. Since the structures of GNATs are unquestionably related to their functions and many GNAT functions are underexplored, my laboratory has been working to expand our knowledge of GNAT structure/function relationships in a variety of bacteria. In this talk, I will highlight examples of how the GNAT scaffold has enabled unique structural and functional characteristics in clinically relevant bacteria and present paths for new exploration.

Track: Celebrating 100 Antibody Drugs

ABS087 | Development of recombinant antibodies for detecting multiple conformational states of glaucoma-associated myocilin

Minh Thu Ma, Athéna Patterson-Orazem, Laura Azouz, Ahlam Qerqez, Jennifer Maynard, Raquel Lieberman
Georgia Institute of Technology (United States)

Throughout biomedical research, antibodies that recognize well-characterized epitopes are key reagents in the reproducibility and interpretation of immunoassays. Antibodies also serve as potent immunotherapies and medical diagnostic agents in the clinic. The antibodies currently used to detect glaucoma-associated myocilin are unable to differentiate among disease-related myocilin conformations, limiting our understanding of its function and misfolding in a biological context. Current myocilin-directed antibodies also only target myocilin from one species, necessitating a different antibody for different types of experiments. Here, we describe our progress in developing new myocilin-directed antibodies with the following desired characteristics: (i) conformationally specific to discern between folded and misfolded states, (ii) recombinant to facilitate large production and stringent quality-control via DNA sequencing, (iii) selective to unique epitopes across the domains of myocilin, and (iv) cross-reactive between human and mouse myocilin to standardize antibody use across multiple model organisms in vision research. We achieved our goals by employing antibody engineering methods: we immunized mice with human and mouse myocilin domain constructs, generated phage display libraries from extracted antibodies sequences, and conducted successive bio-panning rounds against relevant myocilin constructs. Follow up validation included testing detection limits in ELISA and Western blots, among other experiments. The development of a suite of antibodies is crucial to facilitating new insight into the biological function of myocilin in the trabecular meshwork and its misfolding in glaucoma, as well as generating novel diagnostic and anti-aggregation therapeutics for myocilin-associated glaucoma.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS088 | Elucidating the mechanism behind glaucoma-associated myocilin aggregation through biophysical characterization of early and late-stage aggregation events

Emily Saccuzzo, Mubark Mebrat, Yuan Gao, Wade Van Horn, Anant Paravastu, Raquel Lieberman
Georgia Institute of Technology (United States)

Glaucoma is a progressive optic neuropathy and the second leading cause of blindness worldwide. Inherited mutations in the gene encoding for the protein myocilin lead to early-onset elevated intraocular pressure (IOP) and account for 2-4% of the ~45 million cases of open angle glaucoma, the most common glaucoma subtype. ~90% of known disease causing variants are found within the C-terminal olfactomedin domain of myocilin (mOLF). Preliminary results from our lab have revealed that glaucoma-causing mOLF mutations are generally associated with a decreased thermal stability as well as the adoption of a non-native tertiary structure. It is proposed that these non-native, destabilized states adopted by mutant mOLF prime the protein for the formation of aggregates, which are subsequently sequestered intracellularly, as opposed to being secreted. This accumulation of mutant mOLF leads to TM cell death and eventual loss of TM tissue homeostasis, causing increased IOP and accelerated timelines for vision loss. Here, I present our progress utilizing nuclear magnetic resonance (NMR) to probe the regions of mOLF most susceptible to dynamics/improper folding as a result of destabilizing mutations. I also explore the structure of both mOLF oligomeric aggregates and end point fibrils using a combination of solid-state NMR (ssNMR) and negative stain transmission electron microscopy (TEM). The cumulation of this work should enhance our understanding of the mechanism behind mOLF aggregation, and pave the way for new glaucoma therapeutic and diagnostic tools.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS089 | Regulation of Cullin 2-RING ubiquitin ligases

Kankan Wang
Purdue University (United States)

Through recruiting interchangeable substrate receptor modules (SRMs) to the cullin (CUL) core, cullin-RING ligases (CRLs) catalyze the ubiquitination and degradation of diverse proteins that play key roles in a myriad of biological processes. As the founding member of CRLs, the CUL1-based CRL1s are known to be activated by both CAND1, which exchanges the SRMs associated with the common CUL1 core, and NEDD8 conjugation (neddylation), which modifies CUL1 and alters CRL1 conformation to promote substate ubiquitination. Similar to CRL1s, CRL2s comprise the CUL2 core that is homologous to CUL1, and SRMs whose composition differ from CRL1s. Because of the structural difference, it is unclear if neddylation and CAND1 regulate CRL1 and CRL2 in

similar fashions. To uncover mechanisms regulating CRL2s, we studied the activity of CRL2(VHL), the E3 ligase well known for targeting HIF1α for ubiquitination and degradation, and for PROteolysis TArgeting Chimeras (PROTACs) induced degradation of disease-causing proteins. We found that neddylation promoted the in vitro ubiquitination of the full-length HIF1α and the degron peptide of HIF1α to similar extents, demonstrating that neddylation activates CRL2(VHL)-dependent ubiquitination regardless of the substrate sizes. In HEK293 cell-based cycloheximide chase assays, inhibiting neddylation failed to stabilize HIF1α, which was due to VHL-independent degradation of full-length HIF1α, but it stabilized the Carboxy-terminal Oxygen-Dependent Degradation (CODD) domain truncation of HIF1α. This finding suggests that the cellular activity of CRL2(VHL) is better reflected by the degradation of CODD than that of HIF1α, and we thus analyzed the degradation of CODD in HEK293 with or without CAND1-knockout (KO). Surprisingly, CODD degraded faster in the KO cells, and furthermore, PROTAC-induced degradation of CRL2(VHL) target proteins also became faster in the KO cells. This finding suggests that unlike CRL1, CAND1 is an inhibitor for CRL2(VHL), and this inhibitory effect influences the efficacy of VHL-based PROTACs.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS091 | STIM2 regulation by calmodulin and RGS10

Karen Ramirez Quintero, Cynthia Tope, Ramona Bieber Urbauer, Shelley Hooks, Jeffrey Urbauer
The University of Georgia (United States)

The overall goal of our research is to decipher the mechanistic details underlying the complex regulation of STIM (Stromal Interaction Molecules) proteins by calmodulin (CaM) and the regulator of G-protein signaling (RGS) protein RGS10. The ER (Endoplasmic Reticulum) membrane-spanning STIM proteins monitor calcium concentration in the ER. In response to decreased calcium concentration in the ER, STIM proteins (STIM1 and STIM2) dimerize and interact with plasma membrane calcium channel proteins of the SOCE (Store-Operated Calcium Entry) system to promote calcium influx into the cell.

Both STIM1 and STIM2 are regulated by the general calcium regulatory protein CaM, and recently it has been shown that the regulator of G-protein signaling protein RGS10 also regulates STIM2. The mechanism of STIM2 regulation by CaM and RGS10 is further complicated by the fact that CaM also binds and regulates RGS10. Our focus is to elucidate the mechanistic details of CaM and RGS10 regulation of STIM2. Towards this end we are producing various constructs of STIM2 domains for studies to measure CaM and RGS10 binding affinities and for structural studies. For instance, we have produced the cytosolic coiled-coil regions 1 and 2 of STIM2 and used the purified protein to measure its affinity for CaM using intrinsic tryptophan fluorescence. Consistent with earlier studies, CaM binding to this region of STIM2 is calcium dependent and high affinity. We are employing isothermal titration calorimetry to assess the thermodynamics of these interactions, and other spectroscopic methods to characterize structural changes. Our results should assist in understanding the complex means by which calcium concentration is regulated in the cell.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS092 | Probing the mechanism of isonitrile formation by a non-heme iron(II)-dependent oxidase/decarboxylase

Antonio Del Rio Flores, David Kastner, Wenjun Zhang
UC Berkeley (United States)

The isonitrile moiety is an electron-rich functionality that decorates various bioactive natural products isolated from diverse kingdoms of life. Isonitrile biosynthesis was restricted for over a decade to isonitrile synthases, a family of enzymes catalyzing a condensation reaction between L-Trp/L-Tyr and ribulose-5-phosphate. The discovery of ScoE, a non-heme iron(II) and α-ketoglutarate-dependent dioxygenase, demonstrated an alternative pathway employed by nature for isonitrile installation. Biochemical, crystallographic, and computational investigations of ScoE have previously been reported, yet the isonitrile formation mechanism remains obscure. In this present work, we employed in vitro biochemistry, chemical synthesis, spectroscopy techniques, and computational simulations that enabled us to propose a plausible molecular mechanism for isonitrile formation. Our findings demonstrate that the ScoE reaction initiates with C5 hydroxylation of (R)-3-((carboxymethyl)amino)butanoic acid (CABA) to generate C5-OH CABA, which undergoes dehydration presumably mediated by Tyr96 to synthesize

imine CABA in a trans configuration. (R)-3-isocyanobutanoic acid (INBA) is finally generated through radical-based decarboxylation of imine CABA, instead of the common hydroxylation pathway employed by this enzyme superfamily.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS093 | Structure and Binding of an Exogenous Siderophore by a Receptor Protein in a Gut Commensal Bacterium

Randika Perera, Brian Ferrer, Wenhan Zhu, Walter Chazin
Departments of Biochemistry and Chemistry, Center for Structural Biology, Vanderbilt University (United States)

Iron is an essential nutrient for bacterial growth, replication, and metabolism. During an infection, humans limit the availability of free iron for pathogenic bacteria through nutritional immunity. However, this drastic action could lead to complete upset of the microbiome. Commensal bacteria provide a means to stabilize the microbiome during activation of the nutritional immunity response and resist colonization of pathogens. Bacteria have multiple mechanisms to obtain iron from the host environment, including high affinity small molecules termed siderophores. Interestingly, the human commensal gut bacterium Bacteroides thetaiotaomicron does not produce siderophores yet it grows best under iron-limiting conditions. This is achieved by utilizing Enterobactin (Ent), a siderophore that is produced by other bacteria in the gut, such as Escherichia coli and Salmonella.

We identified a receptor protein, XusB for Ent in B. thetaiotaomicron, which is upregulated during Salmonella-induced gut inflammation, and are using biophysical and structural approaches to elucidate its mechanism of action. A 2.6 Å crystal structure of XusB shows it is comprised of a 22 strand β-barrel with loops on the extracellular side of the barrel that contains the putative Ent binding site. XusB binds Ent with high affinity (kd 148 nM). Molecular docking results suggest that Ent binds to a positive charge cluster on the extracellular side of the barrel. Small angle X-ray scattering (SAXS) revealed that the structure of BT2064 becomes more compact upon binding of Ent, as well as reducing overall flexibility, consistent with binding of Ent to loops on the extracellular side of the barrel. In addition to progress towards determination of the structures of XusB in the absence and presence of Ent, the results will be discussed in the context of how this microorganism can thrive during iron-limiting conditions.

Track: Protein Phase Separation in Biomolecular Condensates

ABS094 | Purification and Characterization of Black Seeds (Nigella sativa) Proteins by Edman Sequencing and Mass Spectrometry

Basir Syed, Basir Syed, Kadambri Ayithi, Jonathan Boules, Van Do, Minh Nguyen, Aftab Ahmed
Chapman university (United States)

According to the CDC, cancer deaths in the United States dropped 27% in the past 21 years, but still, it is the second leading cause of death. In traditional folk medicine, compounds extracted from various plant components such as fruit, seeds, leaves, flowers, etc., play a crucial role in treating several ailments, including cancer. Therefore, there is a constant interest in developing more effective, targeted, and cost-effective anticancer drugs. Medicinal plants are an enormously rich source of biologically active peptides and proteins. Nigella sativa, a miraculous medicinal herb commonly known as black seed or Kalonji, belongs to the plant family Ranunculaceae. Our group has previously reported the anticancer activity of proteins extracted from its seeds against the MCF7 breast cancer cell line. The present study focuses on applying two-dimensional liquid chromatography for protein purification and employing automated Edman sequencing techniques for the primary structure elucidation of purified proteins. We also used Q-TOF LC/MS/MS, and De Novo protein sequencing bioinformatics tools to evaluate the Black seeds proteome. The proteins were extracted in PBS precipitated in 80% ammonium sulfate. The HiTrap Heparin column was employed to enrich the heparin-binding proteins/peptides from the crude extract and were further purified by the second-dimensional RP-HPLC. The identity of several proteins was established by complementary techniques, including N-terminal amino acid sequencing and mass spectrometry. Our preliminary findings based on the homology search identified several biologically active proteins, including non-specific Lipid Transfer Protein (ns-LTP), Defensin, and Nigellin-1 proteins. Further, the differential mass spectrometric analysis of tryptic digest of heparin bound and unbound fractions revealed exciting information on black seeds proteome. Work is in progress on determining the complete primary structure of these and other biologically active proteins from the black seeds for their anticancer potentials

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS095 | Dissecting ligand binding and temperature sensitivity in the human TRPV1-VSLD

Aerial Owens, Minjoo Kim, Camila Montano, Wade Van Horn
Arizona State University (United States)

TRPV1 is an integral membrane polymodal cation channel that is involved in a variety of essential biological functions including thermosensing, thermoregulation, and nociception. Discrete TRPV1 activation modes such as ligand, heat, and proton have been challenging to disentangle, however, dissecting the polymodal nature of TRPV1 is essential for therapeutic development. The human TRPV1 (hTRPV1) voltage-sensing like domain (VSLD, transmembrane helices S1-S4) contains the canonical vanilloid ligand binding site and has been shown to significantly contribute to thermosensing by undergoing temperature-dependent conformational changes. Here we present a comprehensive NMR-detected hTRPV1-VSLD binding study that investigates diverse agonists and antagonists beyond canonical vanilloids. Modern unsupervised analysis of NMR binding experiments provides an efficient method for dissecting the energetic contributions to binding of distinct molecular features in a library of TRPV1-specific ligands. Additionally, temperature-dependent binding studies show that the allostery between heat activation and ligand regulation can be quantified and dissected. These results indicate that how ligands alter protein dynamics is a crucial parameter to making TRPV1 mode-selective compounds and is an important consideration for future therapeutic design.

Track: Protein Phase Separation in Biomolecular Condensates

ABS096 | Probing sequence space and non-natural amino acids to uncover molecular interactions driving FUS Liquid-Liquid Phase Separation

Noah Wake, Nicolas Fawzi
Brown University (United States)

Liquid-liquid phase separation (LLPS) is widely accepted as a mechanism by which cells compartmentalize biomolecules and perform cellular functions inside and outside of the nucleus. Fused in Sarcoma (FUS) is an essential RNA binding protein that is implicated in neurodegenerative diseases and leukemias through abberant LLPS. In this work, we probe sequence variants that refine our understanding of the roles of individual amino acid types beyond tyrosine and arginine in FUS phase separation. Using novel biochemical methods, we selectively create natural and non-natural amino acid mutations to demonstrate how amino acid interaction details fine tune FUS LLPS. Solution-state NMR is used to demonstrate that non-aromatic amino acids interact within the dispersed phase, and biophysical assays are used to quantify how changing the identity of non-aromatic amino acids impacts the extent of FUS phase separation. We find that all amino acid types contribute to phase separation and provide additional insight into how different side chains contribute to phase separation.

Track: High Throughput Protein Science

ABS097 | Preparing All Crystal Structure Models in the Protein Data Bank for Broad Use

AJ Vincelli, Firas Khatib
UMass Dartmouth (United States)

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 may be improved to leverage the "quality in, quality out" paradigm, saving time and money on projects utilizing these input proteins. First, we used six industry-standard quality metrics to create an aggregate "Quality Score" from 0% (worst) to 100% (best). Then we 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. Electron density data and crystal contacts 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 Quality Scores to an average of 99.4% $\pm$ 0.8% with sub-Angstrom all-heavy-atom RMSDs from their starting structures, and with a reasonable runtime and near-identical reproducibility across architectures

(manuscript in preparation). This algorithm also increased the Quality Scores of previously-prepared structures in the PDB-REDO database, demonstrating synergistic improvements. We are currently Relaxing all crystal structures in the PDB in partnership with Cyrus Biotechnology, and will release the results and method to the public for broad use upon completion. Our Pareto optimal Relax protocol can be used to prepare X-ray structures as high-quality inputs for protein engineering projects, and the prepared database can serve as a high-quality dataset for machine learning and other big data applications.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS098 | Nanobody as a Tool to Investigate Different Biochemical Activities of UCH37

Yanfeng Li, Lin Hui Chang, Jiale Du, Sandor Babik
University of Massachusetts Amherst (United States)

UCHL5/UCH37 is a fundamental deubiquitinase associated with the 26S proteasome. It serves as a positive regulator of protein degradation by debranching K48 linked ubiquitin chains on the substrate. Our lab has previously discovered a non-canonical K48 ubiquitin chain specific binding site (ncS1′) on the opposite face of UCH37 relative to the canonical S1 ubiquitin-binding site (cS1), which is required for chain debranching and proteasome-mediated degradation of proteins modified with branched chains. While the canonical S1 (cS1) site, which is thought to be the principal Ub-binding site, is dispensable for K48-linkage-specific binding. Instead, cS1 is important for N-terminal ubiquitination removal from the substrate. These results suggest that UCH37 uses more than one site to perform different biochemical activities. However, the mechanism of how UCH37 utilizes the two distinct sites to perform different activities is not fully uncovered, nor are the UCH37 substrates that depend on these acitivities. Here, we use directed evolution coupled with yeast surface display to identify nanobodies that selectively bind to the cS1 or ncS1′ site of UCH37, which could potentially block the interaction with ubiquitin and disrupt the corresponding activities. We believe that these nanobodies could be valuable tools to study the mode of functions of UCH37 by decoupling the
activities of UCH37, and help identify substrates that give insights into the biological significances.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS099 | The Pex6 N1 Domain Contributes to Pex1/Pex6/Pex15 Complex Assembly

Ryan Judy, Dominic Castanzo, Andreas Martin, Brooke Gardner
Department of Molecular, Cellular, and Developmental Biology, University of California Santa Barbara (United States)

Post-translational protein targeting to peroxisomes relies on approximately 35 peroxisome biogenesis factors. Among these are the AAA-ATPases Pex1/Pex6, which form a dynamic complex that recycles components of the peroxisomal translocation complex in an essential step for transporting proteins into the peroxisome. Defects in Pex1 and Pex6 are the most common cause of peroxisomal biogenesis disorders, yet despite Pex1/Pex6's essential role in peroxisome biogenesis, the mechanisms by which the complex assembles into a functional motor and interacts with adaptors are poorly understood. Using X-ray crystallography, we determined the structure of the yeast Pex6 N1 domain, a predicted substrate and co-factor binding domain. Guided by cryo-electron microscopy and AlphaFold2 predictions, we performed photo-crosslinking experiments that explain how the Pex6 N1 domain carries out a dual role in Pex1/Pex6/Pex15 complex assembly. The N1 domain of Pex6 interacts with an otherwise unstructured loop from the Pex1 ATPase domain. Structure predictions show that this loop is present across eukaryotes, suggesting a conserved role in Pex1/Pex6 assembly. Additionally, Pex6 N1 binds to Pex1/Pex6's adaptor protein and in vitro substrate, Pex15. These experiments contribute to understanding how active and conformationally dynamic motors can form stable assemblies.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS100 | Protein linked domain inhibitor design and development for Ube2D E2 conjugating enzyme

Zara Bukhari, Li Gu, Anneroos E Nederstigt, Kim Harvey, Joseph S. Harrison, Derek L Bolhuis, Nicholas G. Brown
University of the Pacific (United States)

Ubiquitin is a post-translational modification system that regulates the cellular machinery by targeted degradation

via the three-tiered enzymatic cascade. The nexus of the enzymatic cascade is the E2 conjugating enzyme that directs the conjugation chemistry, ubiquitin chain type, and the type of E3 ligase that has access to the primed ubiquitin. However, little is understood about the promiscuous UBE2D-E2 conjugating enzyme which plays a critical role in the regulation of tumor suppressor protein p53. Further adding to the complexity is the presence of four different isoforms of Ube2D on different chromosomes for humans. To address this, we used a protein engineering approach to design Ube2D specific inhibitors. A family of linked domain inhibitors was created that targets simultaneously the backside binding domain (Ubl) which provides a noncovalent allosteric interaction site and a high degree of sequence divergence, along with the E3 interacting domain. We established that these inhibitors had affinities for Ube2D in the range of 10 -6 M to 10-9 M using ITC,1:1 stoichiometry of binding was orthogonally confirmed by size exclusion chromatography. The specificity of these inhibitors to Ube2D was corroborated by utilizing yeast to hybrid system to test against other E2 conjugating enzymes. We also demonstrated that these inhibitors were active in cell culture and revealed that these inhibitors sensitize the Hela cells to cisplatin thereby indicating the inhibitor applicability in chemotherapies. Therefore, we were able to achieve our overarching goal to equip the Ubiquitin society with a tool to study Ube2D via the novel linked domain inhibitor having nanomolar affinity and specificity towards Ube2D.

Track: Protein & Ligand - A New Marriage Between an Old Couple

ABS101 | How the EGF receptor generates ligand-dependent differential signaling outputs

Yongjian Huang
HHMI and University of California, Berkeley (United States)

In this study, we provided solid evidence for a molecular mechanism by which two related, high-affinity growth factors, binding in exactly the same site, can achieve differential signaling outputs through a dimerized receptor tyrosine kinase.

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that couples the binding of extracellular ligands, such as EGF and transforming growth factor-α (TGF-α), to the initiation of intracellular signaling pathways. EGFR binds to EGF and TGF-α with similar affinity, but generates different signals from these ligands. To address the mechanistic basis of this phenomenon, we have carried out cryo-EM analyses of human EGFR bound to EGF and TGF-α. We show that the extracellular module adopts an ensemble of dimeric conformations when bound to either EGF or TGF-α. The two extreme states of this ensemble represent distinct ligand-bound quaternary structures in which the membrane-proximal tips of the extracellular module are either juxtaposed or separated. EGF and TGF-α differ in their ability to maintain the conformation with the membrane-proximal tips of the extracellular module separated, and this conformation is stabilized preferentially by an oncogenic EGFR mutation. Close proximity of the transmembrane helices at the junction with the extracellular module has been associated previously with increased EGFR activity. Our results show how EGFR can couple the binding of different ligands to differential modulation of this proximity, thereby suggesting a molecular mechanism for the generation of ligand-sensitive differential outputs in this receptor family.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS103 | Dissecting and engineering allosteric activation of ATP phosphoribosyltransferase: link between protein dynamics and active-site electrostatic preorganisation

Marina Corbella Morato, Gemma Fisher, Rafael G. da Silva, Shina Caroline Lynn Kamerlin, Magnus S. Alphey
Uppsala University (Sweden)

ATP phosphoribosyltransferase catalyses the first step of histidine biosynthesis, such reaction is controlled via a complex allosteric mechanism where the regulatory protein HisZ enhances the activity of the catalytic dimer HisGs while also mediates its allosteric inhibition upon histidine binding. Activation by HisZ was proposed to position Arg56 from HisGs to stabilise the transition state and departure of the leaving group, however, active-site mutants of HisGs with impaired reaction chemistry can be allosterically restored upon HisZ binding. Here we use MD simulations to dissect HisZ allosteric activation, indicating that HisZ regulatory protein constrains the dynamics of HisGs to favour a preorganised active site where key residues R56 and R32 are poised to stabilise transition state and leaving-group departure. We further engineered HisGs to mimic allosteric activation in the absence of the regulatory protein HisZ, by proposing HisGs mutations capable of restoring the dynamical communication pathway generated upon HisZ binding.

ATP phosphoribosyltransferase catalyses the first step of histidine biosynthesis, such reaction is controlled via a complex allosteric mechanism where the regulatory protein HisZ enhances the activity of the catalytic dimer HisGs while also mediates its allosteric inhibition upon histidine binding. Activation by HisZ was proposed to position Arg56 from HisGs to stabilise the transition state and departure of the leaving group, however, active-site mutants of HisGs with impaired reaction chemistry can be allosterically restored upon HisZ binding. Here we use MD simulations to dissect HisZ allosteric activation, indicating that HisZ regulatory protein constrains the dynamics of HisGs to favour a preorganised active site where key residues R56 and R32 are poised to stabilise transition state and leaving-group departure. We further engineered HisGs to mimic allosteric activation in the absence of the regulatory protein HisZ, by proposing HisGs mutations capable of restoring the dynamical communication pathway generated upon HisZ binding.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS105 | How Do Enzymes Impart Catalytic Superpowers to Amino Acids?

Suhasini Iyengar, Kelly Barnsley, Rholee Xu, Aleksandr Prystupa, Mary Jo Ondrechen
Northeastern University (United States)

Enzymes catalyze reactions under mild conditions that would otherwise require extreme conditions such as high temperature or strong acidity or basicity. To achieve this, enzymes often impart reactive chemical properties to amino acid sidechains that are far less reactive in the absence of the protein environment. In the enzymatic environment, active site amino acid sidechains that are weak Brønsted acids or bases in a small peptide can transform into a strong acid or base. The primary amine side chain of lysine, which would normally be protonated at neutral pH, can be deprotonated to serve as a nucleophile. We present a set of 20 enzymes that represent all six major EC classes and a variety of fold types for which experimental studies of the catalytic residues' mechanistic roles have been reported in the literature. For these 20 enzymes the computed electrostatic and proton transfer properties are investigated. The catalytic aspartates and glutamates are shown to be strongly coupled to at least one other aspartate or glutamate residue, and frequently to multiple other carboxylate residues, with intrinsic pKa differences less than ~1 pH unit. These catalytic acidic residues are sometimes coupled to a histidine, wherein the intrinsic pKa of the acid is higher than that of the His. Anion-forming residues, Tyr or Cys, with intrinsic pKa higher than that of the lysine, are found strongly coupled to all catalytic lysines in the set. Some catalytic lysines are also coupled to other lysines with intrinsic pKas within ~1 pH unit. Some basic principles about the design of enzyme active sites are discussed. The interactions described here provide important clues about how side chain functional groups that are weak Brønsted acids or bases for the free amino acid can become strong acids, bases, or nucleophiles in the enzymatic environment. Supported by NSF CHE-1905214.

Track: Machine Learning in Protein Science

ABS106 | De Novo Design of Site-Specific Protein Binders Using Surface Fingerprints

Alexandra Van Hall-Beauvais, Pablo Gainza, Sarah Wehrle, Andreas Scheck, Anthony Marchand, Zander Harteveld, Casper Goverde, Dongchun Ni, Tan Shuguang, Priscilla Turelli, Didier Trono, Henning Stahlberg, Geroge Gao, Michael Bronstein, Bruno Correia
EPFL (Switzerland)

Protein-protein interactions (PPIs) play crucial roles in most cellular functions. The ability to disrupt or create a PPI offers many potential applications for the development of therapeutics and sensors, among others. However, de novo design of PPIs is a difficult challenge, despite advancing design methods. A broadly applicable design pipeline with high success rate would be invaluable for numerous applications.

MaSIF (Molecular Surface Interaction Fingerprinting) software has been developed by our lab to identify complementary molecular surfaces, using learned fingerprints, and design de novo binders using only a given target structure. This method has been applied to two disease-relevant targets, PD-L1 and the SARS-CoV-2 spike protein. MaSIF identified putative binding fragments to each target, which were then transferred to protein scaffolds, giving the fragments stability and the potential for additional contacts to the target.

PD-L1 is an important cancer immunotherapy target. Using MaSIF, we designed two binders that engage the flat surface of PD-L1 with high affinity. Crystallography of the complexes showed interface RMSDs of 1.1 Å compared to the MaSIF-predicted structures.

The SARS-CoV-2 virus infects cells by receptor binding domain (RBD) binding to ACE2 on the human cells. We designed a binder to RBD that overlaps with the ACE2

site, thereby blocking infection. After in vitro evolution, the binder has nanomolar affinity for various spike proteins (including WT and omicron) and effectively neutralizes omicron SARS-CoV-2 in live virus assays. A cryo-EM structure was obtained which verified that the binder was binding to the predicted site.
Overall, we show a highly useful method for designing de novo binders for therapeutically relevant targets. The binders are specific to the sites predicted using MaSIF and disrupt the native binding interactions.

Track: Machine Learning in Protein Science

ABS108 | De Novo Design of Viral Epitope Mimetics with Non-Regular Structural Features

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Proteins are central to numerous fundamental biological functions. Beyond proteins found in nature, advancements in understanding protein folding and structure-function relationships has made it possible to design novel protein sequences that adopt defined three-dimensional structures. Using computational tools, protein designers can construct proteins whose structural and biophysical properties have not been identified in nature. However, producing functional de novo proteins remains a challenge. One notable application of protein design is development of vaccine immunogens. Several pathogens evade traditional vaccine strategies, including respiratory syncytial virus (RSV). Previous work in our lab generated computationally designed immunogens which focus the immune response to antigenic sites capable of generating neutralizing antibodies against RSV. A recently characterized epitope of RSV, site-V, displays an uncommon curved ß-sheet fold for which previous methods proved unsuitable. Here, we successfully utilized two unique de novo design approaches to present the site-V epitope. The first approach used Rosetta design and prioritized control of torsional irregularities vital for curved ß-sheet formation to promote folding and maintain appropriate presentation of the epitope. The second approach employed a recently described deep learning design method generating "hallucinated" protein scaffolds. Both methods generated thermodynamically stable proteins that show specific binding to a site-V monoclonal antibody. Optimized computational sequence design and experimental affinity maturation is ongoing. The immunogens will be used in immunization studies to assess their ability to elicit neutralizing antibodies. Overall, we propose de novo scaffolds for engaging a structurally complex viral epitope which was not previous amendable to established design methods.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS109 | Making non-available enzyme available: Chemical-assisted refolding of formate dehydrogenase from Candida boidinii

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NADH-dependent formate dehydrogenase from Candida boidinii (CbFDH) has been reported as the most stable for carbon recycling and is at the center of intense research, due to the "green" advantages the bioconversion can offer. However, production cost and stability issues may preclude its use in laboratories and industry. Efforts have been made to produce cbFDH in E coli, however only 20-50% FDH is obtained in the soluble form. It is, therefore, necessary to develop a refolding process to obtain active cbFDH from the inclusion bodies (IBs) thereby increasing its availability for various biotechnological applications including high throughput structural biology projects.
In this study, we have developed a systematic refolding screen to evaluate the impact of pH, buffer system, time, redox couples as well as a range of additives on the cbFDH refolding process. We compared both rapid and slow dilution methods of refolding. The refolding screen comprised sucrose, glycerol, arginine, GSH: GSSG, and ZnCl2. For slow dilution, the multichannel pump was used to deliver solubilized IBs (sIBs) into the refolding buffer in a controlled manner by adjusting the flow rate of addition. The refolding efficiency and the quality of the refolded protein were analyzed by functional enzymatic assay, CD spectroscopy, and tryptophan fluorescence. These screening experiments showed that the slow addition of sIBs at a flow rate of 5ul/min into the refolding buffer of pH 7.5 containing glycerol and arginine improved the refolding yield by 46%. Our results confirmed the importance of glycerol and arginine in improving the refolding yield. This study provided a rapid and inexpensive method of recovering insoluble cbFDH which is otherwise ignored when overexpressed in E coli, thus increasing its availability for research in protein science and engineering.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS110 | Biochemical Properties of Novel 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 species that aggregate 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 model of non-eukaryotic IAPs for in vitro molecular studies. A recent bioinformatic study uncovered over 1000 putative IAP sequences in archaeal and bacterial genomes. Here, we report recombinant expression, purification, solution characterization by small angle neutron scattering (SANS), and enzymatic activity of selected new IAPs. These new IAPs express well and are from noted archaea such as Haloferax 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.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS111 | Dynamics, ensembles, and solvent interactions: the complex energy landscape of chorismate mutase allostery

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Saccharomyces cerevisiae chorismate mutase (ScCM) is a key homodimeric enzyme in the shikimate pathway responsible for the generation of aromatic amino acids, where it is allosterically inhibited and activated by Tyr and Trp, respectively. X-ray crystallography experiments previously characterized ScCM bound with two Tyr (ScCM:YY) and two Trp (ScCM:WW) ligands. We used NMR relaxation and calorimetry methods to further explore the free energy landscape and conformational dynamics of ScCM in the absence and presence of Tyr and Trp. NMR chemical exchange saturation transfer (CEST) experiments and isothermal titration calorimetry (ITC) experiments further revealed that ScCM populates states in which only one allosteric ligand is bound (i.e. ScCM:Y and ScCM:W) or both allosteric ligands are bound (i.e. ScCM:YW). These states were previously uncharacterized but make substantial contributions to the function of ScCM under physiologically relevant concentrations of Tyr/Trp. NMR relaxation dispersion methods further demonstrated that binding of Tyr and Trp abrogate and activate motions on the microsecond-to-millisecond timescale, respectively. Conformational dynamics on the picosecond-to-nanosecond timescale were also investigated through determination of methyl-axis order parameters. Upon binding either Tyr or Trp, ScCM experiences a quenching of motions on this timescale, which we related to a loss of protein conformational entropy. Further ITC and NMR studies were consistent with the Tyr-bound form of ScCM being associated with more water molecules compared to the Trp-bound form and Tyr binding being associated with a less positive solvent entropy change. More recent experiments have suggested site-specific chemical shift changes in the presence of organic co-solvents, potentially pointing towards specific protein-solvent interactions that might differentially affect ScCM function in the presence of allosteric ligands. Our studies highlight the complex dynamic behavior of ScCM and provide new tantalizing insights into how protein-water interactions may modulate enzyme function and allosteric regulation.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS112 | Structural Dynamics of Orexin 2 Receptor and G protein Complex using Molecular Dynamics Simulations

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Meiji University (Japan)

Orexin2 receptor (OX2R) is classified as a class A G-protein-coupled receptor (GPCR) and belongs to the group of orexinergic systems that are composed of two peptide

ligands (i.e., orexin-A and -B) and two GPCRs (i.e., OX1R and OX2R). The OX2R is involved in the regulation of feeding behavior and sleep-wake rhythm to give some examples. Such kinds of neurological processes are caused by GPCR activation. Through the activation of GPCRs by ligand binding, GPCRs change their conformation and then downstream signal transduction is carried out via a trimeric protein called G-protein that consists of three subunits (Gα, Gβ, and Gγ) and via G-protein-independent pathways through GPCR kinase-mediated phosphorylation and arrestin coupling. Although the structure of the OX2R has been clarified by structural analysis, the atomic-level mechanisms of GPCR activation, G-protein activation unknown. Here, we performed and analyzed several microsecond-scale molecular dynamics (MD) simulations of OX2R using AMBER MD software. We performed not only simulations of OX2R, but also performed simulations of the complex of OX2R and G-protein. In this poster, we first show the results of the MD simulations and investigate the dynamics of OX2R and G-protein complex. Then, we discuss implications for the activation mechanism of OX2R and for the binding mechanism of G-protein.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS113 | Structural basis for the action of heparan sulfate and other periodic polyanions as ubiquitous amyloid agonists

Emily Lewkowicz, Olga Gursky
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Amyloidoses, such as Alzheimer's disease or inflammation-linked amyloid-A (AA) amyloidosis, are incurable diseases in which normally soluble proteins or peptides deposit as insoluble amyloid fibrils in various organs. These diseases affect ∼500,000,000 patients worldwide but have limited treatment options. A better mechanistic understanding of amyloid fibril formation and stabilization is needed in order to make advances in the therapeutic targeting of amyloidoses. Besides proteins, ex vivo amyloid deposits contain other components such heparan sulfate (HS) proteoglycans. Notably, HS has been shown to promote amyloid formation for various unrelated proteins both in vivo and in vitro, and is known as an obligatory cofactor for tau fibril deposition. Our goal is to provide the structural basis for understanding why HS and other polyanions act as amyloid agonists.

To date, all high-resolution structures of protein amyloid fibrils reveal a common structural motif of stacks of parallel in-register cross-β-sheets that produce periodic arrays of identical residues approximately 4.7 Å apart. Charged arrays present in amyloid fibrils are energetically unfavorable unless compensated by a complementary periodic array. We propose that HS, a polyanionic array with ∼5Å charge-charge separation, binds to positively-charged basic arrays to nucleate and stabilize amyloid fibril formation.

To test this, we used flexible docking of a heparin tetrasaccharide to high resolution ex vivo amyloid fibril structures from both neurodegenerative and systemic amyloidoses. The results reveal that negatively-charged sulfate and carboxylate groups of heparin coordinate positively-charged arrays of basic residues in amyloid fibrils via charge-charge interactions. We have identified many potential heparin/HS binding sites in this docking study that correspond to extra densities in high resolution cryoEM maps. Ongoing molecular dynamics simulations of the human SAA fibril with heparin show charge-mediated heparin-fibril interactions. This binding mode provides insight into the essential role of cofactors in promoting the nucleation and growth of amyloid fibrils.

Track: Miniproteins: Are There Really That Many Additional Genes That We Have Been Missing?

ABS114 | Is it a mistake in the genome sequencing or a spare part protein?

Mary Andorfer, Lindsey Backman
MIT (United States)

Proteins of 127-amino acids can act as ‘spare part proteins’ to restore activity to oxygen-damaged glycyl radical enzymes (GREs). GREs are abundant in the human microbiota, catalyzing reactions involved in primary and secondary metabolism. When oxygen damages a GRE, the enzyme is literally cut in two at the site of the glycyl radical species. Facultative anaerobes have evolved a fascinating solution: a repair protein, YfiD, can bind to the cleaved enzyme (pyruvate formate lyase (PFL) in the case of YfiD) and restore activity. In this presentation, we will consider the mechanism of repair using structural, biochemical and biophysical methods. We will probe the basis by which YfiD identifies a PFL that need repair and how it distinguishes between PFLs that are too heavily damaged for repair and ones that can be saved. We also consider whether other GREs have spare part proteins or if PFL is unique: is that short stretch of DNA a

sequencing mistake (merely a GRE fragment) or a spare part protein?

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS115 | In Vitro Characterization of the Intra-Melanosomal Domain of Human Recombinant Tyrp1 Protein and its OCA3-Related Mutant Variants

Monika Dolinska, Yuri Sergeev
NIH/NEI (United States)

Tyrosinase Related Protein 1 (Tyrp1) is the most abundant melanosomal protein of the melanocyte and is necessary to synthesize the black/brown eumelanin, catalyzing the oxidation of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) to 5,6-quinone-2-carboxylic acid (IQCA) in the melanin pathway. Mutations to the TYRP1 gene can result in oculocutaneous albinism type 3 (OCA3), a rare autosomal recessive disorder characterized by reduced synthesis of melanin pigment in skin, hair, and eyes with some visual function abnormalities. To investigate the effect of genetic mutations on the human Tyrp1 structure, function, and stability we expressed and purified the intra-melanosomal domain of Tyrp1 (residues 25-537), and its mutant variants mimicking OCA3-related changes, C30R, R87G, H215Y, D308N, and R326H. Proteins produced in the whole insect Trichoplusia. Ni (T. ni) larvae were purified by immobilized metal affinity chromatography (IMAC) followed by size-exclusion chromatography (SEC), and analyzed by enzymatic, biochemical, and biophysical methods. Our in vitro studies have shown that the D308N and R326H variants keep the conformations close to the native. The mutations, which are located on the periphery of the protein, do not directly affect the protein stability and its enzymatic activity. In contrast, C30R and R87G variants are unstable and aggregate during the purification process indicating the protein misfolding. This suggests an essential role for the Cys-rich domain affected by these mutations. H215Y variant, which disrupts the binding of Zn2+ in an active site shows on a gel a decreased band of the properly folded protein. The disruption may result in allosteric alterations to the active site, reducing the stability of the enzyme/substrate interactions. Our results, consistent with the clinical and in silico studies, can help to understand the mechanism of protein malfunction due to different mutations, and their link to the phenotype of the disease, and will aid in finding a potential treatment for OCA3 patients.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS116 | Identification and Crystallization of Clathrate Binding Proteins Found Through Metagenomic Analysis of Deep Sea Sediments

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Clathrates are crystalline ice cages that contain gas molecules, and some, including methane clathrate, are capable of supporting life. We hypothesize that clathrate-dwelling organisms produce clathrate binding proteins (CBPs) to enable survival in such a harsh environment in a manner analogous to organisms that make antifreeze proteins (AFPs) to survive in freezing conditions. Through the metagenomic analysis of deep sea sediments found off of the coasts of Oregon and Japan, genes harboring an amino acid motif (TxxxAxxxAxx) found in Type I AFPs were identified as potential CBPs. The corresponding proteins were then expressed and purified and five hypothetical clathrate-binding proteins were produced. Four of these proteins (CbpA2,3,5,6) were shown to bind to and change the morphology of THF clathrate, a model system for gas clathrates. Excitingly, the CbpAs also inhibited the growth of the more biologically relevant methane clathrate. A fragment of CbpA3 active against methane clathrate was then crystallized, and the structure was solved using X-ray crystallography. The protein structure provides a starting point to tease apart the mechanism of activity of this new class of proteins and will give insight into the survival strategies of clathrate-dwelling organisms. In addition, CbpAs can be utilized as green gas clathrate inhibitors, as gas clathrate inhibition is a billion dollar industry.

Track: High Throughput Protein Science

ABS117 | Molecular Recognition of Pathological Tau Fibril Conformations

Emma Carroll, Kelly Montgomery, Taia Wu, Jason Gestwicki
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Tau is an intrinsically disordered protein that binds microtubules in healthy neurons but forms pathological aggregates (fibrils) that drive neurodegenerative diseases

known as tauopathies. Structures from patient brain tissues revealed that fibrils adopt unique tauopathy-dependent conformations that differ from those of heparin-induced recombinant tau fibrils in vitro. An understanding of the cellular factors driving conformation-specific fibrillization is hampered by a lack of tools for determining fibril conformation and an incomplete understanding of molecular crosstalk with tau posttranslational modifications. Here, we utilize fibrils induced with diverse polyanions to identify recombinant tau/polyanion combinations that may generate distinct, inducer-specific fibril forms. We use this conformationally diverse fibril library to develop tools for molecular recognition of pathological tau fibril conformations and explore how posttranslational modifications alter the fibrillization process. We envision that this toolkit can be used for systematic characterization of how a cellular factor of interest modulates tau's conformational landscape to induce disease-associated fibril conformations.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS118 | BT-Benzo-29 Binds to The Catalytic Site of FtsZ from Streptococcus pneumoniae to Exhibit Its Anti-FtsZ And Consequently, Anti-Pneumococcal Action

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The master protein of cell division, FtsZ, polymerizes to form a central Z-ring that leads to cytokinesis in bacteria. Targeting bacterial cell division by small molecule inhibitors of FtsZ is a potential strategy for anti-bacterial therapy. BT-benzo-29, a benzimidazole derivative, inhibits the proliferation of the model gram-positive bacteria, Bacillus subtilis by targeting FtsZ. In this study, we have explored the potential of BT-benzo-29 against a clinically relevant respiratory pathogen, Streptococcus pneumoniae. BT-benzo-29 displays highly potent anti-proliferative activity (minimum inhibitory concentration, MIC of 80 nM) against S. pneumoniae, comparable to commercial antibiotics like erythromycin (MIC = 90 nM) and penicillin (MIC = 20 nM). Several lines of evidence from tryptophan fluorescence studies, electron microscopy, and GTPase activity measurement suggest that BT-benzo-29 inhibits the polymerization of FtsZ from S. pneumoniae (SpnFtsZ). We demonstrate that BT-benzo-29 inhibits SpnFtsZ assembly by delaying the nucleation phase, preventing bundling, and reducing the GTPase activity. Further, the binding of BT-benzo-29 to SpnFtsZ was explored using molecular docking complemented with fluorescence spectroscopic studies. Docking results showed BT-benzo-29 binding at the domain interface of N- and C-termini of SpnFtsZ, near the T7 loop. When BT-benzo-29 binds to SpnFtsZ, the fluorescence of SpnFtsZ reduces. SpnFtsZ contains two tryptophan (W294 and W378) residues in its sequence. W294 is present in close proximity to the T7 loop of the protein, a region undergoing conformational change to allow cooperative polymer formation. Using mutants of SpnFtsZ bearing one tryptophan each, we observed that the fluorescence of only W294 reduces upon BT-benzo-29 binding, while W378 fluorescence remains unchanged. The reduction of tryptophan fluorescence indicates a change in the environment of this region, leading to hindrance of monomer-monomer association. Together these results suggest that BT-benzo-29 binds at the catalytic site of FtsZ, inhibits its polymerization, and displays strong anti-pneumococcal action.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS119 | Probing of Caspase-6 Structure, Inhibition, and Dynamics for the Development of Neurodegeneration Therapeutics

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Cysteine aspartate proteases, caspases, are a family of apoptotic and inflammatory human proteases implicated in a variety of diseases. Specifically, increased expression and activity of caspase-6 in human neurons has been associated with common neurodegenerative disorders including Alzheimer's Disease (AD) and Huntington's Disease (HD). In AD, caspase-6 cleavage of microtubule-associated proteins tau and tubulin can lead to axonal degeneration and subsequently the observed cognitive decline. The role of caspase-6 in these degenerative pathways, as well as its minimal importance in homeostatic functioning, suggest it may be an optimal drug target. In our research, we delve into structural aspects of caspase-6 that may render it susceptible to selective inhibition over other caspases. Previous structural biology investigation as well as high-throughput screening coupled with structure-activity relationship optimization have uncovered unique structural interconversions and nanomolar caspase-6 inhibitors. Here, I expand upon our understanding of caspase-6 structure and regulation utilizing

NMR to gain mechanistic insight. Using modern isotopic-labeling strategies we can further elucidate the 130's helix-strand interconversion associated with substrate's binding to the proteolytic active-site. Understanding the manipulation of the enzyme into its active or inactive conformations may provide one route of selective inhibition (Fig. 1). Complementarily, the sensitive detection afforded by NMR can determine the structural orientation of our previously identified inhibitor within its allosteric pocket. Collectively these endeavors contribute to our growing understanding of the biochemical role of caspase-6 in AD pathophysiology and progress toward development of treatments for neurodegeneration.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS120 | The Expression, Purification, and Site-Directed Spin-Labeling of KCNE4

Alison Bates, Indra Sahu, Carole Dabney-Smith, Gary Lorigan
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Accessory subunits belonging to the KCNE family modulate voltage-gated potassium (Kv) channels such as Kv1.1, Kv1.3, Kv2.1, and Kv7.1. Incorrect modulation of potassium current through these channels have been linked to several diseases for example, long QT syndrome, allergic rhinitis, and acute lymphoblastic leukemia. Increased understanding of the molecular underpinnings of KCNE modulation of potassium channels would help expand our understanding of the etiology of these diseases. KCNE4 is unique in the KCNE family as it has been shown to inhibit current in Kv channels. Previously, the role of KCNE4's tetraleucine motif has been tied to its inhibitory effects. In this study, we describe a new expression and purification protocol for KCNE4 to enhance our ability to obtain structural and dynamic information using electron paramagnetic resonance (EPR) spectroscopy. Site-directed mutagenesis was used to attach a nitroxide spin-label at residues of interest. This technique in combination with line-shape analysis of continuous-wave EPR spectroscopy (CW-EPR) was used to observe changes in spin-label side-chain mobility as KCNE4 interacts with different membrane mimetic systems. As the spin label on the protein encounters the membrane, the line-shape of CW-EPR spectra broaden indicating a decrease in mobility of the spin label. The expression, purification, and site-directed spin-labeling of KCNE4 developed in this study will be used in the future to determine the role of the tetraleucine motif in protein structure and function to elucidate modulation of Kv channels in native and disease states.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS121 | Development of a Genetic Code Expansion System 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. Therefore, it is important to study this pathogens proteome and characterize new drug targets. Genetic code expansion uses a biorthogonal amino acid tRNA synthetase/tRNA pair to site-specifically incorporate a non-canonical amino acid into a protein at an amber stop codon. This technique would broaden researchers' abilities to study the proteome of C. glabrata, however, genetic code expansion has never been implemented in the species. We are working to demonstrate proof of concept for genetic code expansion in C. glabrata by incorporating p-benzoylphenylalanine (pBpa) into superfolder GFP (sfGFP) using an E. coli tyrosyl-tRNA synthetase/tRNA pair (EcTyrRS/tRNA-CUA). Incorporation of pBpa by the EcTyrRS/tRNA-CUA pair is quantified by expression of full-length sfGFP when position 149 is mutated to the amber stop codon. Successful development of this method will expand the tools available to investigate C. glabrata's proteome, and future studies in our lab will use pBpa to photocrosslink interacting proteins and map protein-protein interactions in their native environment.

Track: Protein Science Addressing Health Disparities

ABS122 | Identification of the TRPV1 ion channel heat sensor and insights into polymodal function

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Arizona State University (United States)

The ability to sense and respond to environmental temperature is among the most fundamental traits in biology. The transient receptor potential vanilloid 1 (TRPV1) ion channel functions as the canonical heat sensor in

mammals. In addition to temperature activation, TRPV1 is a polymodal receptor activated by low pH (protons) and chemicals, like capsaicin, the pungent vanilloid compound from spicey peppers. More than 50 clinical trials have evaluated TRPV1 antagonists for efficacy in various types of pain. A vexing issue for TRPV1 therapies has been on-target adverse events in themosensing and thermoregulation. One significant challenge has been to identify the regions of TRPV1 that directly sense heat, as opposed to allosterically regulating temperature-sensing. Informed by bioinformatics and known TRPV1 isoforms, we have generated a minimally functional TRPV1 construct. Recombinant expression and purification of the minimal construct (TRPV1-mc) show that it is tetrameric by electron microscopy and retains the key functional attributes of TRPV1 polymodal activation by heat, protons, and ligands by single-channel planar bilayer electrophysiology. The polymodal function of TRPV1-mc is validated in cells by a new method we recently co-developed, whereby purified TRPV1-mc protein is delivered to mammalian cells, and subjected to whole-cell patch-clamp electrophysiology. Building on these results, we further dissect the TRPV1 transmembrane region into evolutionarily conserved domains to identify and quantify TRPV1 thermosensitivity by solution NMR and other biophysical methods. These studies identify the core thermosensing regions of TRPV1 and quantify their respective contributions to heat activation. Additionally, these studies help to understand the polymodal function of TRPV1, which is anticipated to be of therapeutic relevance.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS123 | Characterizing the regulation of carrier domain conformational dynamics in pyruvate carboxylase

Amanda Laseke, Martin St. Maurice, Josh Hakala
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Pyruvate carboxylase (PC) is a central metabolic enzyme that produces oxaloacetate from pyruvate and bicarbonate in an ATP-dependent manner. The catalytic activity of PC contributes to glucose homeostasis and has been implicated in enhanced tumorigenesis and metastasis in certain cancers. PC is a swinging-domain enzyme, requiring a large carrier domain translocation between distantly located catalytic domains to facilitate the transfer of the reaction intermediate between the two active sites. We are studying the movement and allosteric control of this dynamic carrier domain with the goal of providing new insights into carrier domain enzyme function. Although carrier domain translocation in PC has traditionally been assumed to follow an induced-fit mechanism, biophysical observations demonstrate that the enzyme exhibits conformational flexibility in the absence of ligands and, thus, follows a conformational selection model. Additionally, our kinetic studies have revealed a wide range of catalytically productive carrier domain motions, where substrates and allosteric ligands shift the equilibrium positioning of the carrier domain. To further study this positional equilibrium, mutations were generated to facilitate crosslink-based trapping of the carrier domain in specific conformations. The rate of crosslinking, which is a function of carrier domain positioning, can be determined in the presence of various substrates by monitoring changes in intrinsic tryptophan fluorescence. We have used this system to investigate mutations in PC with altered catalytic profiles, yielding insights into how changes in carrier domain positional equilibrium correspond to changes in catalytic turnover. This approach contributes to a more detailed kinetic description of the carrier domain translocation and regulation in PC by directly observing dynamic conformational changes in the absence of catalytic turnover.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS124 | Polyethylene glycols and protein stability

Claire Stewart, Gil Olgenblum, Daniel Harries, Gary Pielak
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Concentrated solutions of synthetic polymers are often used to stabilize proteins, but there are few informed models that explain the consequences of polymer-induced crowding. Effects arise from two types of crowder-protein interactions: hard-core repulsions and soft interactions. Simple hard-core repulsions are only stabilizing, while soft (aka chemical) interactions can be, stabilizing (if repulsive) or destabilizing (if attractive). Several crowding models are based on spherical, colloid-based solution theories. These models are athermal and only account for steric effects, ignoring soft interactions. We investigated the temperature dependence of protein stability in polyethylene glycols (PEGs) as a function of polymer size and concentration. We used the 7-kDa metastable N-terminal SH3 domain of Drosophila signal transduction protein drk (SH3) as the test protein. SH3

was fluorine labeled at its single tryptophan enabling observation of the folded and unfolded states using 19F nuclear magnetic resonance spectroscopy. The temperature dependence of the free energy of unfolding ($\Delta Gu^{\circ\prime}$), as well as the entropy ($\Delta Su^{\circ\prime}$) and enthalpy ($\Delta Hu^{\circ\prime}$), were used to establish a new, informed crowding model that accounts for the temperature-dependent soft interaction effects. This more nuanced understanding of macromolecular crowding will lead to better predictions of how polymers stabilize protein-based drugs and how more biological relevant molecules impact proteins in the crowded cellular environment. This work is supported by the National Science Foundation (MCB-1909664, to G.J.P.), the United States-Israel Binational Science Foundation (BSF 2017063, to G.J.P. and Daniel Harries).

Track: Protein Phase Separation in Biomolecular Condensates

ABS125 | Protein-Protein Interactions of KCNQ1 (100-370) and KCNE1 Observed Via SDSL-EPR Line Shape Analysis

Rebecca Stowe, Gunjan Dixit, Alison Bates, Indra Sahu, Lauryn Cook, Carole Dabney-Smith, Gary Lorigan
Miami University (United States)

KCNQ1, also known as Kv7.1, is a voltage gated potassium channel that associates with the KCNE protein family. Mutations in this protein has been found to cause a variety of diseases including Long QT syndrome, a type of cardiac arrythmia where the QT interval observed on an electrocardiogram is longer than normal. This condition is often aggravated during strenuous exercise and can cause fainting spells or sudden death. KCNE1 is an ancillary protein that interacts with KCNQ1 in the membrane at varying molar ratios. This interaction allows for the flow of potassium ions to be modulated to facilitate repolarization of the heart. The interaction between these two proteins has been studied previously with methods such as cysteine crosslinking. Electron paramagnetic resonance (EPR) spectroscopy line shape analysis in tandem with site directed spin labeling (SDSL) was used to observe changes in label mobility as KCNE1 interacts with KCNQ1. The line shape of a spin label EPR spectrum broadens as the mobility of the spin label decreases, so as the spin labeled residue comes into contact with the membrane bilayer and other proteins the mobility decreases, and the line shape broadens. KCNE1 was labeled at different sites that were found to interact with KCNQ1 based on previous literature, along with sites outside of that range as a control. Once labeled KCNE1 was incorporated into 3:1 PC:PG vesicles, KCNQ1 (helices S1-S6) was titrated into the vesicles. Any differences in line shape once KCNQ1 is added is due to interactions between the labeled site on KCNE1 and KCNQ1.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS126 | Modeling and mutational evidence that the human papillomavirus L2 capsid protein contains a conserved -sheet essential for infectivity

Patrick Buckley, Changin Oh, Justin Chang, Bayan Galal, Daniel DiMaio
Yale University (United States)

Human Papillomavirus (HPV) causes approximately 5% of human cancer worldwide. Despite the existence of highly efficacious prophylactic vaccines, HPV remains a serious public health challenge due to low vaccine uptake. HPVs are non-enveloped viruses with double-stranded DNA encapsidated by 360 copies of L1 major capsid protein and up to 72 copies of L2 minor capsid protein. Previous work showed that the L2 protein penetrates through the endosomal membrane during virus entry, allowing essential interactions with cellular proteins in the cytoplasm and proper trafficking of the virus to the nucleus. The structure of L2 in solution or in the HPV capsid remains undetermined. We predicted the structure of the L2 protein with the latest versions of AlphaFold and RoseTTAFold, open-source modeling programs based on neural networks. Modeling predicts that most of L2 is unstructured, but that it contains a central anti-parallel β-sheet conserved in highly divergent HPV types. We then designed and tested HPV16 pseudoviruses containing mutations in the predicted β-sheet region. Mutations predicted to disrupt the β-sheet led to a dramatic reduction in infectivity, whereas mutations predicted to preserve this structure were tolerated. These data support the predictive power of computational models of L2 and suggest the presence of a previously unknown structured region in L2 that is conserved across a diverse range of papillomaviruses and required for infection. Our results also demonstrate the utility of AlphaFold and RoseTTAFold in the analysis of proteins that are resistant to traditional methods of structural characterization.

Track: Protein Science Addressing Health Disparities

ABS127 | The Study of the Orientation of Gramicidin A in Both Diblock and Triblock PMOXA-PDMC Copolymers Using EPR Spectroscopy

Emma Gordon, Emma Gordon, Gary Lorigan, Joel Fried
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Gramicidin A is a hydrophobic pentadecapeptide antibiotic produced by Bacillus brevis. This membrane protein, consisting of alternating L- and D- amino acids, forms an ion channel that is selective for small univalent cations. The orientation of this peptide and its head-to-head dimer formation are of special interest. This can be studied via synthetic polymer membranes and EPR spectroscopy. Diblock and triblock copolymers are used as mimetic systems in place of lipid bilayers. CW-EPR spectroscopy coupled with site directed spin labeling (SDSL) can provide important structural information that is traditionally difficult to obtain in systems such as biological membranes. Evidence of Gramicidin A head-to-head dimer formation has been found in both the liposome and the polymersome mimetic membranes using EPR. Moving forward, this technique can be used to study membrane proteins in hybrid vesicles.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS128 | Topological Examination of the Bacteriophage Lambda S Holin by EPR Spectroscopy

Andrew Morris, Rehani Perera, Indra Sahu, Gary Lorigan
Miami University (United States)

The S protein from bacteriophage lambda is a three-helix transmembrane protein produced by the prophage which accumulates in the host membrane during late gene expression. It is responsible for the first step in lysing the host cell at the end of the viral life cycle by multimerizing together to form large pores which permeabilize the host membrane to allow the escape of virions. Several previous studies have established a model for the assembly of holin into functional holes and the manner in which they pack together, but it is still not fully understood how the very rapid transition from monomer or dimer to multimeric pore occurs with such precise timing once the requisite threshold is reached. Here, site-directed spin labeling with a nitroxide label at introduced cysteine residues is used to corroborate existing topological data from a crosslinking study of the multimerized holin by EPR spectroscopy. CW-EPR spectral lineshape analysis and power saturation data are consistent with a three-helix topology with an unstructured C-terminal domain, as well as at least one interface on transmembrane domain 1 which is exposed to the lumen of the hole, and a highly constrained steric environment suggestive of a tight helical packing interface at transmembrane domain 2. These preliminary results are largely in agreement with the established model and open a path for detail EPR structural studies of canonical holin system.

Track: Protein Science Addressing Health Disparities

ABS129 | Targeting the Interaction of Huntingtin Oligomers with Lipid Membranes as a Therapeutic Strategy

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Numerous systemic and neurodegenerative disorders, including Alzheimer's disease (AD), Huntington's disease (HD), and Parkinson's disease (PD), are characterized by protein aggregation. While a hallmark of disease-associated protein aggregation is the formation of in β-sheet-rich fibrils termed amyloid, a variety of globular oligomers may represent the most potent toxic species. In addition, many of these proteins and their aggregate forms directly interact with and damage lipid membranes. With regard to HD, an expansion of a polyglutamine (polyQ) domain within the huntingtin (htt) protein beyond a critical threshold triggers the aggregation process. The first 17 N-terminal amino acids that directly precede the polyQ domain facilitates the formation of oligomers and functions as a lipid-binding domain. Here, the role of the aggregation state of a mutant htt-exon1 fragment on its ability to bind and damage lipid membranes was investigated. Based on colorimetric lipid binding assays and in situ atomic force microscopy, htt oligomers were the most membrane active species, followed by monomers and then fibrils. While the ability of htt monomers to bind lipid membranes was heavily influenced by electrostatic interactions, conformational flexibility played a key role in the htt oligomer/membrane interaction. Stabilizing htt oligomers via chemical crosslinking abolished their ability to bind membranes, resulting in reduced cellular toxicity. Based on this, a screen to discover small molecules that block the ability of htt to

bind membranes was performed. Two lead compounds were identified, and these compounds reduce htt-induced toxicity in a C. elegans model of HD.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS130 | Spectroscopic Studies of the Envelope Protein of SARS-CoV-2 in a reconstituted native lipid environment

Andrew Morris, Andrew Morris, Gary Lorigan
Miami University (United States)

The envelope protein from SARS-CoV-2 (E) is a single pass transmembrane protein which accumulates in the endoplasmic reticulum-golgi intermediate compartment in host cells, where it acts as a viroporin and an ion channel which plays a critical role in viral budding and assembly. A recent study produced a structure of the pentameric transmembrane domain (TMD), but no structural information is available for the entire protein, and the conformation and dynamics of the C-terminal domain (CTD), which is presumed to be a soluble region, is unknown. Here we present a preliminary investigation into the full length of the protein in a native lipid environment utilizing solid state NMR to examine lipid dynamics and power saturation EPR spectroscopy with site specific introduction of nitroxyl spin-labels as well as Double Electron-Electron Resonance EPR (DEER-EPR) to examine topology of the protein with respect to the lipid bilayer. These studies are the first examination of the E protein from SARS-CoV-2 in its full length, as well as the first to examine the organization of the CTD.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS131 | Lipid Vesicle Size Affects Formation of Styrene-Maleic Acid Lipid Particles (SMALPs)

Yazmyne Richardson, Emma Gordon, Laurence Haddad, Indra Sahu, Gary Lorigan
Miami University (United States)

Membrane proteins account for about 30% of the human genome but are underrepresented in the protein database. X-Ray crystallography is the dominant structural determination method for proteins, and it is difficult to crystallize membrane proteins in native lipid-like environments using detergents. Styrene Maleic-Acid (SMA) copolymers provide a detergent-free membrane mimetic system that facilitates the formation of disc shaped SMA lipid nanoparticles (SMALPs). SMALPs are proving to be effective at solubilizing a wide range of membrane proteins without loss of stability or lengthy optimization. Here we study how lipid vesicle size affects the formation of SMALPs. Lipid vesicle samples were extruded to different sizes using an extruder apparatus. These samples were titrated with an SMA solution leading to a homogeneous SMALP system 20 nm in diameter. The resulting SMALPs were characterized using a combination of dynamic light scattering (DLS), transmission electron microscopy (TEM). DLS showed that smaller lipid vesicle samples formed homogeneous SMALPs systems at lower lipid to SMA weight ratios. TEM provides a visualization of the lipid vesicles before and after SMALP formation.

Track: Protein Phase Separation in Biomolecular Condensates

ABS132 | Gelation of a Tardigrade Desiccation-Tolerance Protein

Jonathan Eicher, Julia Brom, Shikun Wang, Sergei Sheiko, Joanna Atkins, Gary Pielak
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Protein-based pharmaceuticals are increasingly important, but their inherent instability requires a 'cold chain', which entails costly refrigeration during production, shipment, and storage. Drying can overcome this problem, but most proteins require the addition of stabilizers, and some can never be successfully formulated. Thus, there is a need for new, more effective protective molecules. Cytosolically abundant heat soluble proteins from tardigrades are a promising source of inspiration because these disordered, monodisperse polymers form hydrogels whose structure protects client proteins during drying. We used Fourier transform infrared spectroscopy, differential scanning calorimetry, and small-amplitude oscillatory shear rheometry to characterize gelation. A 5% (v/v) gel has a strength comparable to human skin, and melts cooperatively and reversibly near body temperature with an enthalpy comparable to globular proteins. We suggest that the dilute protein forms α-helical coiled coils, and increasing the concentration drives gel formation via intermolecular β-sheet formation.

This work was supported by NIH grant R01GM127291 (GJP) and NSF grants DMR 1921835 and DMR 2004048 (SSS).

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS133 | Indibulin Binds to Tubulin at the Colchicine Site and Displays Low Affinity Towards -III Tubulin Isotype, A Possible Reason for its Reduced Neurotoxicity

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Indibulin is a microtubule-depolymerizing agent with excellent tumor-suppressing activity and low neurotoxicity. Indibulin exhibited less sensitivity towards differentiated neuronal cells as it failed to depolymerize the microtubules in these cells. To further elaborate on this, we have explored the binding characteristics of indibulin and tubulin and investigated indibulin's differential activity in neuronal cells. Indibulin bound to tubulin with a dissociation constant of 3.3 ± 1.7 μM. Molecular docking analysis revealed that indibulin accommodates itself in the colchicine-binding site on the αβ-tubulin heterodimer. This was further confirmed in an in vitro competitive assay, where indibulin competed with colchicine and significantly inhibited the colchicine-tubulin interaction suggesting that indibulin is a colchicine-site binder. Next, we investigated the effect of indibulin in neuroblastoma cells. Indibulin was highly neurotoxic towards undifferentiated cells, whereas it was ineffective in differentiated cells. Differentiated neuronal cells express higher levels of β-III-tubulin isotype than undifferentiated cells. Taking this into consideration, we modeled the 3D structures of four β-tubulin isotypes, β-I, β-II, β-III, and β-IV, using homology modeling and docked indibulin. Surprisingly, both indibulin and colchicine bound to β-III-tubulin isotype with the lowest affinity. The binding energy of indibulin for β-III-tubulin was increased to -9.39 kcal/mol from the average -10.6 kcal/mol for other isotypes, indicating lowered affinity. To correlate this, the efficacy of indibulin in β-III-depleted differentiated cells was determined. Indibulin significantly reduced the viability of β-III-depleted differentiated cells however was ineffective in β-II-depleted differentiated cells. Colchicine, too, exhibited increased neurotoxicity in β-III-depleted differentiated cells. In contrast, vinblastine did not show any preference for different isotypes, suggesting that only colchicine-site binders are sensitive to the β-III-tubulin levels in cells. Together, our data provide an insight into the possible role of β-III-tubulin isotype in modulating the neurotoxicity induced by colchicine-site binders.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS134 | Targeting PfUCHL3 Using Chemically Constrained Peptides

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University of Cambridge (United Kingdom)

PfUCHL3 is a highly conserved, dual specificity deubiquitinating and deNeddylating enzyme acting within the ubiquitin proteasome system (UPS) of Plasmodium falciparum, the deadliest and most prominent Plasmodium species responsible for the transmission of malaria to humans. Malaria kills on average 450,000 people per year across 90 different countries, and there is at present no broadly effective vaccine against the disease. PfUCHL3 is essential to P. falciparum cell viability, and through the use of the RaPiD peptide discovery system (Yamagishi Y et al, 2011), 10 novel, non-natural, cyclic peptides were selected for against PfUCHL3. The peptides all displayed tight dissociation constants for PfUCHL3 in the low nanomolar range (6-35 nM) and 4 of the 10 peptides were shown to inhibit the activity of PfUCHL3 in cleaving its ubiquitin substrate in vitro. The peptides were all shown to have selectivity for PfUCHL3 over HsUCHL3, the human homologue of the enzyme, and through NMR analysis we found that all 4 of the inhibitory peptides bind to the substrate recognition region of PfUCHL3. Thus, the peptides act by interfering with ubiquitin's ability to bind to PfUCHL3 and represent the first reported inhibitory molecules for PfUCHL3. Work is currently underway to determine the cell permeability properties of the peptides and their ability to kill plasmodium parasites in vivo with the long-term aim of taking these peptides forward for the generation of a novel class of anti-malarial therapeutics.

(Yamagishi Y et al, 2011) - Yamagishi Y, Shoji I, Miyagawa S, Kawakami T, Katoh T, Goto Y, Suga H. Natural product-like macrocyclic N-methyl-peptide inhibitors against a ubiquitin ligase uncovered from a ribosome-expressed de novo library. Chem Biol. 2011 Dec 23;18(12):1562-70. doi: 10.1016/j.chembiol.2011.09.013. PMID: 22195558.

Track: Protein Science Addressing Health Disparities

ABS135 | Elucidating the interaction of Hsc70 with the SNARE protein, SNAP-25

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The complex mechanism of synaptic vesicle fusion with the plasma membrane and neurotransmitter release is initiated with the formation of the SNARE complex at the presynaptic terminal. The SNARE complex is composed of four helices contributed by three proteins: one helix from syntaxin (anchored in the plasma membrane), one helix from synaptobrevin (anchored in the synaptic vesicle membrane), and two from SNAP-25 (one N-terminal and one C-terminal, with the protein anchored in the plasma membrane by palmitoylation of a linker between these). SNAP-25 is an intrinsically disordered protein that displays a propensity to aggregate in vitro. Synaptic vesicle fusion is tightly regulated and requires the constitutively expressed Hsp70 chaperone (Hsc70) and its co-chaperones CSPα (J protein) and Hsp110 (nucleotide-exchange factor). However, the role of the Hsc70 system in SNARE complex formation remains unknown. We hypothesize that Hsc70 and CSPα cooperate to chaperone SNAP-25 to keep it in a state that is competent for SNARE complex formation, and this interaction helps to bring the vesicular and plasma membranes into proximity for exocytosis. To test these hypotheses, we are employing an in vitro bottom-up approach to study the interaction between Hsc70 and CSPα with SNAP-25. Using a peptide array spanning the sequence of SNAP-25, we identified three potential Hsc70-interacting sequences and designed peptides containing these sequences to test their binding to Hsc70 in solution. We are characterizing the interaction of SNAP-25 peptides with Hsc70 and CSPα using a combination of biochemical and biophysical techniques, such as binding assays by fluorescence anisotropy, activation of Hsc70 ATPase activity, chemical crosslinking, hydrogen-deuterium exchange mass spectrometry, and nuclear magnetic resonance. Preliminary peptide binding data suggest that the Hsc70-binding sites identified in the array are indeed good Hsc70 binders in solution, with relative affinities consistent with the intensities of Hsc70 interactions observed in the array.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS136 | The conformational heterogeneity of the SARS-COV-2 reveal molecular insights into viral fitness and immune system evasion

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Since the onset of the SARS-COV-2 pandemic, many biochemical structures of its spike protein, the protein associated with virus-receptor binding and cell entry, were experimentally determined using cryo-electron microscopy. Such studies reported two main conformations of the spike protein: a closed conformation with all receptor binding domains in the down position or an open conformation where at least one receptor binding domain is in the up position. The majority of such structures showed anisotropic resolution with less resolution accuracy in the N-terminal domain (NTD) and receptor binding domains (RBD) which contain diverse mutations that increase the immune evasion potential of these variants of concern. Our microsecond simulations of full atomic structures that obtained from a deep learning approach, RosettaFold reveal NTD exhibits two sub-conformations in the closed state and three other sub-conformations in the open state. Every sub-conformation exhibits a different combination of N-terminal domain positions relative to the receptor binding domains and only one of the closed sub-conformations exhibits true 3-fold rotational symmetry. Our findings could pave the road towards a fixed conformation of the spike protein, which could lead to better immunization and the development of a more potent vaccine.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS137 | Structural Characterization of Selective and Potent Allosteric Inhibitors of Caspase-6 for Neurodegenerative Disease Treatment

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The cysteine aspartate protease, caspase-6 has been shown to be involved in several neurodegenerative processes such Alzheimer's (AD) and Huntington's Diseases (HD). Overactivity 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 (Figure 1). 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. Preliminary analysis of x-ray data from co-crystals of caspase-6 and KT-60 shows extra density around Cys 264, and model building and refinement 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.

Track: High Throughput Protein Science

ABS138 | Reconstructing the mammalian brain protein interactome using high-throughput proteomics

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Understanding the complexity of the mammalian brain has been a long-standing challenge, not least because of the many specialized proteins and assemblies required for proper neuron function and connectivity. In particular, protein-protein interactions (PPIs) are critical to the proper functioning of neural proteins, and neurons from different mammals share many stable macromolecular protein complexes that perform conserved cellular functions. However, such data are only partially known due to the challenge of defining PPIs at high throughput directly from mammalian brains. In this project, we determined neuronal PPIs and "protein neighborhoods" at large scale by applying a tag-less proteomics technique called co-fractionation/mass spectrometry (CF-MS) to primary brain tissues of several mammals, including humans. CF-MS involves non-denaturing extraction of endogenous proteins in their native assembly states, followed by chromatographic separation into biochemical fractions, then mass spectrometry to define co-eluting proteins. Given sufficient independent separations and the appropriate statistical framework, stable protein complexes can be reliably identified based on protein co-elution patterns. We will describe our progress in combining this integrative proteomic approach with available brain PPI data and information from sequence orthology across mammals in order to define a large-scale map of conserved protein complexes in the mammalian brain.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS139 | Characterizing Tubulin Interactions with the Proline Rich Region of Tau

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Tau is a microtubule-associated protein that is generally thought to function in regulating microtubule stability and dynamics. Normally, tau plays an important role in modulating axonal microtubules in neurons, where it is highly expressed. Intracellular tau aggregates are found in a broad class of diseases, including Alzheimer's Disease, termed tauopathies. As an intrinsically disordered protein, tau lacks a stable secondary and tertiary structure, and this disorder is maintained even when binding to soluble tubulin and microtubules. Multiple tau-tubulin binding sites have also been identified, spanning the proline-rich region (PRR), microtubule binding repeats (MTBR: R1–R4), and pseudo-repeat, R′. Although dozens of post-translational modifications have been proposed or identified on tau, it is phosphorylation, and specifically hyperphosphorylation of tau that is correlated with disease and alterations to microtubule binding. Intriguingly, potential phosphorylation sites also cluster with high frequency within the PRR. Our lab has recently identified that the isolated PRR is capable of both binding to tubulin as well as having tubulin polymerization capacity. Here, we use fluorescence correlation spectroscopy (FCS) in conjunction with bulk polymerization measurements

to characterize the impact of phosphomimic mutations in the PRR on tubulin binding and polymerization. We find that single phosphomimics affect tubulin binding nominally, with T231E having the most significant impact. However, as phosphorylation events rarely occur in isolation on tau, we also tested combinatorial effects. We found that multiple phosphomimics alter microtubule polymerization. Furthermore, we used mass spectrometry to delineate the regions on both PRR and tubulin responsible for these interactions. Together these measurements provide insight into previously overlooked relevance of tau's PRR in functional interactions with tubulin.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS140 | An integrative approach reveals the structure of elusive ciliary protein complexes and transient interactions.

Caitlyn McCafferty, Ophelia Papoulas, Gabriel Hoogerbrugge, David Taylor, John Wallingford, Edward Marcotte
University of Texas (United States)

As cryo-electron microscopy continues to push the molecular resolution boundaries of purified, single particle samples, its promise for examining more complex mixtures and in situ samples becomes within reach, but not without their own challenges. Specifically, cryo-electron tomography (cryo-ET) has become a powerful tool in illuminating the structure and function of biological complexes in cellular context, however elusive complexes and complexes with transient interactions are still difficult to capture. The integration of cryo-ET with complementary techniques can help to address some of these challenges. In cilia, intraflagellar transport is the passage of ciliary proteins from the basal body to the tip of the cilia and is mediated by the IFT-A and IFT-B complexes and is notoriously difficult to study due to its transient nature. We combine cryo-ET with shotgun-EM, in-situ chemical crosslinking, and integrative modeling to assemble the IFT-A complex and its association with the IFT-B complex. This approach has uncovered a number of IFT cargos from cation channels to proteins essential in cell division. In doing so, we discovered a novel protein complex with an uncharacterized domain structure. We use our combination of techniques to assemble the near-native structure of this complex, enabling us to uncover its function and explain its divergent ancestry.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS141 | Using Crystallographic Ensemble Refinement for Computational Design of Highly Active De Novo Enzymes

Behnoush Seifinoferest, Rojo Rakotoharisoa, Joshua Rodriguez, Niayesh Zarifi, Michael Thompson, Roberto Chica
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The creation of artificial enzymes is an example of the vast applications of protein design; however, most in-silico designed de-novo enzymes have low activity. Directed evolution is known as a common method for increasing the catalytic activity of these new enzymes. Although directed evolution has proven efficient for increasing the catalytic activity of de-novo enzymes, it may be beneficial to avoid extensive and costly laboratory work and have a mostly computational method for designing high-activity enzymes. Previous studies (Broom et al. 2020) suggested that enzymes with catalytic rates similar to natural enzymes can be designed via computational enzyme design with input from X-ray crystallography, with no need for further modification by directed evolution. More precisely, Broom et al. suggested refining the conformational ensemble of the low-activity design against room-temperature x-ray diffraction data, followed by an in-silico ensemble-based enzyme design as a method for increasing enzyme activity. Here, we have tested this idea, using the computationally-designed KEMP Eliminase HG3. Starting from the conformational ensemble of HG3, determined by crystallographic ensemble refinement, we performed a second round of ensemble-based computational design to further rigidify the enzyme active site and stabilize the transition state. These designed mutants were first filtered by kinetic calculations and MD simulations, and then laboratory-tested for their activity. Several of these designs demonstrated a more than 100-fold increase in catalytic activity relative to the original HG3 sequence. X-ray crystallography on three of the most active designs revealed good agreement between the computational designs and the crystal structures and demonstrated a correlation between active site structure and enzyme activity, consistent with Broom, et al. These results validate our new strategy for rigidifying the active site and increasing enzyme activity using a combined approach that combines crystallography and computational design, circumventing the need for extra lab work and directed evolution.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS142 | Probing Functional Protein Methyl-Side Chain Dynamics on the Sub-Microsecond Time Scale by Nanoparticle-Assisted NMR Spin Relaxation (NASR)

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The conformational dynamics of proteins are critical for performing their biological activities. Nuclear magnetic resonance (NMR) spectroscopy in solution is powerful in quantifying the protein dynamics and flexibility over a broad range of time scales. However, limited experiments are sensitive to internal motions on the intermediate nanosecond to microsecond (ns-μs) time-scale range. We recently developed the nanoparticle-assisted NMR spin relaxation (NASR) method that solves this long-standing challenge. NASR utilizes the transient and non-specific interactions between folded proteins and slowly tumbling spherical nanoparticles (NPs). It is sensitive to motions up to the overall tumbling correlation time of the NPs, typically in the range of μs. Here, we extended the NASR method to study methyl-side chain dynamics. We measured both 2H-relaxation and 13C-relaxation on isotopically labeled colicin E7 immunity protein (Im7) and human ubiquitin in the presence and absence of anionic silica-nanoparticles. We found that the dynamics of methyl-side chains in ubiquitin are predominantly sampled on the sub-ns time scale. In contrast, Im7 exhibits additional dynamics on the intermediate ns-μs time scale in the first loop, which forms the binding interface with its cognate colicin, consistent with the backbone NASR results. The method allows quantitative characterization of the amplitudes of methyl-side chain internal motions on the sub-μs time scale.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS143 | The Molecular Basis for the Synergistic Aggregation of Tau by -Synuclein

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University of Pennsylvania Chemistry Dept (United States)

Age-related neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease (PD) take an overwhelming toll on individuals and society. Traditionally, misfolding and aggregation of a single protein (tau in AD and α-synuclein, or αS, in PD) was thought to be responsible for causing these different pathologies. However, there is increasing evidence that the pathologies of these two diseases overlap, and the individual proteins primarily associated with each disease promote each other's aggregation. Both tau and αS are intrinsically disordered proteins (IDPs), lacking stable secondary structures under physiological conditions. Here, we complement ensemble aggregation studies with Fluorescence Correlation Spectroscopy (FCS) to investigate the interactions between tau and αS. Specifically, we measure interactions between full-length tau and constructs corresponding to its proline rich region (PRR) and microtubule binding domain (K18) with monomeric and aggregated forms of αS. We find only weak interactions between any of the tau variants and monomeric αS. Binding to αS aggregates, however, is enhanced relative to monomer, particularly by the PRR. The presence of the C-terminal tail of αS enhances this interaction. Interestingly, neither full-length nor truncated αS seeding has a significant impact on tau aggregation under the conditions of our experiments. The findings of our study draw attention to a central role of the PRR in mediating protein-protein interactions and underscores the complexity of the homotypic and heterotypic interactions underlying pathological protein aggregation.

Track: High Throughput Protein Science

ABS144 | Towards a Comprehensive Receptor Interactome for the Human Immune System

Jarrod Shilts, Yannik Severin, Francis Galaway, Nicole Müller-Sienerth, Zheng-Shan Chong, Sophie Pritchard, Sarah Teichmann, Roser Vento-Tormo, Berend Snijder, Gavin Wright
Wellcome Sanger Institute (United Kingdom)

In this study, we report the results of a large-scale initiative to systematically discover, quantify, and integrate the interactions between cell-surface proteins on human immune cells. These surface proteins include receptors that are fundamental to driving immune responses and are the basis of immunotherapy treatments. Deciphering the full set of interactions that these surface receptors participate in is therefore essential for developing new therapies and resolving how immune cells communicate with one another.

We began by developing a high-throughput method to rapidly screen protein-protein interactions using purified

recombinant human cell surface proteins. As many cell surface interactions are highly transient, special consideration was taken to make the method sensitive enough to detect even very weak interactions. This approach allowed us to test hundreds of thousands of potential interactions with a fraction of the resources required by similar prior methods.

Each interaction discovered from our screening was individually validated by orthogonal techniques and had its binding kinetics measured, resulting in a unique high-confidence and quantitative view of the receptor contacts between immune cells. We could integrate this "interactome" with proteomic and single-cell transcriptomic data to investigate principles of surface protein function, as well as mathematically model leukocyte adhesion. Finally we employed a recently-developed multiplex immune cell imaging method to directly test the therapeutic potential of purified recombinant surface proteins identified from our screening. This work has revealed promising new pathways for targeting immune cells with protein-based therapeutics, provides useful new tools and techniques for the protein science community, and advances our understanding of basic receptor biology.

Track: Celebrating 100 Antibody Drugs

ABS145 | DARPins as SARS-cov2 therapeutics

Vikas Chonira
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Despite the transforming power against a plethora of diseases, antibodies suffer an important limitation as therapeutics for global pandemics such as COVID. Antibody production requires sophisticated mammalian cell culture and is consequently expensive and antibody supply is limited by global manufacturing capacity. As an alternative, we report the engineering of an ultra-potent and broadly neutralizing synthetic protein – FSR16m – as a nasally delivered therapeutic for treating SARS-CoV-2 infection. FSR16m is a trimeric fusion protein composed of DARPin SR16m and T4 foldon. The IC50s of FSR16m against the authentic beta B.1.351 and delta B.1.617.2 variants were 1.76 ng/mL (47 pM) and 2.33 ng/mL (30 pM), respectively, on par with the Regeneron antibody cocktail. Affinity studies confirmed that FSR16m can efficiently bind a panel of 21 receptor-binding domain (RBD) mutants, including many that are resistant to the antibody therapeutics currently authorized for emergency usage. Intranasally administered FSR16m effectively protected mice infected with the authentic delta B.1.617.2 variant resulting in significantly reduced weight loss and 10-100-fold reduction in viral RNA load in lungs, hearts and nasal washes. The strong neutralization potency, combined with its broad neutralization spectrum, high expression yield (>100 mg per liter of E. coli culture in shaker flasks) and storage stability (<10-fold activity loss after 1 month's storage at room temperature) render FSR16m a promising candidate as intranasally delivered therapeutic for treating and preventing SARS-CoV-2 infection.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS146 | Engineering and Design of Minimal Metalloproteinase Inhibitor Scaffolds

Maryam Raeeszadeh-Sarmazdeh, Linh Do
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Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases with key roles in extracellular matrix degradation and remodeling. MMP overexpression is long known for its contribution to several diseases such as cancer, neurological disorders, and cardiovascular diseases, making MMPs great targets for developing novel therapeutics. Tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors of MMPs that bind through several flexible interacting loops. TIMPs, a family of four in human, have great similarities in sequence and structure. However, TIMPs have a wide spectrum of binding affinity and selectivity to different MMPs.

Employing yeast surface display and DNA shuffling techniques among the TIMP family, we generated a scrambled TIMP (ScTIMP) library to identify the dominant minimal MMP inhibitory domain and fragments among TIMPs. We first analyzed the levels of TIMP expression and MMP binding of yeast displayed full-length and the inhibitory N-terminal domain of human TIMPs using flow cytometry. Full length TIMPs and N-TIMP proteins showed a spectrum of yeast display and MMP-3 catalytic domain (MMP-3 cd). Additionally, we screened our ScTIMP library for protein expression and MMP-3cd binding via fluorescent-activated cell sorting (FACS). Interestingly, several of the ScTIMP fragments as short as 20 amino acids were isolated after three rounds of FACS screening, with maintained or improved binding to MMP-3cd (up to 4-fold). We also sought to determine the inhibition profile of these isolated clones towards soluble MMP-3cd. Our study allowed us to explore the TIMP motifs that are involved in binding to MMP. We expect this study will shed light on understanding of molecular mechanism of TIMP-MMP binding and inhibition to develop the next class of MMP inhibitors with more flexibility, and tissue-penetration.

Track: High Throughput Protein Science

ABS148 | Rescuing from the DEAD: Analysis of the DEAD-Box Motif in a Polymerase Clamp Loader

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Recent advances in high-throughput DNA synthesis and sequencing provide an unprecedented opportunity to use deep mutagenesis to leverage structural information into functional insights. We have been applying high-throughput mutagenesis to DNA polymerase sliding clamp loaders. Clamp loaders are pentameric AAA+ ATPase that rely on ATP hydrolysis-guided movement between subunits to load ring-shaped proteins, clamps, around DNA in preparation for DNA replication. The clamp loader contains a highly conserved DExx motif, known as the DEAD box in certain ATPase clades, near each ATP-binding site. The function of the final D residue of the DEAD-box motif is unknown but is speculated to be involved in substrate-coupled hydrolysis. Despite the need for highly coordinated movements for clamp loader function, we have found that the clamp loader is remarkably tolerant to point mutagenesis. The presented work builds off this discovery by probing the clamp loader's ability to repair damage to a speculated hub of these coordinated events via deep mutagenesis. Through X-ray crystallography, Cryo-EM and biochemical assessment, we find evidence for the substrate-sensing and catalytic roles of the final DEAD box aspartic acid residue. Finally, deep mutagenesis reveals many sites of repair, most of which are located far away from the nucleotide binding site. Most interestingly, nearly all repair mutations exist in regions of the protein that are highly tolerant to mutagenesis and accept many different chemical identities towards recovering function. Such results point towards potential modes of evolutionary selection against deleterious mutations, as well as elucidate defined structural states of clamp loader function.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS149 | Understanding the Differences of Danusertibs Residence Time in Aurora Kinases A/B: Dissociation Paths and Key Residues Identified Using Conventional and Enhanced Molecular Dynamics Simulations.

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The unbinding of drugs from their molecular targets can take several minutes to hours, and it is measured by the dissociation rate constant (koff) and, therefore, the residence time (RT=1/koff). In order to study this event in a computational time scale and describe it at molecular level, it is necessary to use computational techniques such as molecular dynamics (MD) simulations and MD advanced techniques for sampling conformational space. Well-Tempered Metadynamics (WT-MetaD) is an enhanced sampling method that has been used in different studies to explore the drug's unbinding pathways and measure the change in the free energy associated with the dissociation process. In this work, it is described the dissociation process, through MD and WT-MetaD simulations, of the drug Danusertib, which is a potent type I inhibitor of Aurora A and B kinases that presents similar Kd(M) values but different RT (s) values against these kinases, without a clear molecular and energetic explanation so far. First, several MD replicas for Danusertib-AuroraA/B complexes were performed and it is reported their molecular and energetic stability. Then, WT-MetaD simulations were applied to describe the dissociation paths of the drug and its computational RTs for the unbinding process in both systems. Several measurements of Danusertib's relative RT (in nanoseconds) and their statistical significance, are reported for Aurora A and B, which are in agreement with experimental data. Additionally, it is highlighted the key and differential amino acids that can contribute to the drug's RT during the dissociation process from Aurora A and B. Tentative dissociation paths along the dissociation routes were also depicted.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS150 | The Effect of Interdomain Interactions on the Structure and Stability of the Eleventh Type 3 Domain from Human Fibronectin

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Fibronectin is an extracellular matrix glycoprotein that plays a major role in cell adhesion, cell growth and tissue organization. Fibronectin matrix assembly involves the formation of insoluble fibrils through noncovalent

interactions between fibronectin dimers; however, the mechanism of fibril formation has not been completely elucidated. Fibronectin contains several fibronectin type 3 (FN3) domains, which have a β-sandwich structure composed of three-stranded (β-strands A, B and E) and four-stranded (β-strands C, D, F and G) β-sheets. FN3 domains are susceptible to mechanical unfolding. It has been hypothesized that their unfolding may lead to exposure of cryptic binding sites during fibril formation. The 11th FN3 domain (11FN3) from human fibronectin binds to anastellin, a fibronectin fragment known to induce fibril formation in vitro. The position of β-strand G in isolated 11FN3 (PDB ID 5DFT) deviated from the position expected based on sequence alignment with other FN3 domains with known structures. To determine the effect of flanking residues and neighboring FN3 domains on the structure and stability of 11FN3, X-ray crystallography, nuclear magnetic resonance spectroscopy (NMR) and denaturant titration experiments were performed on various protein constructs containing 11FN3. We found that β-strand G of 11FN3 in the multi-domain constructs is consistent with the expected position based on sequence alignment. It is shifted by one residue as compared to β-strand G in isolated 11FN3. Multiple conformations were observed for the multi-domain constructs containing 11FN3 by NMR. In addition, changes in the sequence in the C-terminal portion of 11FN3 due to alternative splicing resulted in dramatic changes in the 1H-15N HSQC NMR spectra and free energy of unfolding. The extra domain A (EDA) at the C-terminus of 11FN3, which is only present due to alternative splicing, also affected its stability. These results suggest that flanking residues and domains affect the structure and stability of 11FN3.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS151 | Computational Design of an NADPH-dependent Sulfite Reductase to use a Noncanonical Redox Cofactor

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Cells use redox-active nicotinamide cofactors to transfer electrons between diverse oxidoreductases, with some cells using these cofactors to couple hundreds of reactions in parallel. The complexity of these natural metabolic networks makes it challenging to redirect metabolic flux to synthetic pathways without disrupting electron flow to native oxidoreductases. To overcome this challenge, redox enzymes can be engineered to use alternative cofactors that enable orthogonal electron transfer within a closed metabolic circuit. Recent studies have leveraged computational approaches, such as RosettaDesign, to rapidly engineer redox enzymes with altered cofactor specificity. Here, we apply Rosetta design tools to engineer the E. coli assimilatory sulfite reductase to utilize the noncanonical cofactor nicotinamide mononucleotide (NMN+) by computationally redesigning the cofactor binding site and then screening designs based on sulfur production in vitro. To enable utilization of NMN+, we first disrupted an allosteric specificity gate conserved in the flavoprotein oxidoreductase family, enabling broader cofactor specificity. We then used the Rosetta CoupledMoves protocol to redesign the native NADPH binding interface, removing interactions with the native NADPH cofactor while simultaneously predicting mutations that form new interactions with NMN+. Finally, we designed C-terminal extensions to form additional interactions with NMN+ and further increase binding affinity. Ongoing efforts are examining the cofactor specificity profiles of these designed proteins. The design principles revealed by our effort should be applicable to other nicotinamide derivatives as well as the broader family of flavoprotein oxidoreductases, including commercially valuable cytochrome reductases and photosynthetic redox enzymes.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS152 | Synthetic mutation acts as global suppressor in TEM -lactamase

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Protein evolution is constrained by thermodynamic stability, but the molecular basis for how mutations that change stability impact fitness is unclear. We have demonstrated that a prevalent stabilizing mutation in TEM β-lactamase, M182T, increases fitness by reducing proteolysis in vivo. We also show that a synthetic mutation, M182S, can similarly act as a global suppressor, and its absence from natural populations is likely due to genetic inaccessibility rather than differences in the protein's structure or activity. First, we measured the stabilities of TEM variants containing G238S, M182T, and M182S substitutions or a combination (G238S/M182T and G238S/M182S) and found that G238S is destabilizing and M182S restores stability in a similar manner to M182T. To determine whether changes in stability have an impact on cellular contexts, we performed quantitative immunoblotting

experiments to measure the respective abundances of the soluble, functional form and the insoluble, aggregated form of TEM. It was observed that both M182T and M182S increase TEM abundance for both soluble and insoluble protein and that this increase occurs to similar extents in the wild-type andG238S backgrounds. Interestingly, differences in abundance can only be partially explained by differences in stability—whether thermodynamic or kinetic. Next, we determined whether variants containing M182T or M182S stabilizing mutations offered fitness advantages by measuring the minimum inhibitory concentrations (MIC's) of BP and CFX and performing head-to-head competition assays. The fitness data reveal that M182T and M182S interact with G238S to increase resistance, and changes in fitness correlate well with in vitro activities of the variants against benzylpenicillin (BP) and cefotaxime (CFX).

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS154 | A Knowledge-Based Model of Circular Dichroism for Proteins

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We present a model of far-UV circular dichroism (CD) for proteins, which is based on the DeVoe classical theory of optical activity. The main input of the model is a set of atomic complex polarizabilities that are determined from the analysis of a series of synchrotron radiation CD spectra together with their corresponding 3D structures from the Protein Data Bank. The results of this knowledge-based model of circular dichroism (KCD) compare favorably with the predictions of other models. Among them, only the KCD model is able to describe the effect of D-amino acids. Under
favorable conditions, our model can be used to evaluate reconstructions of incomplete protein structures as well as assess structural changes.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS155 | Impact of the Endoplasmic Reticulum Membrane Protein Complex (EMC) on the Mutational Tolerance of Rhodopsin

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The proteostasis network features a spectrum of partially redundant chaperones and insertases that assist nascent membrane proteins (MPs) as they establish their orientation within the membrane (topology) and form their native structure. However, it remains unclear how preferential chaperone interactions are specified by the molecular features of MP substrates and how mutations can remodel these interactions. Recent investigations of the endoplasmic reticulum membrane protein complex (EMC) suggest it facilitates the cotranslational membrane insertion of the first transmembrane domain (TMD) of certain GPCRs and that it can function as a holdase for misassembled TMDs in polytopic MPs. To explore the nature of these interactions, we have employed deep mutational scanning to compare the plasma membrane expression of ∼1,200 rhodopsin variants in HEK293T cells and in ΔEMC6 HEK293T cells. Consistent with emerging perspectives, trends within TMD1 suggest EMC dependence is sensitive to changes in solvation energetics. However, we also find that EMC impacts the expression of numerous TMD 2 & 7 variants. A statistical analysis shows the depletion of EMC enhances the expression of temperature- and retinal-sensitive variants that disrupt a native helical kink in TMD2 or perturb the topological energetics of TMD7. Together, these findings reveal how EMC engages this substrate and how this relates to its impact on mutational tolerance.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS156 | Title: Investigating the Rap1A-Raf kinase Interaction

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Activation of Raf kinase is the critical first step in the Mitogen-Activated Protein Kinase (MAPK) signaling pathway which leads to cell survival and proliferation. This depends on the small GTPase Ras binding to the

Ras-binding domain (RBD) and cysteine-rich domain (CRD) of Raf to activate the kinase. The related protein, Rap1A can also activate the MAPK pathway under certain conditions. Ras and Rap1 share ~50% sequence conservation, including nearly identical Switch I regions that bind the RBD. Sequence deviations between the two GTPases occur at the CRD binding site and at the catalytic position 61 in Switch II, where Ras has a glutamine and Rap1 contains a threonine. Ras is a well-studied proto-oncogene, while Rap1 has more recently been shown to be activated through cAMP-related signaling and more rarely plays a role in oncogenesis. Although dimerization of the C-terminal kinase domain has been understood to be involved in Raf activation for the past decade, our group has more recently shown the binding of RBD to Ras triggers robust dimerization of Ras molecules on the membrane leading to increased allosteric connections. Here we explore the interaction between Rap1A and Raf kinase using molecular dynamics (MD) simulations and use community network analysis to study allosteric networks for comparison with Ras/Raf networks we recently discovered. Whereas MD simulations of the Ras-CRafRBD show the RBD as three distinct communities of interacting residues that reduce to a single community upon Ras dimerization, our MD simulations of the Rap1A-CRafRBD show a single community already exists in the monomeric state. Likewise, Rap1A in the complex has a similar number of communities as Ras in the Ras-Raf dimer complex. Thus the role of dimerization, if any, in increasing allosteric networks in Rap1A does not appear to be as critical as it has been shown to be for Ras.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS157 | Refinement of the data-driven model of the KRas4B/Calmodulin complex

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KRas4B (henceforth called KRas) is a small peripheral membrane GTPase that regulates activation of a variety of signaling pathways, including the Raf/MEK/ERK pathway, leading to cell proliferation, survival, and differentiation. Activation of these signaling pathways is dependent on the nucleotide-bound state of KRas: GTP-bound KRas "turns on" signaling while GDP-bound KRas "turns off" signaling. Interestingly, KRas is the only one of the three Ras isoform that interacts with Ca2+-bound calmodulin (CaM). However, attempts to crystallize the KRas/CaM complex have proven difficult due to the flexible nature of the hypervariable region (HVR) of KRas as well as the linker region of CaM. Previously, we presented a model of the complex containing unprocessed full-length KRas in complex with CaM, guided by small angle x-ray scattering (SAXS), NMR nuclear overhouser effects (NOEs) and chemical shift perturbations. This KRas/CaM complex fit our SAXS envelope well (X=1.97). To further refine and study this model, it underwent molecular dynamics simulations with NOE constraints for a total of 1 microsecond each (three simulations total). From these simulations, a model was determined that maintained an open conformation as seen in the SAXS envelope, with the C-terminus bound to the CaM C-lope and the HVR interacting with the CaM linker. Most NOEs between the KRas HVR and CaM linker region were maintained throughout the simulation with a partial retainment of the HVR helical conformation. The average model from this simulation fit well in our SAXS envelope (X=1.95).

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS158 | Do all SARS-CoV-2 Spike experimental structures look the same?

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We analyzed all SARS-CoV-2 Spike structures (more than 400 structures) available in the Protein Data Bank (PDB) to identify structural differences among them. We found that besides Open or Closed Receptor Binding Domains, Spike structures are different in other features that might facilitate or indicate a Spike conformational change pathway. We identified the coupling between several of those features. Also, we found that even though SARS-CoV-2 viral entry can take place either at the surface of the host cell membrane at normal pH or at the surface of endocytic vesicles at acidic pH, most of the experimental structures are resolved at normal pH, with very few determined at acidic pH. By analyzing these limited acidic pH structures and comparing them with normal pH structures, we identified a possible pH dependent region - Fusion Peptide Proximal Region (FPPR) - that samples 3 different states at acidic pH and 2 states at normal pH with one state being more common than the other.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS159 | Extremophilic Specialization in Bacterial Topoisomerases Revealed through Bioinformatic Analyses

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Gyrase is an essential bacterial enzyme that supercoils circular DNA. It is particularly useful as a model system for studying molecular machines because it has multiple macromolecular interfaces and catalytic centers, and makes multiple large-scale conformational changes as part of its function. In addition, it is present across nearly all bacteria, including extremophiles, making it an interesting case study to investigate principles of protein engineering of biological catalysts. The goal of our work is to ascertain whether we can detect differential specialization in extremophilic enzymes by studying gyrase homologs across diverse bacterial species. Using a sliding window-based amino acid frequency analysis on partitioned sequence alignments, we found that there are specific motifs in the C-terminal DNA binding domain of psychrophilic gyrase homologs. These enrichments were not detectable using overall enrichment analyses, supporting the hypothesis that clusters of particular amino acid enrichments may drive flexibility in psychrophiles and support their function in extremely cold environments.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS160 | Deciphering Mechanisms of K-Ras Signaling through B-Raf Kinase

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The assembly of multivalent protein clusters at the cell membrane has been shown to be essential for the effective activation of the mitogen-activated protein kinase (MAPK) pathway. Packer et al. demonstrated that CRaf-RBD (Ras Binding Domain) promotes Ras dimerization on supported lipid bilayers and that the structure of Ras/-Raf-RBD dimer in solution is consistent with the published crystal structures of the complex. Moreover, it has been reported that heterodimers of BRaf/CRaf predominate in normal Ras-dependent signaling and possess a significantly increased kinase activity compared to the respective homodimers. The current study focuses on studying allosteric networks within the KRas/BRaf-RBD monomer and dimer complexes observed, comparing them with CRaf and heterodimers of the BRaf/Ras/Ras/CRaf complex. Biochemical and biophysical characterization of homodimers with BRaf and heterodimers with BRaf and CRaf in solution is presented. In the future, we plan to obtain crystal structures containing a monomer/dimer of KRas/BRaf-RBD and a heterodimer of BRaf-RBD/KRas/KRas/CRaf-RBD for molecular dynamics (MD) simulations and analysis. The findings from this study will provide insight into the molecular mechanisms through which Ras interacts with Raf, advancing our understanding of Ras signaling in a way that may translate into novel strategies to target Ras-driven cancers.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS163 | Tracing Prokaryotic Evolution Through Chloride Channel Structure

Lily DeBell, Shivansh Mahajan, William Clemons
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ClC proteins are a superfamily of chloride/hydrogen antiporters, including both chloride channels and transporters. These proteins play a central role in many biological processes by allowing for transport of chloride ions into cells across a proton gradient. Studies of ClC structure also provide critical insights into the evolutionary history of bacteria and archaea. The structure of E. coli ClCs consists of an inverted-repeat topology in which the N- and C- terminal subdomains are identical and embedded in the membrane but flipped relative to each other. Such topology is believed to have originated from an ancient gene duplication. We aim to determine if there exist bacteria and archaea which possess "half-repeat" ClCs which lack inverted topology and therefore the gene duplication. Multiple sequence alignment between E. coli ClCs and bacterial and archaeal genomes revealed many ClC candidates. Computational predictions of ClC candidate structure determined that several ClC candidates closely resemble E. coli proteins. Notably, some ClC candidates appeared to have half-repeat subunits dimerized to form the full-length inverted-topology ClC. Ongoing work focuses on performing expression, purification, and structural determination of half-repeat ClC candidates to validate the computational results and demonstrate the existence of half-repeat ClCs.

Track: Imaging & Tracking of Proteins in Space and Time

ABS164 | Non-invasive bioluminescent imaging of kinase inhibition in mouse brain

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Aberrant kinase activity is a common contributor to many human diseases including cancer, thus there is an intense interest in developing novel kinase inhibitors or repurposing existing drugs for treating difficult-to-treat diseases, such as brain tumors. However, assessing drug activity in preclinical animal models remains a key bottleneck in the drug development process, as current methods are time-consuming, expensive, and do not allow longitudinal observations in the same subject. Here, we describe a new technology, kinase-modulated bioluminescent indicators (KiMBIs) that allow non-invasive visualization of kinase inhibition in genetically tagged cells. In both subcutaneous and intracranial tumor xenograft mouse models, KiMBIs enabled monitoring drug activity quantitively and longitudinally, and providing insights into the pharmacokinetics and pharmacodynamics. Furthermore, in intracranially AAV-infected mice, KiMBI could discriminate between brain-permeable and -impermeable kinase inhibitors. Overall, KiMBIs represent a powerful tool for rapid screening kinase-modulating agents in vivo and for expedient dose and schedule optimization in drug development.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS165 | Molecular Architecture of NuRD Subcomplexes using Integrative Structure Determination

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The Nucleosome Remodeling and Deacetylase (NuRD) complex is a chromatin-modifying assembly that regulates gene expression and DNA damage repair. Despite its importance, limited structural information is available on the complex and a detailed understanding of its mechanism is lacking. We investigated the molecular architecture of three NuRD sub-complexes: MTA1-HDAC1-RBBP4 [MHR], MTA1(N)-HDAC1-MBD3GATAD2(CC) [MHM], and MTA1-HDAC1-RBBP4-MBD3-GATAD2 (NuDe, Nucleosome Deacetylase) using Bayesian integrative structure determination with IMP (Integrative Modeling Platform, https://integrativemodeling.org/), drawing on information from SEC-MALLS, DIA-MS, XLMS, negative-stain EM, X-ray crystallography, NMR spectroscopy, secondary structure, and homology predictions. The structures were validated by their fit to input information, jack-knifing tests omitting random subsets of data, as well as by information not used in modeling, including independent cross-links, cryo-EM maps, biochemical assays, and known cancer-associated mutations. Localization of the full-length MBD3, a methyl-CpG DNA binding protein that connects the deacetylase and chromatin-remodeling modules in NuRD, was not previously characterized. Our models indicate two different localizations for MBD3 in NuRD, suggesting a mechanism by which MBD3 in the presence of GATAD2 asymmetrically bridges the two modules in NuRD. Further, our models indicate three previously unrecognized subunit interfaces in NuDe: HDAC1(C)-MTA1(BAH), MTA1 (BAH)-MBD3, and HDAC1(60-100)-MBD3. We observed that a significant number of cancer-associated mutations mapped to protein-protein interfaces in NuDe. Our approach also allows us to localize regions of unknown structure, such as HDAC1(C) and MBD3(IDR), thereby resulting in the most complete and robustly cross-validated structure of the NuRD complex so far.

Track: Protein Science Addressing Health Disparities

ABS166 | Structural insights into the Chemokine Inhibition by Tick Evasins

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Inflammation is a normal response to any injury or infection. However, sustained inflammation can cause numerous diseases. A hallmark of inflammation is leukocyte recruitment to the affected tissue, regulated by small secretory proteins called chemokines, which bind and activate chemokine receptors expressed on the leukocyte surface. As a strategy to suppress detection and prolong their feeding and residence time, "ticks", hematophagous arachnids, produce chemokine-binding proteins called 'evasins', which bind to host chemokines and block chemokine receptor activation, preventing leukocyte migration. As a natural selective chemokine-inhibitory protein, evasins have excellent potential as therapeutics for inflammatory diseases. Evasins are classified into two

superfamilies, Class A and B, CC and CXC chemokine binders, respectively. In our study, we discovered a novel evasin class "A3". We expressed and purified several A3 evasins including EVA-M and EVA-A, which bind to all human CC chemokines, but none of CXC chemokines. We solved EVA-M and EVA-A crystal structures in a complex with several chemokines (CCL7, CCL11, CCL16 and CCL17), which revealed the mode of chemokine binding and selectivity. The absolute selectivity to CC chemokines determined by an extensive backbone to backbone hydrogen bonding between the evasin 1st □-sheet and the chemokine CC motif; is conserved across all the structures. Furthermore, the broad-spectrum binding of A3 evasins originated from the distinct hydrophobic pocket on the core, which accommodates N- loop first amino residue of the CC chemokines. Using structural and mutational information, we engineered EVA-M and EVA-A variants with different selectivity to that of wild types. We rationalized the selectivity difference of engineered A3 evasins by solving five crystal structures of EVA-M variants complex to different chemokines. Overall, this study elucidates the relationship between the structure and function of A3 evasins and paves a pathway for the engineering of potent anti-inflammatory molecules.

Track: Protein Phase Separation in Biomolecular Condensates

ABS167 | Proteins Are Solitary! Pathways of Protein Folding and Aggregation in Protein Mixtures

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We present a computational and experimental study on the folding and aggregation in solutions of multiple protein mixtures at different concentrations. We show how in protein mixtures, each component can maintain its folded state at densities higher than the one they would precipitate in single species solutions. We demonstrate the generality of our observation over many different proteins using computer simulations capable of fully characterising the cross-aggregation phase diagram of all the mixtures. Dynamic light scattering experiments were performed to evaluate the aggregation of two proteins, the bovine serum albumin (BSA) and the consensus tetratricopeptide repeat (CTPR), in solutions of one or both proteins. The experiment confirms our hypothesis and the simulations. These findings elucidate critical aspects of the cross-regulation of expression and aggregation of proteins exerted by the cell and the evolutionary selection of folding and not-aggregating protein sequences, paving the way for new experimental tests.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS168 | Microbially-derived Natural Products inhibit Japanese encephalitis virus RNA dependent RNA Polymerase: A Molecular Dynamics Study

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Japanese encephalitis (JE) is an infection of the brain, and one of Asia's most common viral encephalitis, caused by the Japanese encephalitis virus (JEV). Around 68,000 JE cases occur annually, with a ∼30% mortality rate, and ∼50% of survivors develop permanent neurological sequelae. JEV was first reported in Japan in 1871, transmitted across several Asian countries and is now striking the Australasian region and Southern Europe [1, 2]. JEV RNA dependent RNA Polymerase (JEVRdRp) protein has been characterized as a promising therapeutic target because of its central role in viral replication [3]. Natural compounds have high chemical diversity and less toxicity compared to synthetic compounds. Hence, in search of novel compounds, this study screened 32,552 microbially derived natural products from the Natural Products (NP) Atlas library against the adenosine triphosphate (ATP) binding pocket of the JEVRdRp (PDB ID: 4HDH) via three accuracy levels: High Throughput Virtual Screening, Standard Precision, and Extra Precision (XP) using the Glide module of the Schrödinger suite. The best-docked poses were filtered based on the binding free energy obtained via molecular mechanics generalized born surface area (MM/GBSA) calculation. Consequently, 43 compounds were obtained with binding free energy between -5.46 to -66.74 kcal/mol. Based on the interaction and binding affinity, the four best compounds, viz. Phellibaumin B, Termstrin B, Erythrolic acid C, and Stachybotrys microspora triprenyl phenols-3 (SMTP-3) were considered for molecular dynamics simulations. Notably, all the docked complexes showed significant intermolecular interactions and dynamic stability as a function of 100 ns interval. Furthermore, absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis favoured the selected compounds for drug development. Interestingly, SMTP was documented for plasminogen modulating properties [4], and the microbial sources of the remaining three compounds

possess medicinal properties. Collectively, the results advocate the selected compounds as promising hit candidates for therapeutics against JEV disease.
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Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS169 | Engineered tick evasins revealed co-operativity between the structural components for human chemokine selectivity and binding affinity

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Chemokines are critical mediators in the inflammatory cascade. Binding of chemokines to their receptors, expressed on the surface of leukocytes, results in leukocyte migration towards injured or infected tissues (1). Evasins are tick salivary proteins that bind to chemokines and prevent activation of chemokine receptors to subvert the host immune response (2). To understand the principles governing the CC chemokine recognition and selectivity of class A evasins, we have characterised the new evasins named EVA-P974, from Amblyomma cajennense, and EVA-RPU-02, from Rhipicephalus pulchellus, and compared them to the archetypal class A evasins, EVA-1 and EVA-4. The X-ray diffraction studies from the crystal structures of EVA-P974 complexed to two different chemokines, C-C motif chemokine ligand 7 (CCL7) and C-C motif chemokine ligand 17 (CCL17) together with extensive mutational analysis, revealed the role of the specific amino acid residues in N-terminal region in chemokine binding affinity and selectivity (3). To test the hypothesis that swapping the N-terminus between evasins would alter their chemokine selectivity, we designed chimeric evasins by interchanging the N-terminal regions between EVA-1, EVA-4, EVA-P974 and EVA-RPU-02. We expressed them in mammalian cells, purified and examined their chemokine binding properties. In line with our hypothesis, we found that the chemokine binding selectivity and affinity of these chimeric evasins differed from those of the parental evasins, but not in the predicted manner. The chimeric evasin, CHI-4R, consisting the N-terminus of EVA-4 and the core region of EVA-RPU-02 was absolutely selective for the chemokine CCL8 (Kd = 64.4 ± 0.1 nM). Likewise, another chimeric evasin, CHI-1R that contains the N-terminus of EVA-1 and core region of EVA-RPU-02, was able to bind to CCL14 selectively. Our findings revealed that different evasins achieve binding to similar group of chemokines by utilizing different residues. Overall, our study showed that the chemokine binding specificity and affinity of evasins can be altered by interchanging their N-terminal regions. This study provides proof of principle that evasins can be engineered to target specific chemokines.

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Track: Protein Science Addressing Health Disparities

ABS171 | Small Molecule Enhancers of Thyroxine-binding Affinity of Transthyretin : an Insight Towards Therapeutic Intervention of

Pre-eclampsia
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Transthyretin (TTR) is an evolutionary conserved 55kDa serum and CSF protein, synthesized mainly in the liver, retinal epithelium, choroid plexus, placental villi and uterus. As the nomenclature indicates, the main function of the protein is transport of the thyroid hormones T4 (thyroxine) & T3, and Retinol-binding protein (RBP). Infact, it is the only known thyroxine transporter in brain and across placenta which makes it crucial in different disease pathologies. Thus, functional regulation of TTR has colossal utility in various physiological complications involving hypothyroidism and hyperthyroidism, especially in neuronal and gestational impairments. During pregnancy, TTR is synthesised from the placental villous syncytiotrophoblast cells at the placental-maternal interface and the native TTR tetramers aids in the transport of maternal thyroxine to developing foetus and placenta which is crucial for proper placenta formation and maturational processes of foetal nervous system. Additionally, TTR initiates the process of uterine spiral artery remodelling. Dysregulation in TTR levels or functions leads to major pregnancy complications including but not limited to preclampsia. Preclampsia is associated with reduced placental and serum TTR levels, hypothyroidism and dissociation of the TTR tetramers into partially unfolded monomers which oligomerize into amyloid fibrils. The deposition of these fibrils in the placental tissue as well as maternal vasculature facilitates the establishment of the disease. In the present article, we identified some naturally occurring small molecules i.e. betaine and taurine as enhancers of thyroxine-binding affinity of TTR. Further, the structure, thermodynamic stability, internal dynamics, tetrameric fraction and other aspects of the modified protein was probed to understand the mechanism behind this functional modulation. Both these molecules also proved to be effective against the aggregation propensity of TTR. This highlights the importance of betaine and taurine administration in pregnancy, mainly pre-eclamptic patients.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS173 | The gut metabolite, Trimethylamine N-oxide, inhibits protein folding by affecting cis-trans isomerization and induces cell cycle arrest

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Trimethylamine N-Oxide (TMAO) is an important metabolite, which is derived from choline, betaine, and carnitine in various organisms. In humans, it is synthesized through gut microbiota and is abundantly found in serum and cerebrospinal fluid (CSF). Although TMAO is a stress protectant especially in urea-rich organisms, it is an atherogenic agent in humans and is associated with various diseases. Studies have also unveiled its exceptional role in protein folding and restoration of mutant protein functions. However, most of these data were obtained from studies carried on fast-folding proteins. In the present study, we have investigated the effect of TMAO on the folding behavior of a well-characterized protein with slow folding kinetics, carbonic anhydrase (CA). We discovered that TMAO inhibits the folding of this protein via its effect on proline cis-trans isomerization. Furthermore, TMAO is capable of inducing cell cycle arrest. This study highlights the potential role of TMAO in developing proteopathies and associated diseases.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS174 | The evolution of the (dis)assembly of photocatalytic LPOR regulated by NADPH, NADP+ and MGDG

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Light-dependent protochlorophyllide oxidoreductase (LPOR) is a unique example of an enzyme with a light-driven catalysis that was recently shown to form helical assemblies on lipid membranes. The enzyme is involved in the penultimate reaction of the chlorophyll biosynthetic pathway, has two substrates and can be found in all oxygenic phototrophs, including bacteria, algae and land plants. In this study we explore the natural diversity of the properties of the enzyme originating from all of these organisms to uncover the evolutionary history of LPOR. We focused our research on the interaction with lipids, substrates binding and products release.

We found that the bacterial variant of the enzyme requires MGDG for an efficient product release, while in plant variants, MGDG promotes oligomerization of the enzyme in darkness. Interestingly, depending on the redox state of the nucleotide bound within the complex, the oligomers can form either linear helical assemblies or large spherical assemblies composed of branched tubes. After light exposure, the complexes composed of NADPH and protochlorophyllide undergo the reaction, however, the disassembly of these complexes depend on NADPH

concentration. We found that the formation of linear helical assemblies on lipid membranes can inhibit the product release from these complexes due to the interaction with NADPH. The analysis of several plant variants of the enzyme originating from different phylogenetic branches revealed the differences in the oligomerization properties and in the interaction with NADPH and NADP+. The relation between the properties, structure and amino acid sequence of the variants is discussed, as well as the physiological significance of the findings. Moreover, we compare the behavior the enzyme in vitro and in vivo conditions in three plant species.

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Track: Protein Science Addressing Health Disparities

ABS175 | Engineering of Tick Evasins as Chemokine-targeted Anti-inflammatory Agents

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Inflammatory diseases account for numerous deaths and morbidity worldwide. A hallmark of inflammatory diseases is the excessive influx of white blood cells into the affected tissues, which is coordinated by pro-inflammatory mediators called chemokines. Humans have ~50 chemokines divided into two major classes – CC and CXC chemokines. A specific subset of chemokines are associated with an inflammatory disease; for example, the chemokines CCL2, CCL7 and CCL8 are involved in atherosclerosis. As natural chemokine inhibitors, evasin proteins produced in tick saliva are potential therapeutic agents for numerous inflammatory diseases. These evasins are classified into two major classes – class A and B – with absolute specificity to CC and CXC chemokines, respectively. Engineering evasins to block the desired chemokines and avoid off-target side effects requires structural understanding of their target selectivity. Here, structures of the class A evasin EVA-P974 bound to the human CC chemokine ligands CCL7 and CCL17 and to a CCL8-CCL7 chimera reveal that the specificity of class A evasins for chemokines of the CC subfamily is defined by conserved, rigid backbone-backbone interactions, whereas the preference for a subset of CC chemokines is controlled by side chain interactions at hotspots in flexible structural elements. Hotspot mutations alter target preference enabling the enhanced inhibition of selected chemokines. The structures of engineered EVA-P974 bound to several chemokines including CCL2, a non-binder to wild types, reveal an underlying molecular mechanism of Class A evasins target preference. This structural understanding enabled us to rationally engineer evasins with modified chemokine selectivity, providing a foundation for future engineering of evasins as targeted anti-inflammatory therapeutics.

Track: Celebrating 100 Antibody Drugs

ABS178 | Molecular Basis of Contribution of Intramolecular Interactions between Complementarity Determining Region 3 (CDR3) and Framework Region on Biophysical Properties in Camelid Single-Domain Antibody

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The Camelid single domain antibody, referred to VHH or Nanobody, is considered as a versatile tool for various biotechnological and clinical applications because of its favorable biophysical properties such as small size, strong affinity, or high stability. Compared to conventional human or murine antibodies, VHHs have 3-4 residues longer complementarity determining region 3 (CDR3) loops, which are also more divergent in both sequence and structure. Such diversity of CDR3 and a single-domain nature of VHHs enables to form a unique convex paratope. To take advantage of the characteristics and for further application, various research on VHH engineering have been encouraged and conducted.

In this study, we investigated the impact of CDR-Grafting using a humanized VHH in clinical trials, in terms of affinity, thermal stability and structure. The chimeric VHH, which was generated by exchanging the CDRs, exhibited a significant decrease of affinity and thermal stability, and a large conformational change in the CDR3. To elucidate the molecular basis of this impact, we employed MD simulations, focusing on the intramolecular interactions between the bended CDR3 and framework2, and identifying the residues in framework2 involved in the intramolecular interactions with CDR3. Mutational analysis of these residues showed a decrease of thermal stability and affinity, suggesting that the important role of intramolecular interactions between CDR3 and framework2. Subsequently, we performed

back-mutational analyses on the Chimeric VHH, with substitutions on the framework2 residues, resulting in an increase of thermal stability and affinity. The results suggested that back-mutations could restore the intramolecular interactions, resulting in the gain of thermal stability and affinity.

In light of these observations, we discuss the molecular basis of contributions of CDR3-framework2 intramolecular interactions on VHHs and further designability of the framework region of VHHs without modifying the CDRs.

Track: Celebrating 100 Antibody Drugs

ABS179 | Proteolytic Antigen Cleavage Mediated Amplification (PACMAN) Catalysis as a potential game changer in antibody therapy against amyloid diseases

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Diseases caused by the amyloid aggregation of small peptides are widely spread such as Alzheimer's disease, Parkinson's disease, and Diabetes mellitus type 2 (T2DM). In the case of T2DM the involved peptide is islet amyloid polypeptide (IAPP) or amylin. IAPP is a 37-amino-acid secreted hormone, connected to gastric emptying and is co-secreted with insulin. However, in case of T2DM IAPP is found as amyloid deposits which contribute to β-cell-death in T2DM patients. Therefore, IAPP aggregates are a promising candidate for therapeutic intervention.

One promising idea of diminishing the cause of β-cell-toxicity is antibodies with proteolytic activity against IAPP aggregates. These special antibodies unite the high specificity of antibodies and the proteolytic activity of proteases. The key advantage of these antibodies is the immediate cleavage reaction which circumvents adverse inflammation reactions caused by the activated immune system.

PACMAN is a method to select these proteolytic antibodies from donated human blood. An antibody library will be tested for its proteolytic activity in an emulsion-based bead-display setup with an in vitro transcription and translation system (IVTT). Based on different state-of-the-art techniques like emulsion-polymerase-chain-reaction (emPCR), IVTT, next generation sequencing (NGS), and fluorescence-activated-cell-sorting (FACS) it is possible to enrich promising antibody variants and find antibody sequences which can be used against distinct targets.

The proof of concept of PACMAN was achieved via a model system in which the proteolytic antibody was mimicked by the protease of Tobacco Etch Virus (TEV-protease) and IAPP by a TEV-target. PACMAN could serve as a game changer in antibody drug discovery in T2DM, Alzheimer's and Parkinson's disease.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS180 | Multi-State Design to Stabilize Viral Class I Fusion Proteins

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Life-threatening viruses such as Ebola, influenza, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), and the pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) use class I fusion proteins to induce the fusion of viral and cellular membranes and infect the host cell. During this process, class I fusion proteins irreversibly refold from a metastable prefusion state to a stable postfusion state to provide the energy that catalyzes the membrane fusion reaction. While both pre-and postfusion conformations are usually immunogenic, the labile prefusion state has been shown to promote more potent immune responses. Consequently, stabilizing the prefusion conformation has become imperative for developing vaccine immunogens based on class I fusion proteins. In this work, we aimed at designing an automated computational approach to identify amino acid substitutions stabilizing the prefusion state of class I fusion proteins. Accordingly, we have developed an algorithm that uses the structural information of both pre-and postfusion conformations to identify amino acid changes able to reduce the total free energy of the prefusion state while increasing the energy of the postfusion state. We hypothesized that the transition from one state to another is precluded if the energetics of the conformational switch is inverted. As a proof of concept, we have stabilized the prefusion protein of RSV, hMPV, and SARS-CoV-2. Remarkably, only four protein variants were experimentally tested for each virus before finding a successful design. Our designs were characterized by size-exclusion chromatography, antibody binding with prefusion-specific antibodies, negative-stain electron microscopy, and crystallography studies (RSV and SARS-CoV-2). In conclusion, we have developed a robust computational approach to stabilize class I fusion proteins.

Given the clinical significance of these proteins, we believe that our algorithm can substantially impact the vaccine development field by reducing the time and experimental validation resources needed to optimize these potent immunogens

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS181 | Protein dimer formation in vitro and in cells

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The cellular interior is crowded with macromolecules. These macromolecules affect proteins and their complexes via hard repulsions and chemical interactions (1), yet few studies focus on chemical interactions. Hard repulsions are always stabilizing, while chemical interactions can be stabilizing (repulsive) or destabilizing (attractive). We characterized formation of the GB1-based domain-swapped dimer in buffer and Escherichia coli cells by using nuclear magnetic resonance spectroscopy (2). In buffer, the monomer exists in a partially folded state (3), but the partially folded monomer is absent in cells. In-cell studies using urea show the absence of the partially folded monomer but the presence of the unfolded monomer. The data suggest that attractive chemical interactions in cells unfold the partially folded monomer. Our results lead to the conclusion that the intracellular environment can not only modulate protein complex- stability (4), but can also change the mechanism of protein-complex formation, reinforcing the idea that chemical interactions are more important than hard repulsions in cells.

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Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS182 | In cell structural biology of neurodegenerative disease associated proteins is enabled by sensitivity enhanced solid state NMR

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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.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS183 | A 2.7Å cryo-EM reconstruction of transthyretin (55kDa) covalently bound to a small molecule

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Transthyretin (TTR) is a 55kDa tetrameric protein that carries holo retinol binding protein and thyroid hormone (T4) in vertebrate extracellular fluids. Tetramer dissociation and unfolding of monomeric TTR results in aggregation and amyloid formation, leading to TTR amyloidosis linked to neurodegeneration and cardiomyopathy. The prominent FDA-approved treatment for this disease, tafamidis, binds in the same pockets as T4 and prevents TTR unfolding by stabilizing its native structure. The two symmetric binding sites are located at monomer interfaces on

one of the D2 symmetry axis of TTR, and are therefore each composed of two identical, symmetry-related, surfaces. Consequently, asymmetric molecules like tafamidis can bind in two alternative symmetric conformations. This, compounded by TTR's tendency to form crystals where the asymmetric unit is half of the tetrameric complex with the binding site at the interface, has precluded the generation of models with a single drug conformation. We show this issue can be overcome by single-particle cryoEM. Using a Talos Arctica microscope, we obtained a 2.7Å resolution reconstruction of TTR (Figure 1) covalently modified by a ligand-mimicking small molecule (A2) on both binding sites, without enforcing symmetry. While averaging of two ligand conformations is still discernible in one of the binding sites, the density we obtained allows confident modeling of an asymmetric ligand pose. In contrast, this was impossible to attain from 1.8Å resolution crystal structure data of the same complex. Sample preparation of TTR, a 55kDa protein with tendency to denature are the air-water interface, was the most challenging aspect of this project and required the use of gold-support grids modified with single-layer graphene. The experimental methods and data analysis workflows here presented will help other researchers use cryoEM for drug discovery on small protein targets.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS184 | Rewriting the Rules of Molecular Competition: Transcriptional Regulation by Intrinsically Disordered Proteins

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Intrinsically disordered proteins (IDPs) play important regulatory roles in cellular signaling processes, yet the mechanisms by which disordered proteins use their unique physicochemical properties to achieve biological control are not fully understood. IDPs participate in complex interaction networks and often must compete for binding of shared interaction partners to carry out their cellular functions. Under conditions of oxygen deprivation, the disordered activation domains of the hypoxia-inducible transcription factor HIF-1α and its negative regulator CITED2 control transcription of critical adaptive genes by competing for binding to a partially overlapping surface on the folded TAZ1 domain of the general transcriptional coactivators CBP and p300. We have previously shown that competition between HIF-1α and CITED2 proceeds by a unidirectional allosteric mechanism that enables precise switch-like control of transcription. To gain further mechanistic insight into this process, we have used a combination of structural and biophysical techniques, including NMR relaxation methods and fluorescence approaches, to obtain a detailed molecular description of how disorder and conformational dynamics of IDPs enable efficient switch-like competition for a common binding partner. We find that competition between HIF-1α and CITED2 for TAZ1 binding is not only dependent on the flexibility of the IDPs themselves but also on the conformational dynamics of TAZ1. CITED2 binds to the TAZ1:HIF-1α complex to form a transient ternary complex and drives displacement of HIF-1α by a facilitated dissociation mechanism. Multivalency in both HIF-1α and CITED2 is a key determinant for unidirectional switch-like displacement of HIF-1α by CITED2, and regulatory efficiency is lost when binding motifs of CITED2 are altered by truncation or mutation. Taken together, our results highlight the complexity of competition processes involving IDPs, identify the molecular features critical for effective regulation of the hypoxic response, and illuminate new avenues for harnessing the functions of disordered proteins for therapeutic applications.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS185 | Engineered Enzyme-Cofactor Pairs for Selective Chemical Tracking of Epigenetic Writers in Mammalian Cells

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Enzymatic methylation of cytosine to 5-methylcytosine in DNA is a fundamental epigenetic mechanism involved in mammalian development and disease. DNA methylation is brought about by collective action of three independently expressed and regulated AdoMet-dependent proteins (Dnmt1, Dnmt3A and Dnmt3B). However, the catalytic interactions and temporal interplay of these epigenetic "writers" in establishing and maintaining genomic DNA methylation profiles characteristic to each cell type are poorly understood. To achieve selective tracking of the catalytic action of an individual Dnmt enzyme, we used structure-guided engineering of the mouse Dnmt1

(residues 291–1620) for the transfer of bioorthogonal 6-carbon linear moieties containing a functional azide group onto DNA from a synthetic cofactor analog, Ado-6-N3 [1]. Examination of the produced Dnmt1 mutant pool in vitro yielded a variant that conferred a 8400-fold improvement in cofactor selectivity as compared to the WT enzyme. To establish endogenous expression of the cofactor-engineered version of the enzyme in mouse embryonic stem cells, we installed the corresponding codons in the Dnmt1 alleles using CRISPR-Cas9 genome editing. Further, we found that pulse-internalization of the Ado-6-N3 cofactor by electroporation into the engineered cells enabled selective catalysis-dependent azide-tagging of Dnmt1-specific targets in vivo. The deposited chemical groups were exploited as 'click' handles for reading adjoining sequences and precise mapping of the tagged methylation sites in the genome using TOP-seq [2]. Altogether, we demonstrate the first general approach that produces high-resolution genome-wide temporal "tracks" of the Dnmt1 catalysis in live mammalian cells [3] during the cell cycle or differentiation to somatic lineages, offering unprecedented inroads into studies of genomic methylation in a wide range of eukaryotic model systems.

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Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS186 | Monitoring RNA-Protein Interactions in Living Bacteria in Real-Time using LigandTracer

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RNA Binding Proteins (RBP) act as modulators in key cellular functions by binding RNA and affecting its translation. Therefore, different techniques have been developed to study these interactions both in vitro and in vivo. Here we present a new application to study interactions between Musashi-1 and RNA in living bacteria over time. Musashi-1 is an RBP involved in cell differentiation and proliferation and its interaction with RNA is complex as it has two RNA binding sites and its RNA binding, in turn, can be modulated by allosteric binders.
The employed method detects fluorescence and therefore bacteria were transformed with a reporter system based on two plasmids; one encoding Musashi-1 and the other one an RNA sequence close to the promoter of a green fluorescent protein (GFP) sequence so that GFP expression is downregulated upon the binding. We will describe the method, its reliability and results obtained from various RNA sequences and allosteric inhibitors. The observed variation in GFP expression profile is correlated with data obtained from in vitro techniques such as NMR and SPR.
In conclusion, Musashi-1-RNA interactions lead to strong and reproducible variations in GFP expression levels that correlate with in vitro binding properties.

Track: High Throughput Protein Science

ABS187 | High-throughput profiling of tyrosine kinase specificity reveals the effects of phosphosite-proximal mutations

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Protein tyrosine kinases play a crucial role in cell signaling by phosphorylating tyrosine residues on downstream target proteins. For proper signal transduction, tyrosine kinases must selectively discriminate among a multitude of phosphorylation sites inside the cell. Tyrosine kinases engage phosphosites in a manner that is dependent on the amino acid sequence surrounding the tyrosine residue. Despite extensive work in this area, predicting endogenous substrates based on sequence motifs remains difficult, and the impact of phosphosite-proximal mutations on kinase selectivity is largely unexplored. To address this problem, we developed a scalable platform that utilizes genetically encoded peptide libraries and deep sequencing to profile the phosphorylation efficiencies of thousands-to-millions of sequences by any tyrosine kinase. As a case study, we used this platform to characterize the cytoplasmic tyrosine kinase c-Src. We first screened a peptide library consisting of ∼3,000 human phosphosites and ∼5,000 disease-associated variants or natural polymorphisms of these phosphosites. The signals for individual peptides in these screens correlate linearly with phosphorylation rates measured using an orthogonal in vitro enzymatic assay. From this screen, we identified 127 statistically significant mutations that impact the phosphorylation of 97 different phosphosites by c-Src. We hypothesize that these mutations could lead to aberrant signal transduction. Following this, we screened c-Src against a library of >106 random 11-residue sequences with a central tyrosine. Using data

generated from these screens, we show that we can predict phosphorylation efficiencies of substrates and design highly efficient peptide substrates for c-Src. Lastly, we applied this method to study other tyrosine kinases, including c-Abl, Fer, JAK2, and HER2. We observe varying substrate specificities as well as differences in how mutations affect phosphorylation efficiency. Collectively, these experiments demonstrate the utility of our platform for rapid profiling of tyrosine kinase specificity and robustly assessing the effects of phosphosite-proximal mutations on substrate recognition

Track: High Throughput Protein Science

ABS188 | Systematic Quantification of Newly Synthesized Proteins and Their Dynamics in Human Cells

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Protein synthesis and degradation are typically well-regulated in cells to maintain proteostasis. Aberrant proteostasis is directly related to numerous diseases such as neurodegenerative and cardiac diseases. Proteins are continuously synthesized in cells during their growth and in response to environmental changes. Based on previous work, ~30% of newly synthesized proteins were not properly folded. These improperly folded proteins need to be promptly degraded to reduce the burden to cells. The dynamics of newly synthesized proteins is a critically important component for the maintenance of cellular proteostasis. Comprehensive analysis of the dynamics of newly synthesized proteins aids in a better understanding of proteostasis and the molecular mechanisms of many diseases. We integrated metabolic labeling, bioorthogonal chemistry and multiplexed proteomics to comprehensively investigate newly synthesized proteins and their dynamics. Because many newly synthesized proteins have low abundances, it is essential to enrich them for their global analysis by mass spectrometry-based proteomics. The integrative method allowed us to selectively enrich newly synthesized proteins and distinguish them from existing ones. Parallel experiments were performed to study the degradation of newly synthesized proteins in human cells and measure their half-lives with or without the inhibition of each major degradation pathway. Over 4000 proteins were quantified and their half-lives spanned a wide range. Systematic and quantitative analysis of the dynamics of newly synthesized proteins first revealed that many proteins were degraded through the ubiquitin-proteasome pathway or the autophagy-lysosome pathway. Bioinformatic analysis demonstrates that proteins degraded through two major pathways have distinct properties and functions. Proteins degraded through the ubiquitin-proteasome pathway contain more disordered structures, whereas those through the autophagy-lysosome pathway have significantly higher hydrophobicity. Systematic and quantitative investigation of the dynamics of newly synthesized proteins provides unprecedented and valuable information about protein degradation, which leads to a better understanding of protein properties and cellular activities.

Track: Protein Phase Separation in Biomolecular Condensates

ABS189 | Offbeat Properties and Self-Assembly Behavior of Integral Proteins of Bacterial Microcompartment

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Background: 1,2-propanediol utilization microcompartments (PduMCPs) are proteinaceous organelles that assist certain bacteria, such as Salmonella enterica, in using 1,2-propanediol as an alternative carbon source in low-energy environments. PduMCPs are made up of multiple copies of shell proteins and enzymes. The shell proteins form the outer cover encapsulating enzymes, which work in a cascade. Shell protein PduBB' comprises a major portion of the outer shell and has been suggested to bind the signature enzyme, diol dehydratase PduCDE.
Objective: To study the effect of PduBB'-PduCDE interaction on the stability of the enzyme and elucidate the forces governing their self-assembly in solution phase.
Methods: A combination of complimentary spectroscopic, imaging, biochemical and computational techniques have been employed to study the self-assembly behavior of shell protein PduBB' and its impact on the functioning of enzyme PduCDE.
Results: PduBB' conserves the catalytic activity and native structure of PduCDE at higher temperatures. PduBB' and its variants (PduB and PduB') all interact with PduCDE with micro molar affinity; however, only the PduBB' combination influences its activity and stability. The disordered N-terminal region of PduBB' provides solubility to the shell protein. The presence of the disordered N-terminal in subunit D of the enzyme is necessary for strengthening its association with shell protein. PduBB' exhibits fluid-like behavior and undergoes liquid-liquid phase separation in a crowded environment with appropriate ionic strength. While PduB' has a high self-associating property displaying liquid-solid transition,

PduB has the lowest tendency to undergo phase separation. The co-phase separation of shell protein PduBB' and enzyme PduCDE enhances the catalytic efficiency of the enzyme.
Conclusion: PduBB' has a chaperone-like property towards its native enzyme PduCDE due to its naturally optimized self-assembly behavior. Self-assembly of shell-protein and enzyme is triggered by a combination of protein-protein interactions and phase separation.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS190 | Elucidating the inhibition mechanism of antibiotics in bacterial RNA Polymerase using quasi-Markov State Models

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The emergence of antibiotic resistance urges the development of new antibiotics. Bacterial RNA Polymerase (RNAP) is the enzyme performing transcription and is an effective target for antibiotics. RNAP requires the conformational changes of several structural modules to perform its function. There are two pincers in the RNAP, clamp and β-lobe domains, forming a loading gate. During the initiation of transcription, the opening of the loading gate is necessary to load the template DNA. This is followed by closing the loading gate to stabilize the RNAP and DNA complex. Myxopyronin (Myx) is an antibiotic targeting the movement of the clamp domain to inhibit transcription initiation. To study the recognition mechanism of Myx, we constructed quasi-Markov State Models (qMSM) based on extensive molecular dynamics (MD) simulations. We found that the clamp interconverts between four states: closed, two partially closed, and open states. Myx can only bind to a partially closed state, which suggests that the binding of Myx follows the conformational selection mechanism. Importantly, we found that the opening of the β-lobe only is sufficient for the loading of DNA, highlighting the critical role of the β-lobe and its potential for future antibiotics development. qMSM is a great tool to obtain the slow timescale dynamics of complex conformational change using shorter MD simulations compared to conventional MSM. It is also useful to analyze the recognition mechanism of antibiotics to the target protein. Thus, we will also use qMSM to study the trigger loop (TL) domain of RNAP. TL folding is crucial to stabilize the NTP substrate in the active site for the subsequent catalysis. CBR is a class of antibiotics that inhibit the folding of TL. Studying how TL folds will be important to understanding the recognition mechanism of antibiotics, CBR, and future antibiotics design targeting TL.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS191 | Bioinformatic Analyses of Paralogous RNA Methyltransferases Reveal Determinants of Substrate Specificity

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Antibiotic resistance has become a serious issue, resulting in an estimated 35,000 deaths each year. Resistance to erythromycin, a macrolide antibiotic, constitutes a concerning threat, with over 5000 infections per year and rising. One source of macrolide resistance is the erythromycin-resistance methyltransferases, Erms, which dimethylate A2058 of 23S RNA. This modification inhibits the binding of macrolides as well as lincosamides and streptogramin, resulting in broad-spectrum resistance. The precise interaction between Erm and its substrate RNA has not been visualized at high resolution, hampering efforts to understand and target this interaction. In contrast to Erm, KsgA, a housekeeping ribosomal methyltransferase involved in ribosome biogenesis, has been extensively visualized in complex with its target ribosomal subunit. KsgA has sequence homology with Erm, but catalyzes dimethylation at different positions (A1518 and A1519 of 16S RNA), and is therefore likely to engage its RNA target through a distinct set of interfacial interactions. Here, we have used a sliding-window protocol to detect differential clustering of charged residues in partitioned multiple sequence alignments of a broad set of KsgA and Erm homologs. Our analysis successfully identifies regions of KsgA and Erm known to be critical for specificity and further identifies previously unexplored regions of Erm that may be important drivers of substrate selectivity.

Track: Protein Phase Separation in Biomolecular Condensates

ABS192 | Purification of Membrane Protein Aggregates of C99 without detergents, biophysical characterization, and initial cellular studies

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Membrane proteins have hydrophobic regions associated with lipid bilayers. Lipid-like molecules such as detergents, polymers, and specialized amphipathic proteins are used to study membrane proteins in vitro. We have developed a method to purify single-pass membrane proteins without lipid-like molecules in the purified protein. We call these protein aggregates Detergent Limited Particles (DLPs). DLPs seem to satisfy their hydrophobic needs by forming oligomers that manifest as spherical particles, with hydrodynamic radii of 6-15nm. One of the proteins that has this property is C99, the transmembrane domain of the Amyloid Precursor Protein and immediate precursor to amyloid-β. Unlike classical amyloids, DLPs are reversibly solubilized into monomeric C99 with the addition of detergent. C99 DLPs are stable in aqueous solution for up to 3 months at 4°C and can be frozen for long-term storage. DLP stability and toxicity to mammalian cells are dependent on the membrane protein used to form the particles. Mammalian cells readily internalize DLPs from complete media. We are currently exploring if these particles can be used to deliver functional protein. Regardless of the outcome, DLPs appear to offer an attractive and "minimalist" way of delivering recombinant protein, potentially in fluorescently-tagged or otherwise-chemically modified form, into living cells for any one of a variety of possible applications. In addition, DLPs may allow for studies on initial cellular steps of protein aggregate formation and how cells first approach these aggregates.

Track: Protein Science Addressing Health Disparities

ABS193 | Mechanistic insights and potential for the therapeutic rescue of a chaperonopathy: Limb Girdle Muscular Dystrophy (LGMDD1)

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Molecular chaperones, or heat shock proteins (HSPs), play a crucial role in maintaining cellular homeostasis by protecting against the toxic misfolding and aggregation of proteins. As such, mutations or deficiencies within the chaperone network can lead to several protein conformational degenerative diseases. In fact, dominant mutations in DNAJB6 (Hsp40/Sis1), an Hsp70 co-chaperone, leads to a protein aggregate myopathy termed Limb-Girdle Muscular Dystrophy Type D1 (LGMDD1). DNAJB6 client proteins and co-chaperone interactions in skeletal muscle are not known. Previous studies from our lab suggest that the LGMDD1 mutants in Sis1 not only show substrate specificity but also conformation-specific effects and that the deleterious effect of the G/F domain of DNAJB6 mutants is HSP70-dependent. Here, we used the yeast prion model client in conjunction with in vitro chaperone activity assays to gain mechanistic insights, and found that LGMDD1 mutants affect Hsp40 functions. Strikingly, the mutants changed the structure of client protein aggregates, as determined by altered distribution of prion strains. They also impair the Hsp70 ATPase cycle, dimerization, and substrate processing and consequently poison the function of wild-type protein. These results provide mechanistic insights for this class of myopathy in which mutations alter chaperone activity. Further, these results inform our path to therapeutic intervention for LGMDD1.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS194 | Ligand binding remodels protein side chain conformational heterogeneity.

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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 both the anharmonic and harmonic contributions to conformational heterogeneity. Here, through multiconformer modeling of time- and space-averaged electron density, we measure 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. Among ligand properties, we observe increased protein flexibility as the number of hydrogen bonds decrease 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 NMR studies suggesting that residual side chain entropy can modulate affinity and point to the need to

integrate both static conformational changes and conformational heterogeneity in models of ligand binding.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS195 | Structure-Based Drug Design of Small Molecule Inhibitors of Pyruvate Carboxylase

Nicholas Schneider, Kendra Gilreath, Ellie Lokken, Subhabrata Chaudhury, William Donaldson, Martin St. Maurice
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Pyruvate Carboxylase (PC) is an anaplerotic enzyme that replenishes the TCA cycle intermediate oxaloacetate (OAA). OAA is a precursor molecule for pathways such as gluconeogenesis, fatty acid synthesis, and amino acid synthesis. PC also contributes to glucose-stimulated insulin release in pancreatic beta cells and glucose production in the liver and kidney. Due to its heavy involvement in cellular homeostasis, it is unsurprising that altered PC expression and activity is associated with a wide range of diseases such as type-2 diabetes, bacterial infections, and several types of cancers. Additionally, RNAi studies have further validated PC as a potential target for pharmacological intervention. Currently, there are no effective pharmacological tools available to assess and manipulate the activity of PC in a variety of diseases. This project is focused on discovering, characterizing, and optimizing small molecule effectors of PC. We have used a combination of structure-based drug design and in silico screening to discover two classes of compounds (α-hydroxycinnamic acids and imidazolidinetriones) that inhibit PC at low micromolar concentrations. Competition and computational docking experiments strongly suggest that these compounds bind within or near the catalytic active site in the carboxyltransferase domain of PC. These compounds are also reasonably selective for PC. The imidazolidinetriones offer a promising new direction because they have enhanced cell permeability and reduced metal chelation properties relative to the α-hydroxycinnamic acids. We report on the structure-activity relationships for both compound classes with respect to potency, selectivity, and permeability. These compounds represent a major step in the development of potent and selective small molecule effectors of PC. Highly potent and selective small molecule effectors of PC will expand the pharmacological toolkit available for studying the metabolic reprogramming that accompanies type-2 diabetes and cancer.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS196 | Functional Ancestral Reconstruction of the Cold and Menthol Sensor TRPM8

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Transient receptor potential melastatin 8 (TRPM8) is a polymodally gated ion channel activated by a variety of stimuli including menthol, cold, and pH. TRPM8 has become a potential drug target as it has been implicated to be involved in diverse pathophysiologies such as pain, obesity, and cancer. However, clinical trials targeting TRPM8 have generally caused adverse symptoms in thermal dysregulation. It is expected that an understanding of the molecular underpinnings between the activation modes will aid the development of mode-specific TRPM8 therapies. Here, we use ancestral sequence reconstruction, a computational molecular evolution tool, to explore that ability to disentangle TRPM8 activation modes, which presumably emerged distinctly over evolutionary time. Based on the extant sequences of TRPM8, ancestral reconstruction was used to determine the DNA sequences of the last common primate, mammalian, and vertebrate ancestors. Resurrection and experimental analysis of these ancestral TRPM8 proteins show them to be functional in heterologous mammalian cells. Whole-cell patch-clamp electrophysiology and calcium mobilization assays confirm our hypothesis that the cold and menthol responses differs in terms of emergence and sensitivity between the ancestral constructs suggesting a new method to dissect polymodal protein behavior.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS198 | A Bioinformatic Pipeline for the detection of Amino Acid Enrichments in Three Dimensions: A Case Study of DNA Polymerase I

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Thermophilic versions of DNA polymerases are some of the most well-studied and widely utilized extremophilic enzymes in use today. Their application in the polymerase chain reaction makes them critical to biotechnology, and their presence in a wide array of extremophilic organisms, including halophiles and psychrophiles, make

them an attractive model system for studying extremophilicity in protein-DNA interactions. In this study, we used bacterial Pol I as a model system in the development a bioinformatic pipeline to examine how particular amino acids are differentially enriched in catalytic or ligand-binding sites across large sets of orthologous proteins. Our analysis reveals that compared to their thermophilic counterparts, psychrophilic Pol I homologs are enriched in glycines at positions near bound DNA, a result that is expected from prior work on cold-tolerant enzymes. Our analysis also detected an unexpected preponderance of large hydrophobic and aromatic amino acids in psychrophilic DNA binding sites. Our work demonstrates how small shifts in local amino acid composition may help drive the specialization of enzymes for particular environmental niches.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS199 | Characterizing cross-talk between cold temperature sensitivity and ligand binding in human TRPM8

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The ability to sense and respond to temperature is crucial for mammalian survival. Transient receptor potential melastatin 8 (TRPM8) is a polymodal calcium-permeable ion channel that functions as the principal cold sensor and is the cognate receptor for cooling compounds like menthol. TRPM8 has been implicated in various diseases, including multiple pain states, tumor progression, and metastasis. Thus, TRPM8 has garnered interest as a therapeutic target. However, the administration of TRPM8 antagonist was found to cause severe on-target side effects that have limited their progression to the clinic. . Understanding how different modes of activation are allosterically coupled is important for identifying mode selective antagonists. However, the exact mechanism by which chemical and cold temperatures activate the channel has not been fully elucidated. Functional and structural studies of TRPM8 show that the voltage-sensing-like domain (VSLD, transmembrane helices S1-S4) as the binding site for cooling compounds including menthol, icilin, and menthol analog WS-12. While functional measurements show full-length TRPM8 is highly thermosensitive, however, the location of the thermosensor has not been established. Using 15N-HSQC and 19F-NMR spectroscopy, we investigate human TRPM8-VSLD thermosensitivity and ligand sensitivity. These methods allow us to probe the binding site, structure, and dynamic states of the protein. Here, we show that the hTRPM8-VSLD contributes significantly to the full-length channel cold-activation and there is cross-talk between menthol binding and temperature activation.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS200 | Multi-Disciplinary Approach to Untangle the Complex Mode of Interaction of a Multi-Domain RNA Recognition Motif Protein: The Case of Human Musashi-1

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Because of their ability to interact with RNA, RNA Recognition Motifs (RRMs) are known to be key factors in the transcriptional and post-transcriptional gene expression in eukaryotic cells, and any dysregulation can lead in a number of human disorders, including cancer and neurodegenerative diseases.
In this regard, studying the binding mechanism of proteins containing multiple RRM domains with RNA is needed. However, it is not trivial to unravel the mechanism of action, due to their versatile capability in their RNA recognition that often leads to complex systems. Nowadays, integrating different structural biology techniques is an approach that can be useful to understand how multiple RRMs recognize and bind specific RNAs.
Here, we exploited this approach to study the interaction of a multi-domain RRM protein, the human Musashi-1 (MSI-1). In particular, we have analysed the binding against ssRNAs and have deepen on how the formation of secondary structures in the RNA can affect this binding. We have used Nuclear Magnetic Resonance (NMR) to determine the binding site, Surface Plasmon Resonance (SPR) and SwitchSense to determine the kinetic constants, and Size-exclusion chromatography with multi-angle light scattering (SEC-MALS) for a deeper stoichiometric analysis of the complexes. Altogether, those techniques have been used to study the protein-RNA interaction to get a broader and more complete understanding of the system.
This approach, has allowed us to detect the competition between the two RRMs present in the protein when recognizing the same RNA sequence, leading to a more complex and dynamic interaction than expected.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS201 | Structure-based design of a photoswitchable affibody scaffold

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Photo-control of affinity reagents offers a general approach for high-resolution spatiotemporal control of diverse molecular processes. In an effort to develop general design principles for a photo-controlled affinity reagent, we took a structure-based approach to the design of a photoswitchable Z-domain, among the simplest of affinity reagent scaffolds. A chimera, designated Z-PYP, of photoactive yellow protein (PYP) and the Z-domain, was designed based on the concept of mutually exclusive folding. NMR analysis indicated that, in the dark, the PYP domain of the chimera was folded, and the Z-domain was unfolded. Blue light caused loss of structure in PYP and a two- to sixfold change in the apparent affinity of Z-PYP for its target as determined using size exclusion chromatography, UV-Vis based assays, and enyzme-linked immunosorbent assay (ELISA). A thermodynamic model indicated that mutations to decrease Z-domain folding energy would alter target affinity without loss of switching. This prediction was confirmed experimentally with a double alanine mutant in helix 3 of the Z-domain of the chimera (Z-PYP-AA) showing >30-fold lower dark-state binding and no loss in switching. The effect of decreased dark-state binding affinity was tested in a two-hybrid transcriptional control format and enabled pronounced light/dark differences in yeast growth in vivo. Finally, the design was transferable to the αZ-Taq affibody enabling tunable light-dependent binding both in vitro and in vivo to the Z-Taq target. This system thus provides a framework for the focused development of light switchable affibodies for a range of targets.

Track: Protein Science Addressing Health Disparities

ABS202 | An engineered disulfide-free antibody against norfloxacin

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Antibodies are indispensable immunological molecules that play an important role in research, therapy, and diagnostics. Although antibody variants like single chain fragment variable (scfv) are good candidates for clinical applications, their poor solubility, yield, stability, and shelf life are continuous challenges to address. In this study, we aimed to create a stable scfv in bacterial system with improved expression, solubility, and binding affinity. To accomplish that, we engineered, expressed, and purified a 28 kDa scfv against norfloxacin by rational design to create a disulfide free variant of scfv in E. coli. The disulfide free variant was expected to have better expression, stability, and binding. The far-UV CD spectrum of both the wildtype and engineered protein was found to adopt a well folded structure with characteristic negative peaks between 210 and 220 nm. Also, the RaptorX predicted secondary structures showed conserved structure with characteristic greek-key motif. The disulfide free variant was more thermostable with a Tm of 59 °C as compared to wild type with a Tm of 54.8 °C. The fluorescence-based binding studies showed a similar binding profile between both proteins. We plan to further characterize the binding of the molecule by ELISA and by isothermal titration calorimetry and stability by equilibrium denaturation studies. Our results show that we've established an economical and efficient method to generate novel and functional scfvs in bacteria, providing a foundational platform to further design other clinically useful molecules. This immunoglobulin fragment can be used as a scaffold to generate scfvs for different antigenic targets that can be used in imaging, diagnostics, and precision therapeutics.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS203 | Elucidating the Unusual Disease-Predisposing T118M Variant of Peripheral Myelin Protein 22

Geoffrey Li, Katherine Stefanski, Justin Marinko, Charles Sanders
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Peripheral myelin protein 22 (PMP22) is a tetraspan integral membrane protein predominantly expressed in the Schwann cells of the peripheral nervous system. Genetic variation of PMP22 leads to a hereditary neuropathy known as Charcot Marie Tooth (CMT) disease. Among the CMT disease mutant forms of PMP22, the T118M variant is unusual in that it is not 100% penetrant, i.e. not all heterozygous carriers manifest CMT or other related form

of neuropathy. Here, we investigated the cell biological and biophysical properties of T118M to elucidate why T118M predisposes carrier to CMT with incomplete penetrance. Studies with mammalian cell lines showed that the cell surface trafficking efficiency of T118M is between the wild type (WT) and the other mutant forms that cause severe neuropathy. Coexpressing both WT and T118M in mammalian cells did not alter the trafficking efficiency of either protein, implying the absence of a strong interaction between the WT and T118M forms of the protein. Silent mutations of the rare T118 codon to more common threonine codons did not significantly affect the cell surface trafficking efficiency, suggesting that ribosomal pausing is not essential for proper folding and trafficking. NMR spectroscopy revealed that T118M adopts the same folded state and exhibits comparable backbone amide hydrogen exchange rate as the wild type. Moreover, its thermal stability and unfolding rate in the presence of denaturing detergent were also comparable to wild type. Nevertheless, its thermodynamic stability is reduced relative to WT and it is more prone to intracellular retention. Overall, these results suggest that T118M is misfolded to only a modest degree that does not always result in notable neuropathy symptoms for heterozygous carriers.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS204 | Bioinformatic Analysis of GyrB Genes Reveals Localized Differential Enrichments in Psychrophiles and Thermophiles

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Extremophilic proteins must remain both stable and active at their organisms' growth temperatures. Sites on proteins that contribute to extremophilicity may need to be tuned for flexibility in some regions, but stability in others. The goal of our work is to detect local differential specialization across extremophilic clades. Gyrase, an enzyme that negatively supercoils DNA, has many macromolecular interfaces and moving parts and is well distributed among all bacteria. As such, this enzyme is an excellent model system for studying extremophilicity. GyrB, a subunit of gyrase, is of particular interest because it houses the tetrameric enzyme's ATPase domain, which is necessary for the dimerization-driven catalytic cycle of the enzyme as a whole. Here, we present an analysis of GyrB sequences from a phylogenetically diverse set of over 1000 bacteria. A quarter of these genes were hand-annotated to resolve ambiguities in automatic annotations. Multiple sequence alignments, partitioned by the temperature tolerance of the organism, were analyzed for differential conservation using an in-house sliding window protocol. We find differential enrichments of glycines in the DNA binding and cleavage region of psychrophilic GyrB genes, which we hypothesize support flexibility in this highly dynamic region. In contrast, an enrichment of prolines near the ATP-binding pocket of thermophilic GyrB genes suggests a need for greater stability. Our analysis can be used to predict which regions of GyrB drive temperature-dependent protein flexibility, stability, and catalysis.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS205 | Measuring the free energy landscape of individual proteins using nanopores

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Protein conformational dynamics have been conventionally studied as a function of variables such as concentration of chemical denaturants, temperature, and pressure. In principle, the electric field can be another new perturbation parameter to control protein conformational dynamics. We have developed a new method to electrically control the conformational dynamics of the individual proteins during their translocation through solid-state nanopores. This method enables a new way to quantitatively explore the number of metastable/intermediate states, free energy gaps between each state, short-lived hidden conformational sub-states, transition-path times between states, and separation between protein isoforms. The method requires only a localized electric field without any additional requirements such as enzymes, oligo tags, or chemical denaturants. A simple model will be presented to describe the reciprocal relationship between protein folding and protein translocation.

Track: Miniproteins: Are There Really That Many Additional Genes That We Have Been Missing?

ABS206 | Bases that Code Residues Critical for Humanin Function Are Invariant Suggesting a Link between Function and Mitochondrial Ribosome Integrity

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Humanin belongs to a set of peptides known as MDPs, mitochondrial-derived peptides, involved in metabolic signaling and anti-apoptotic roles. The peptides are encoded by mitochondria, but interact outside mitochondria, both intra- and extra-cellularly. The humanin gene MT-RNR2 consists of a short ORF within the mitochondrial 16S ribosomal DNA sequence. Sequence alignment of humanin across all vertebrates revealed two invariant residues, Cys8 and Leu9, which are both critical for humanin function. The bases coding for these two residues are also invariant, implying they are required for ribosome function. In contrast, several other humanin residues show a strong bias for synonymous mutations, indicating selective pressure to conserve these residues, but not the corresponding bases. Intriguingly, despite the evidence of residue conservation, in over a third of vertebrate species, humanin is either a pseudogene or severely truncated. Even in closely related species, rat and mouse for instance, a pseudogene might be present in one species but not the other, suggesting that the pseudogene must have arisen relatively recently. In nearly all species, nuclear versions of humanin genes are also present, known as NUMTs (nuclear mitochondrial DNA). There could be functional redundancy with humanin NUMT peptides as well as other MDPs which could explain the apparent tolerance for pseudogenes, while some sort of recombination process with NUMTs could revert the pseudogene and truncation mutations during evolution. Future work will include examining the other MDPs to see if they show similar patterns of base and amino acid conservation.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS207 | Ultra-potent gene silencing and chromatin biology analysis enabled by chromodomain superbinders

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Dysregulation of protein methylation is implicated in several cancers and developmental defects. Therefore, systematic understanding of protein methylation is necessary to elucidate complex biological processes, identify biomarkers, and enable drug discovery.
Studying protein methylation relies on the use of antibodies, but these suffer from batch-to-batch variations, are costly, and cannot be used in living cells. Chromodomains, modular domains specialized in the recognition of methylated proteins and nucleic acids, are a potential affinity reagent for methylated targets, but their modest affinity severely limits their application.
By combining phage display and rational engineering we developed a general method for greatly enhancing the affinity of all chromodomains of the human Cbx protein family without affecting their binding specificity. Our strategy allowed the development of powerful probes for proteomics, genome-wide binding analysis, and live-cell imaging.
Furthermore, chromodomain superbinders provided a modular strategy to develop ultra-potent CRISPRi repressors that allowed specific and unprecedented gene silencing.
Our results highlight the power of engineered chromodomains to analyze protein interaction networks on chromatin and represent a simple and modular platform for efficient gene silencing.

Track: Protein Phase Separation in Biomolecular Condensates

ABS208 | The functional and molecular determinants of stress-induced mRNA condensation

Hendrik Glauninger, Jared Bard, Caitlin Wong Hickernell, Tobin Sosnick, Edward Wallace, D. Allan Drummond
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Stress-induced condensation of mRNA and protein into massive, heterogeneous clumps is widely conserved across eukarya, yet the function, mechanisms of formation, and how these condensates relate to basal cellular physiology remain largely unresolved. The release of ribosome-free mRNA following stress-induced polysome collapse is considered to be the trigger of stress granule formation by enabling a diverse set of RNA-RNA and RNA-protein interactions with stress granule nucleators. Here, we show that ribosome-free mRNA is insufficient to trigger mRNA condensation, that specific protein interactions with the stalled translation initiation machinery underlie stress-induced mRNA condensation, and that stress-induced transcripts escape condensation and are robustly translated following heat shock. Global translation initiation inhibition, such as following temperature stress or pharmacological depletion of an essential translation factor, causes most mRNA to condense. Reporter transcripts engineered with blocked translation initiation are specifically condensed, even under

unstressed conditions. Additionally, the mRNA of Hac1, the transcription factor underlying the unfolded protein response (UPR), is translation initiation blocked and condensed under unstressed conditions, yet its mRNA escapes condensation, is translated, and the transcriptional UPR is activated following acute heat shock. We find blocking translation initiation via depletion of different translation initiation factors can either trigger condensation or prevent it. Therefore, the generation of ribosome-free RNA is not the key trigger of stress-induced mRNA condensation. Interestingly, the well-translated stress response messages relatively escape condensation during stress, and basal mRNA condensation inversely correlates with ribosome association. In sum, blocked translation initiation causes mRNA condensation even in the absence of stress, which reframes stress granule formation as an accentuation of normal mRNA physiology and suggests a functional role for mRNA condensation in accelerating translation of stress-induced mRNA.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS209 | Reshaping Energy Landscapes to Enhance Protein-Fluorophore Ligand Interactions

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The de novo computational design of proteins with predefined three-dimensional structure is becoming much more routine due to advancements in both force fields and algorithms. However, creating designs with functions beyond folding is more challenging. In that regard, the recent design of small beta barrel proteins that activate the fluorescence of an exogenous small molecule chromophore (DFHBI) is noteworthy. These proteins, termed mini Fluorescence Activating Proteins (mFAPs), have been shown to increase the brightness of the chromophore more than 100-fold upon binding to the designed ligand pocket. The design process created a large library of variants with different brightness levels but gave no rational explanation for why one variant was brighter than another. Here we use quantum mechanics and molecular dynamics (MD) simulations to reveal how molecular flexibility in the ground and excited states determines brightness. We show that the ability of the protein to resist dihedral angle rotation of the chromophore is critical for predicting brightness. Our simulations indicate that the mFAP/DFHBI complex has a rough energy landscape, with an ensemble of macrostates each having widely varying function. This roughness suggests that mFAP protein function can be enhanced by reshaping the energy landscape towards conformations that better resist DFHBI bond rotation. We have found that the presence of specific side chain rotamers is particularly important for determining how well a given macrostate stabilizes the DFHBI. Through directed MD simulations, we have shown that the relationship involves causation and not merely correlation. To enable reshaping of the energy landscape towards highly functional macrostates, we have developed a novel design algorithm coupling MD simulations with Rosetta sequence design calculations. The approach and algorithm are very general, with applications not only in this system but also in prediction of allosterically acting second- and third-shell mutations and eventually enzyme design.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS210 | Binding of the disordered C-terminal Sos domain to its native partner

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Biophysical characterization of disordered proteins is challenging. A common simplification is to the measure the affinity and kinetics of peptides containing only the residues necessary for binding. While disordered proteins lack stable secondary or tertiary structure, we should not assume that transient structure or allosteric effects are absent when looking outside binding sites. Son-of-sevenless, a multidomain signaling protein from Drosophila melanogaster, is critical to the mitogen-activated protein kinase pathway, passing an external signal to Ras, which leads to cell division. Its disordered 24-kDa C-terminal domain is an auto-inhibitor that blocks the activity of the guanidine exchange factor domain (1). The presence of another protein, Drk, is required for Ras activation. Drk contains two Src homology 3 domains, only one of which, the 7-kDa N-terminal SH3 domain, has a modest affinity for the proline-rich binding sites. We utilize 19F nuclear magnetic resonance spectroscopy and isothermal titration calorimetry to quantify the thermodynamics and kinetics of SH3 binding to the strongest sites, individually and together. We compare these results to those for the peptides (2) and find that the full C-terminal domain binds similarly to the peptides. Our approach can be broadly

applied to other systems to allow for the study of other complex, disordered systems.

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Track: Machine Learning in Protein Science

ABS211 | Deep neural language modeling enables functional protein generation across families

Ali Madani, Ben Krause, Subu Subramanian, Benjamin Mohr, James Holton, Jose Olmos, Caiming Xiong, Zachary Sun, Richard Socher, James Fraser, Nikhil Naik
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Generating artificial protein sequences from scratch using artificial intelligence could enable breakthrough solutions for biomedical and environmental challenges. Viewing amino acid sequences as a language, we demonstrate that a deep learning-based language model can generate functional artificial protein sequences across families, akin to generating grammatically and semantically correct natural language sentences on diverse topics. Our protein language model is trained by simply learning to predict the next amino acid for over 280 million protein sequences from thousands of protein families, without biophysical or coevolutionary modeling. We experimentally evaluate model-generated artificial proteins on five distinct antibacterial lysozyme families. Artificial proteins show similar activities and catalytic efficiencies as representative natural lysozymes, including hen egg white lysozyme, while maintaining activity with as low as 31.4% identity to any known naturally-evolved protein. The X-ray crystal structure of an enzymatically active artificial protein recapitulates the conserved fold and positioning of active site residues found in natural proteins. We show our language model's ability to be adapted to different protein families by accurately predicting the functionality of artificial chorismate mutase and malate dehydrogenase proteins. These results indicate that neural language models successfully perform artificial protein generation across protein families and may prove to be a tool to shortcut evolution.

Track: Machine Learning in Protein Science

ABS212 | Computational Optimization of a FLAG-binding Single-Chain Variable Fragment

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The FLAG peptide has been used commercially for decades for the purification of FLAG-tagged proteins, and identification and monitoring of intracellular biomolecules. While the FLAG peptide is one of the most used epitope tags in the scientific community, commercially available antibodies are not ideal for the intracellular fluorescent labeling of FLAG-tagged proteins of interest. A collaborator has previously engineered a single-chain variable fragment (scFv), "FLAG Frankenbody," for single molecule tracking experiments in living cells [1]. An scFv variant with a stronger affinity for FLAG binding would improve performance for viral translation investigation and longer microscopy experiments. Herein, we set out to use computation-guided protein engineering to design scFv variants with predicted higher binding affinity for FLAG. To this end we have been testing computational pipelines that use Rosetta [2] and AlphaFold2 [3]. In the case of Rosetta, we evaluated established "docking" methods [4] as well as $\Delta\Delta G$ prediction [5]. In the case of AlphaFold2, it was possible to predict the binding mode for FLAG by framing peptide binding as a structure prediction problem [6]. Furthermore, we were able to conduct in silico directed evolution where the fitness metric was based on the AlphaFold2 pLDDT confidence scores for the FLAG peptide. Lastly, the most promising anti-FLAG scFv designs are further evaluated using molecular dynamics umbrella sampling.

[1] DOI: 10.1111/gtc.12893
[2] https://doi.org/10.1371/journal.pone.0020161
[3] DOI: https://doi.org/10.1101/2021.08.15.456425
[4] DOI: 10.1002/prot.22716
[5] DOI: 10.1002/pro.2187
[6] DOI: https://doi.org/10.1101/2021.07.27.453972

Track: Imaging & Tracking of Proteins in Space and Time

ABS217 | Engineering ancestral dehalogenases for single molecule imaging

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Across many fields, visualizing complex cellular conditions requires equally sophisticated instrumentation. Recent advances in molecular imaging have enabled the visualization of localized interactions, gene expression, and a multitude of other essential cellular functionalities. Additionally, the development of fluorescent probes, reporter systems, and a range of molecules that enhance these visualization strategies have forever changed the playing field for examining complex microenvironments. However, even some of the brightest and highly specific fluorescent systems are not without their drawbacks, including conflicts with multiplexing, poor visualization of low abundance molecules, and inadequate photophysical properties of the fluorescent device itself. The development of specific ligands in these visualization experiments remains a bottleneck. While recent efforts have focused on improving the fluorescent ligands, a lack of attention has been placed on engineering the protein fusion partner itself, despite its critical role within the protein-ligand imaging assay. In this presentation, we describe a high-throughput approach for engineering haloalkane dehalogenase fusion proteins using libraries of variants based on rigorous phylogenetic analysis, topological features of the enzyme's active site, and computed changes in stability upon mutagenesis. We explore how modification of the enzyme structure, through rationally designed candidate libraries, can harness the evolutionary and biochemical predictions that are essential to evolve this protein-ligand system, specifically for single molecule imaging. While single molecule imaging is continuing to expand as a relatively recent imaging frontier, finding opportunities to improve the experimental design and complexity of imaging experiments is essential to grow our knowledge of disease states, biochemical cascades, regulators of basal cellular functionality, and their unknown facilitators.

Track: Machine Learning in Protein Science

ABS218 | Hallucinating native-like antibodies with deep learning

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Hallucinating native-like antibodies with deep learning
Antibodies recognize and bind an extremely diverse repertoire of antigens via 6 hypervariable loop regions known as the complementarity determining regions or the CDRs. A common goal in antibody engineering to generate a library of CDRs enriched in binders also known as affinity maturation. Experimental methods for affinity maturation are expensive, laborious, and time-consuming and rarely allow the efficient exploration of the full design space. Deep learning (DL) models are transforming the field of protein structure-prediction, engineering, and design.1 While several DL-based protein design methods have shown promise, specialized models for antibody design are highly desirable.2 Our approach aims to fully leverage structural information which is becoming increasingly abundant in the era of highly accurate structure prediction models such as AlphaFold3 (all proteins, multimers) and DeepAb4 (antibodies only). Inspired by the hallucination frameworks that specifically leverage such structure prediction DL models, we propose the FvHallucinator for generating antibody sequences conditioned on a target antibody structure with DeepAb.2,5,6 On a benchmark set of 60 antibodies, the FvHallucinator recovers over 50% of the wildtype CDR sequence on all six CDR loops. At the VH-VL interface, the FvHallucinator designs amino acid substitutions that are highly enriched in human repertoire sequences. Furthermore, when compared to a large experimentally characterized library of CDR H3s of the anti-HER2 antibody,7 trastuzumab, the FvHallucinator designs exhibit high sequence identity to known HER2-binders. We propose a pipeline that screens FvHallucinator designs to obtain a virtually screened library enriched in binders for an antigen of interest. We apply this pipeline to the CDR H3 of the trastuzumab-HER2 complex to generate designs that retain the original binding mode and improve the binding affinity and interfacial properties. Thus, the FvHallucinator pipeline enables fast and inexpensive generation of diverse, structure-conditioned antibody libraries enriched in binders.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS219 | Atypical Structure and Regulation of an Atypical Chemokine Receptor

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ACKR3 is a 7-transmembrane receptor that bears all expected features of a G protein-coupled receptor (GPCR), but does not couple to G proteins and instead is

β-arrestin biased. The receptor shares a common agonist, CXCL12, with the canonical GPCR, CXCR4. Together this trio of proteins drives cell migration in the context of cardiac and CNS development as well as cancer growth and metastasis. Whereas CXCR4 directly promotes migration through G protein activation, one of the best characterized functions of ACKR3 is to scavenge chemokines, which indirectly regulates migration by shaping the extracellular chemokine gradient. Herein we set out to determine the molecular basis for the β-arrestin bias of ACKR3 and the mechanisms of CXCL12 scavenging. In collaboration with the Tesmer lab (Purdue Univ.), we determined the structure of ACKR3 bound to CXCL12 by cryo-EM. The chemokine bound ACKR3 in an unexpected pose and the structures, along with functional experiments, provide insight into the inability of ACKR3 to activate G proteins and its bias for β-arrestin. Scavenging occurs by constitutive internalization and recycling of ACKR3 and concomitant transport of CXCL12 to lysosomes for degradation. Because β-arrestins contribute to internalization of many GPCRs, we investigated their role in scavenging but found that scavenging by ACKR3 was unaffected in β-arrestin knockout cells. Thus any β-arrestin contribution must be to some other fundamental ACKR3-driven process. Instead, we found that GPCR kinase (GRK) phosphorylation was essential. Using GRK knockout cells, we observed that GRK5 acts as the dominant kinase driving chemokine scavenging whereas GRK2 only produced a limited response. However, activation of CXCR4, which liberates Gβγ upon activation of heterotrimeric G proteins, increased the impact of GRK2, which depends on Gβγ for activity. These results suggest a mechanism for GRK-mediated cooperation between ACKR3 and CXCR4 and GPCR-mediated control of ACKR3 activity through GRK2.

Track: Protein Phase Separation in Biomolecular Condensates

ABS221 | The Endo-lysosomal System as a Potential Source for A Oligomers that Potently Induce Tau Missorting

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During amyloid aggregation, different species of multimeric assemblies form and interact with each other by the nature of that process. Due to many of these being transient, but their building blocks staying the same, it is hard to differentiate between the effects the different forms have on the course of the process. It is however established that not all of these forms display the same properties regarding cytotoxicity in their related illness and likely play different roles in the pathogeny of whichever disease they contribute to.

Amyloid-β peptide (Aβ) plays an important role in the pathogenesis of Alzheimer's Disease (AD) and it is widely believed that not the fibrils, which can be found in postmortem brains of AD patients, are the main culprit of Aβ-related symptoms but rather smaller oligomers. We look at a subset of these; metastable, off-pathway oligomers termed AβOs and show that they are more effective than Aβ amyloid fibrils at triggering Alzheimer's disease-related processes as well as propose a physiologically possible formation pathway. To achieve this, a dimeric construct called dimAβ is used which has a higher propensity for oligomerisation than aggregation and therefore allows the isolation of previously difficult to obtain forms.

In addition to Aβ accumulating in the endo-lysosomal vesicles in which the pH is far lower than in the cytoplasm, AβO assembly is accelerated 8,000-fold in these circumstances as monitored by Thioflavin T-kinetics. Due to oligomerisation being a highly concentration dependent process, this means even Aβ42, the most disease relevant form of Aβ, can reasonably form AβOs under physiological conditions. We could also demonstrate that these AβOs target dendritic spines, induce tau missorting and impair neuronal activity, which are all AD-related characteristics.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS222 | Native Ion-Mobility Mass Spectrometry Reveals the Influence of Membrane Mimetics on Protein Structure

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University of Michigan (United States)

Membrane Proteins (MPs) make up ∼30% of the human genome and are responsible for a wide variety of key cellular functions. Despite the significance of MPs in biology; they remain dramatically understudied when compared with cytosolic proteins. In general, membrane mimetics must be used to study MPs ex vivo, posing a range of challenges associated with using standard

structural biology tools to extend our knowledge of MP structure and function. Native ion mobility-mass spectrometry (IM–MS) has recently enabled the investigation of MP stoichiometry, interactions, lipid binding, and stability, but the impact of individual mimetics on MP IM-MS data remains relatively unknown. We selected three types of MPs with diverse structures and functions, including: the monotopic Cytochrome P450 (CYP) 3A4, the transmembrane wild-type Peripheral Membrane Protein 22 (PMP22), the L16P mutant of PMP22, and the small multidrug resistance transporter GDX. These four proteins have previously been studied in either detergent micelles or nanodiscs (ND)s, however a comprehensive evaluation of their collision cross-sections (CCSs), stabilities, unfolding pathways, oligomerization, and lipid binding selectivity across membrane mimetic classes has not been previously reported. We have successfully developed and optimized protocols for incorporating all of these MPs in detergent micelles, bicelles, and nanodiscs. Our IM-MS and collision induced unfolding (CIU) stability data reveals structural differences through their unfolding pathways in PMP WT across each mimetic probe. For example, in the case of GDX, our results indicate a preference in nanodiscs for the binding of selected lipids. In the case of PMP22, we have observed significant changes in the monomer-dimer populations when comparing data acquired from protein stored in micelle and bicelle environments. In this presentation, we will further discuss our most recent native IM-MS data on membrane mimetics, their impact on MP structure, and generalized protocols for their implementation for IM-MS studies more broadly.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS223 | Understanding the Function of UCHL3

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Breast cancer is the second leading cause of cancer death in women, and it is closely related to a defect in DNA double-strand break (DBS) repair through homology recombination (HR) pathway. Impaired HR can trigger genome instability and result in cancer progression. Recent studies have shown that one of the key regulators in the HR is Ubiquitin C-terminal Hydrolase L3 (UCHL3). While UCHL3 is overly expressed in breast cancer cells, its precise role in HR is poorly elucidated. Like most signal transduction cascades, HR involves a series of reversible ubiquitin- and phospho-transfer events. In preliminary studies, a cell line deficient in UCHL3 was generated and we observed an aberrant persistence of protein phosphatases, DNA damage proteins, as well as impaired HR. These results have led to the hypothesis that UCHL3 modulates the HR by interacting with HR protein factors, and the interaction is dependent on its enzymatic activity. Using a combination of global proteomics, targeted mutagenesis, unnatural amino acid incorporation, and various biochemical assays, we have identified that phosphorylation is crucial for UCHL3 enzymatic activity. We will then use hydrogen-deuterium mass spectrometry, NMR, and molecular docking to demonstrate how a specific phosphorylation site on UCHL3 affects its conformation and results in a hindered interaction between UCHL3 and its ubiquitinated substrates. Upon completion of this project, we will be able to explore and validate UCHL3-interacting HR protein factors, and understand the fundamental UCHL3 regulation in HR upon its PTM. This information will also shed lights on developing therapeutic treatment for individuals with breast cancer.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS224 | Tertiary motifs as building blocks for the design of protein-binding peptides

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MIT (United States)

Despite advances in protein engineering, the de novo design of small proteins or peptides that bind to a desired target remains a difficult task. Most computational methods search for binder structures in a library of candidate scaffolds, which can lead to designs with poor target complementarity and low success rates. Instead of choosing from pre-defined scaffolds, we propose that custom peptide binding structures can be constructed to complement a target surface. Our method mines tertiary motifs (TERMs) from known structures to identify surface-complementing fragments or "seeds." We combine seeds that satisfy geometric overlap criteria to generate peptide backbones, and score the backbones to identify the most likely binding structures. We found that TERM-based seeds can describe known binding structures with high resolution: the vast majority of peptide binders from 486 peptide-protein complexes can be covered by seeds generated from single-chain structures. Furthermore, we demonstrate that known peptide structures can be

reconstructed with high accuracy from peptide-covering seeds. As a proof-of-concept, we used our method to design 100 peptide binders of TRAF6, seven of which were predicted by Rosetta to form higher-quality interfaces than a native binder. The designed peptides form three structural groups that each interact with a distinct site on TRAF6, including the native peptide-binding site. These results demonstrate that known peptide binding structures can be constructed from TERMs in single-chain proteins and suggest that TERM information can be applied to efficiently design novel target-complementing binders.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS225 | The role of protein oligomerization on CP-receptor signalin

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Calprotectin (CP) is a heterodimer of the S100A8 and S100A9 EF-hand calcium binding proteins, which activates cell surface receptors that signal through the NF-κB inflammatory signaling pathway. In the case of irritable bowel disease (IBD) these pathways are overstimulated, which leads to damage of the gastrointestinal tract and disease symptoms. Our laboratory is investigating the structural mechanism of receptor activation by CP and exploring the potential therapeutic value of targeting CP with small molecule inhibitors. Here we describe studies to determine the role of calcium-induced tetramerization to CP in receptor activation. Like all S100 proteins, calcium-induced conformational changes in CP are required for binding to partner proteins. However, in the case of CP, the addition of calcium correlates with the formation of dimers of dimers (heterotetramers) and other higher order oligomeric states. In order to investigate the biological relevance of CP tetramerization and facilitate in-depth biophysical and structural analysis, we have prepared two single-site and mutations of hydrophobic residues mediating the tetramer interface (Ile60, Ile73) and the corresponding double-site mutant. With the goal of maximally destabilizing the tetramer interface, these Ile residues were mutated to Lys (I60K, I73K). All three mutants were expressed and purified. NMR as well as DLS data confirms that the mutations do not affect heterodimer formation and that addition of calcium does not induce tetramerization. We also report on progress with crystallization and determination of x-ray crystal structures of the mutants to better understand the molecular basis for tetramerization and optimize the properties for analysis of the effects on inflammatory signaling.

Track: Protein Phase Separation in Biomolecular Condensates

ABS226 | Preservation of orphan ribosomal proteins during stress in chaperone-stirred condensates

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Maintenance of a homeostasis of cellular proteome is vital for organismal life which is achieved by a coordinated process of protein synthesis, folding, degradation and trafficking. Cells encounter frequent assaults to the integrity of its proteome either from the fluctuating environment or because of development or disease. Organisms across the tree of life possess an inbuilt capacity to adaptively respond to these primordial stresses. Heat shock response (HSR) has been implicated as an evolutionary conserved cell protective response across the stressors. Recently, nucleolus has emerged as a hub for protein quality control and a nexus of regulation for the HSR. Yet, newly synthesized proteins – presumed to be localized near ribosomes in the cytosol – have been implicated as a major category of HSR activators. Here we reconcile these observations by showing that, upon heat shock and other stressors, newly synthesized ribosomal proteins (nsRPs) condense at the nucleolar periphery and activate the HSR. Mechanistically, we find that nsRPs recruit the J-domain protein Sis1 away from the nucleoplasm, thereby leaving the transcription factor Hsf1 free from repression by high-affinity Hsp70. Remarkably, these peri-nucleolar nsRP condensates are reversible post heat shock, with the nsRPs being exported to the cytosol and get matured to a functional ribosome. We further show that Hsp70/Sis1 system function as molecular stir-bar to keep the stress induced nsRP condensates in highly dynamic liquid form. Acute inhibition of ribosomal protein production prevented Sis1 peri-nucleolar localization and diminished HSR output following heat shock. DnaJB6, a human homolog of Sis1, likewise localized to peri-nucleolar structures upon heat shock in human cells, but not in cells treated with Torin 1 to block ribosomal protein synthesis. We propose that nsRP condensates drive HSR activation across stressors and define peri-nucleolar outposts of protein quality control.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS227 | Optimization of Aerococcus viridans lactate oxidase for direct electron transfer in biosensors

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The use of Lactate oxidase (LOx) for detecting lactate in biosensors has caught attention in recent years, both for clinical diagnosis and the control of fermentative processes. However, disadvantages such as the use of high potentials or the use of redox mediators to measure the reactions limit their efficiency and applicability, appearing as problematic in the lactate sensors that are currently available. In this work, two alternatives have been proposed to optimize Aerococcus viridans LOx (AvLOx) to allow direct electron transfer (DET); the first one consists in opening the active site of the enzyme for the nucleation of platinum nanowires from the active site to the electrode surface. The second one directly anchors the protein on gold surfaces, promoting DET. For the first strategy, arginine 181 (R181) was modified by a hydrophobic residue. At the same time, for the other method, a cysteine was introduced in substitution for a residue on the surface and within 20 Å of the isoalloxazine ring of FMN. Platinum nucleation mutants were R181A, R181I, and R181V; K188C was constructed for attachment to gold surfaces. Mutations were introduced by mutagenic PCR; proteins were expressed and purified in 2 chromatographic steps, obtaining a pure enzyme. Of the R181 mutants, R181V showed the best activity after purification. In addition, a km was obtained in the same order of magnitude as the wild type; however, platinum nucleation has yet to be evaluated. On the other hand, the enzymatic activity of the K188C mutant was comparable to the wild type, and activity was conserved after anchoring to the electrode by a thiol-gold bond. The enzyme-immobilized electrode (K188C) had an amperometric response dependent to lactate concentration without the use of any mediator. These results demonstrate a promising strategy for DET in biosensors.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS228 | Rational Design of Improved and Novel Photodissociable GFPs and RFPs

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Photodissociable Dronpa (pdDronpa) is a synthetic and cofactor-independent photoactive protein that finds use as an optogenetic tool for perturbing and interrogating intracellular signaling pathways. In its bright fluorescent ON-state, pdDronpa is a dimer, while illumination with cyan light leads to cis/trans isomerization, chromophore protonation, and formation of a metastable non-fluorescent monomeric OFF-state. Using microfluidic diffusional sizing, we have quantified the binding affinities of the ON and OFF states of pdDronpa, finding that pdDronpa is efficiently dissociated after isomerization of one protomer unit. Furthermore, by structure-guided redesign of intraprotomer and interdimer H-bond networks we have developed pdDronpa2 that undergoes efficient switching and improves the dark/light affinity change by ∼20-fold for a final Kd(OFF)/Kd(ON) ratio of >1000. Finally, by extrapolating our knowledge from pdDronpa, we have created a novel photodissociable RFP by grafting the dimer interface of pdDronpa onto a stable monomeric RFP (10+ rational mutations) and inserting internal mutations for reversible photoswitching. Our results indicate that formation of a non-protonated metastable trans-isomer of the RFP chromophore results in sufficient interface reorganization to cause dimer dissociation at a level comparable to pdDronpa1. This novel pdRFP extends the color palette of the intracellular optogenetic toolbox and may, for example, find use as a tool for optogenetic multiplexing.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS229 | Engineering a protein crystal scaffold for modular installation of enzymes

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Covalent bonding by the isopeptide formation of SpyTag/SpyCatcher protein linker system has been previously utilized for micro- and nanotechnology applications, including enzyme immobilization and surface display of SARS-CoV-2 Spike glycoprotein. This experimental work is focused on the application of the SpyCatcher-SpyTag system within protein crystal pores. Campylobacter jejuni (CJ) protein crystals possess large (∼13 nm) solvent channels, possess a distinctly honeycomb-like architecture, and can routinely be grown between 360 nm and 0.5 mm

across. While non-covalent installation of enzymes has been demonstrated previously for these crystals (DOI: 10.1039/C8BM01378K, 2019), covalent site-specific installation of a functional guest remains an outstanding challenge. We have demonstrated successful capture of a sfGFP-SpyCatcher003 fusion protein, using CJ crystals doped with a CJ-SpyTag003 fusion protein. Current experimental work focuses on covalently capturing NanoLuciferase-SpyTag and NanoLuciferase-SpyCatcher fusions with CJ-SpyCatcher and CJ-SpyTag-doped crystals, respectively, and demonstrating functionality of the installed guest luciferase. These experimental results will also be recapitulated using doped CJ microcrystals. Due to the inherently modular design, this platform has broad potential for biosensing, and as a recyclable catalyst for multistep biosynthesis.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS230 | Design of highly-specific inhibitors for matrix metalloproteinases

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Matrix metalloproteinases (MMPs) have long been known as key drivers of various diseases, including cancer, neurodegenerative, and cardiovascular, making these enzymes attractive targets for drug design. Engineering selective inhibitors based on tissue inhibitors of metalloproteinases (TIMPs), endogenous human proteins that tightly yet nonspecifically bind to MMP family members, represents a promising new strategy for therapeutic development. TIMP-2 is one of the four humans TIMPs that uses three loops to interact with the highly conserved active site of the enzyme and less conserved surrounding residues. To supply TIMPs with high binding specificity toward one MMP type, we explore a new engineering strategy where we extend one of the TIMP-2's loops, allowing it to interact with non-conserved MMP surface. A library of computationally-designed TIMP2 variants with longer loops was constructed, expressed in the yeast surface display setup and sorted for high binding to the target MMP-14 and low binding to off-target MMP-3. Next generation sequencing (NGS) of the two selected populations followed by comparative analysis of the data yielded hundreds of MMP-14-specific TIMP2 variants. A few most promising variants were expressed, purified and tested for their ability to inhibit MMP-14 and off-target MMPs. Our best TIMP2 variant exhibited 29 pM binding affinity to MMP-14 and 2.4 μM affinity to MMP3, showing 7500-fold improvement in binding specificity compared to WT-TIMP2. Furthermore, the same variant showed 53-fold improvement in binding specificity toward MMP-14 relative to MMP-9, while the later off-target was not explicitly used for negative selection. This study demonstrates that introduction of loop extensions into inhibitors to stretch to the non-conserved surface of the target proteins is an attractive strategy for therapeutic protein design. The strategy presented here could be used not only in design of various TIMP-based MMP inhibitors but also in design of other therapeutic proteins.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS231 | Structural Insight into Halofilicity and Substrate Specificity of Glucose-6-phosphate Dehydrogenase from the Haloarchea Haloferax volcanii.

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Halobacteria archaea thrive in habitats with high salt concentrations, and to date, they are the only group of archaea in which a Glucose-6-phosphate dehydrogenase (G6PDH) has been described that could participate in the Entner-Doudoroff pathway. Structurally, Haloarchaeal proteins have adapted to high salt concentrations by reducing their hydrophobic core and increasing the proportion of negative residues at their surface. However, to date, there are no reports addressing the effect of salt on the dynamics of these halophilic proteins. On the other hand, the only halophilic G6PDH characterized is from Haloferax volcanii (HvG6PDH), which belongs to the short-chain dehydrogenases (SDR) superfamily, and not to the canonical G6PDH family. In this work, we determined that HvG6PDH is a strictly halophilic enzyme whose activity significantly decreases below 1.0 M salt due to a diminution in kcat values, while the KM for both substrates is not affected by different salt concentrations. To have a structural view of this behavior, we modeled the HvG6PDH with glucose-6P and NAD+ at the active site. The model exhibits the common characteristics described for halophilic proteins. Nonetheless, molecular dynamics simulations performed at high (1.5 M) and low (0.12M) KCl concentration showed differences in their conformational dynamics potentially related to the low kcat at low salt concentrations. Simulations at low salt

concentration result in a more swollen protein and regions with higher RMSF when compared to simulations at high KCl. In addition, phylogenetic analysis with close homologs shows that HvG6PDH is related to other sugar dehydrogenases, identifying a characteristic motif (NLTXXH) of G6PDH from Haloarchaea that would be involved in the specificity for Glucose-6P (Fondecyt 1191321).

Track: Protein Science Addressing Health Disparities

ABS232 | An Investigation into How Glycosylation Mediates SARS-CoV-2 Infection

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Objective: To investigate the role of specific SARS-CoV-2 spike glycans on DC-SIGN-mediated trans infection of SARS-CoV-2.

Background: The Covid-19 pandemic remains a significant challenge for society. The mechanism of infection -as well as the discovery and assessment of SARS-CoV-2 entry inhibitors- remains a critical scientific query.

SARS-CoV-2 infects cells by using its surface spike protein to bind to a cellular receptor called hACE2. However, this is seemingly at odds with clinical observations: despite lung tissue only modestly expressing hACE2, the cells of the respiratory system are extremely vulnerable to SARS-CoV-2 infection.

This conundrum was likely solved with the recent demonstration that lectin proteins (such as DC-SIGN) on the surface of certain cells can bind glycans on the SARS-CoV-2 spike and "present" the virus, thereby facilitating efficient infection of cells that poorly express hACE2. This so-called trans infection process is likely how many low-hACE2 cells in the body become infected.

We explored the role of SARS-CoV-2 spike glycans on DC-SIGN-mediated trans-infection by mutating several glycosylation sites and assessing these mutants in pseudoviral infectivity assays. We then identified two likely SARS-CoV-2 inhibitors and tested them on our trans assay system: 1) Griffithsin (Grft), a soluble high mannose binding lectin that potently inhibits a wide variety of viruses, and 2) Mannan, a polymer of high-mannose sugars that acts as a competitive inhibitor of mannose binding.

Results: Removing glycans on SARS-CoV-2 spike protein leads to pseudoviral variants with notable differences in infectivity (in both canonical and trans-infection models), as well as different susceptibilities to mannose inhibitors. Grft also displayed different potencies in inhibiting canonical infection versus trans-infection.

Track: High Throughput Protein Science

ABS233 | Cold spots are universal in protein-protein interactions

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Proteins interact with each other through binding interfaces that differ greatly in size and physico-chemical properties. Within the binding interface, a few residues called hot spots contribute the majority of the binding free energy and are hence irreplaceable. In contrast, cold spots are occupied by suboptimal amino acids, providing possibility for affinity enhancement through mutations. In this study, we identify cold spots due to cavities and unfavorable charge interactions in multiple PPIs. For our cold spot analysis, we first use a small affinity database of PPIs with known structures and affinities and then expand our search to nearly four thousand homo- and heterodimers in the PDB. We observe that cold spots due to cavities are present in nearly all PPIs unrelated to their binding affinity, while unfavorable charge interactions are relatively rare. We also find that most cold spots are located in the periphery of the binding interface, with high-affinity complexes showing fewer centrally located colds-spots than low-affinity complexes. A larger number of cold spots is also found in non-cognate interactions compared to their cognate counterparts. Furthermore, our analysis reveals that cold spots are more frequent in homo-dimeric complexes compared to hetero-complexes, likely due to symmetry constraints imposed on sequences of homodimers. Finally, we find that glycines, asparates, and arginines are the most frequent amino acids appearing at cold spot positions. Our analysis emphasizes the importance of cold spot positions to protein evolution and facilitates protein-engineering studies directed at enhancing binding affinity and specificity in a wide range of applications.

Track: Protein Phase Separation in Biomolecular Condensates

ABS234 | Sequential Activation and Partial Domain Unfolding Control Pab1 Condensation

Tobin Sosnick, Ruofan Chen, Julia Shangguan, Darren Kahan, Joshua Riback, D Allan Drummond

Education (United States)

Poly(A)-binding protein (Pab1 in yeast) is a canonical stress granule marker. Upon heat shock or starvation-induced acidification, Pab1 condenses from the cytosol into foci. In vitro, Pab1 likewise condenses above Tdemix = 40 °C or at acidic pH (Riback, Katanski et al., Cell 2017). Pab1 contains four RNA recognition motifs (RRMs) connected by linkers, followed by an intrinsically disordered P-domain and a C-terminal domain. Previous work found that the P-domain is not required for condensation whereas the RRMs are necessary and sufficient. Here we identify the molecular factors in the Pab1 structure that enable it to transduce cellular stress signals and condense. A central challenge for conducting mechanistic studies of condensates is that obtaining high-resolution structural information is impeded by the condensation process itself. We overcome this issue using Hydrogen-Deuterium Exchange/Mass Spectrometry (HDX-MS) as it can probe the hydrogen bond network at the residue level even in insoluble systems. According to HDX-MS applied to both the heat and pH-induced condensates, the individual RRMs exhibit different levels of partial unfolding with RRM3 remaining largely folded. Additional demixing data from engineered constructs containing multiple copies of each RRM complement the HDX results and indicate that the RRMs do not participate equally in condensation. We propose a "sequential activation" model where each RRM is activated at different temperatures, executes partial unfolding and associates only with activated RRMs on other Pab1 molecules to form the condensate, a mechanism we term "thermodynamic specificity".

Track: High Throughput Protein Science

ABS235 | Sequence Space Neighborhoods of GFP-like Proteins Show Altered Robustness and Diversity over Evolutionary Time

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Evolving proteins traverse sequence space. As they do so, the "neighborhood" of accessible protein functions changes, shaping the evolutionary potential of the evolving protein. Understanding how mutations alter the accessibility of new functions is important, both for understanding natural evolution and for engineering new protein function. To better understand how the functional neighborhoods of proteins change as they evolve, we are studying the evolution of GFP-like proteins from the coral Montastrea cavernosa. Previous work showed the ancestral protein was green, but then acquired a photoconvertible green-red phenotype through substitutions scattered throughout the protein structure. We have developed a protocol that combines random mutagenesis, flow cytometry, and high-throughput sequencing to measure the frequency of different colors—green, orange, and red—up to six mutations away from a central genotype. Using this method, we found that the neighborhood of the constitutively green ancestor contains many more functional proteins than that of a photoconvertible derived protein. Despite being less mutationally robust, however, the neighborhood surrounding the evolved protein showed much greater phenotypic diversity than the ancestral protein. This hints at a tradeoff between accessible phenotypic diversity and robustness. We further found that we could largely recapitulate this change in neighborhood by introducing a single historical substitution, revealing that a single mutation can dramatically change the local neighborhood of phenotypes accessible to an evolving protein. In vitro biophysical characterization has revealed that protein stability only modestly correlates with the composition of these neighborhoods, suggesting these effects are due to specific changes to the environment surrounding the chromophore. Taken together, this work reveals how relatively subtle changes to protein properties can profoundly alter the accessibility of functions available to an evolving protein.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS236 | Archaeal glycogen phosphorylase activity in the absence of pyridoxal 5′-phosphate

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Glycogen phosphorylases (GP) catalyze the breakdown of α-1,4 glycosidic linkages in glucose polymers. All phosphorylases reported to date contain the essential cofactor pyridoxal 5′-phosphate (PLP) linked to a conserved lysine of the active site through a Schiff base. The reaction constitutes the rate-limiting step in glycogenolysis allowing the mobilization of glucose for energy upon nutrient depletion conditions.

The storage of glycogen has been demonstrated in organisms ranging from eukarya to archaea. To analyze the diversity and characteristics of GPs we constructed a

multiple sequence alignment and inferred a phylogenetic tree for the family by the maximum likelihood method. The analysis shows strict conservation of the active site residues in all the phosphorylases sequences analyzed. However, in the methanogenic order Methanococcales of archaea, a mutation of the lysine involved in PLP binding to threonine was observed. To analyze the properties of glycogen phosphorylases from Methanococcales, the enzyme from the organism M. maripaludis (Mm-GP) was characterized. Absorbance and fluorescence analysis indicates that Mm-GP does not contain the PLP cofactor. The enzyme shows generation of the product reaction glucose 1-phosphate in the absence of PLP, has a preference for large glucose polymers like glycogen and a specific activity equivalent to the previously characterized enzymes from archaea and bacteria. 3D structure prediction of Mm-GP with AlphaFold shows a similar fold organization with absence of large segments at the N and C terminal compared to the eukaryal and bacterial structures. Taken together, our analysis indicates that glycogen phosphorylases from Methanococcales catalyze the phosphorolytic breakdown of α-1,4 glycosidic bonds in the absence of pyridoxal 5′-phosphate and raise a key question regarding the reaction mechanism of this enzyme (Fondecyt 1191321).

Track: Protein Phase Separation in Biomolecular Condensates

ABS238 | Protein Refoldability Explains the Role of Disordered Regions and Biomolecular Condensation in Yeast Proteostasis

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Intrinsically disordered regions (IDRs) are portions of proteins that do not form folded globular structures. There are an increasing number of examples demonstrating that one function of IDRs is to support multivalent interaction networks that promote phase separation. In yeast, protein condensation under heat-stress conditions appears to play a major role in maintaining proteostasis. Here, we interrogate the effect of IDRs on a protein's refoldability (capacity to return to native structure following denaturation) and condensability using a combination of limited-proteolysis mass spectrometry (LiP-MS) and fluorescence microscopy. In LiP-MS, we take advantage of a permissive protease that preferentially cleaves at exposed regions to examine structural differences between the native and refolded forms of proteins. We find yeast proteins are surprisingly efficient refolders – significantly more refoldable than typical E. coli proteins – especially those with higher disorder and those that phase separate during heat shock. These observations suggest a model that disordered regions, especially when they separate globular domains, enhance proteins' refoldability. We test this theory further by engineering the inter-domain linkers in two yeast proteins, and observe a correlation between disorder, refoldability, and condensability. These results suggest that one general role of disordered regions is to promote the refoldability of multi-domain proteins in eukaryotic proteomes.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS239 | Kinetic characterization and evolutive trajectory of the allosteric regulation by AMP of archaeal ADP-dependent Sugar Kinases

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It is generally accepted that allosteric regulation is almost absent in archaea and instead enzyme regulation is exerted mainly by transcriptional control. However, some examples of allosteric regulation have been reported in the last years, including a report from our group that the bifunctional enzyme from Methanococcus maripaludis (MmPFK/GK) is activated by its reaction product AMP. This enzyme carried out both glycolytic reactions, named phosphorylation of glucose (GK) and phosphorylation of fructose-6-P (PFK) at the same active site and both activities are activated by AMP. To understand this phenomenon, we performed a comprehensive kinetic characterization, including the determination of the kinetics, substrate inhibition, and AMP activation mechanism of this enzyme. MmPFK/GK has an ordered sequential mechanism where MgADP is the first substrate to bind to the enzyme and AMP is the last product to leave. Activation by AMP of both activities occurs by an increase in the affinity for the sugar substrate being this effect higher in the GK than in the PFK activity. By comparison of the AMP activation in extant homologs enzymes, as well as in the resurrected ancestors of this enzyme family, we determined that AMP activation is an ancestral trait correlated with bifunctionality but is lost during the evolutionary trajectory toward specific GK or

PFK enzymes. Employing bioinformatics protocols based on docking and molecular dynamics we identified putative allosteric sites able to bind and stabilize the AMP-protein interactions, estimate the binding free energy of the complex and recognize key residues for this interaction (Fondecyt 1191321).

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS240 | Determining the structural basis for function of RADX

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Replication fork remodeling is crucial to maintaining the integrity of our genome, as it is required to overcome fork stalling arising from various replication blocks. The ssDNA binding protein (SSB) RAD51 plays a central role in fork remodeling events and, since it is widely involved in DNA replication, recombination and repair, must be tightly regulated. RADX has been identified in the Cortez lab as a strategic factor regulating RAD51 activity, functioning in apposition to BRCA2. In BRCA2 deficient cancer cells, RADX inactivation is sufficient to restore fork protection and confer chemoresistance. The regulation by RADX is context-dependent – in the absence of replication stress it inhibits fork reversal by destabilizing RAD51 nucleoprotein filaments, while generating reversed forks in the presence of persistently stalled forks. In vitro, RADX prevents RAD51-ssDNA filament formation and disassembles existing filaments by accelerating the hydrolysis of RAD51 bound ATP and the subsequent disengagement from ssDNA. Single molecule studies reveal condensation of ssDNA by RADX, which promotes displacement of the ubiquitous SSB RPA and the loading of RAD51.

Our objective is to elucidate the mechanism of action of RADX and its function within the RAD51-RADX-RPA-substrate network. RADX has been shown to interact directly with RAD51 using pull-down assays. NMR analysis revealed the N-terminus of RADX interacts with the RPA70 N-terminal, A and B domains. Using an assay to quantify the effect of different RADX constructs on RAD51-ssDNA filament formation, we found that both the ssDNA and RAD51 binding activities of RADX are essential to its ability to inhibit Rad51 filaments. We are therefore investigating binary and ternary complexes of RADX with RPA and RAD51 on DNA substrates. Understanding the structural basis for the function of RADX gives us insight into a potentially important determinant of chemosensitivity in cancer cells, allowing for the development of more effective cancer therapeutics.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS241 | Discovery of Human and Non-Native Binders of Pro-Apoptotic BAK Reveal Diverse Peptide Sequences Can Directly Activate or Inhibit BAK-Mediated Membrane Permeablilization

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BAK is one of the two pro-apoptotic members that form part of the Bcl-2 protein family. Previous work has shown that binding of certain BH3-only proteins such as truncated BID (tBID), BIM, and PUMA to pro-apoptotic BAK leads to mitochondrial outer membrane permeabilization (MOMP), the release of cytochrome c, and ultimately cell death. The BH3 binding event leads to a series of conformational changes that promote the conversion of BAK from monomer to dimer and subsequently to oligomers that disrupt membranes in a process referred to as activation. Putative intermediate crystal structures, crosslinking data, and in vitro functional tests have provided insights into the activation event, yet the sequence-function relationships that make some, but not all, BH3-only proteins function as activators remain largely unexamined. To address this question, we used computational design, yeast surface-display screening of candidate BH3-like peptides, and structure-based energy scoring to identify ten new binders of BAK that span a large sequence space. Among the new binders are two peptides from human proteins BNIP5 and PXT1 that promote BAK activation in liposome assays and induce cytochrome-c release from mitochondria in HeLa cells. These new activators expand current views of how BAK-mediated cell death can be triggered. We measured the binding kinetics and solved crystal structures of BAK-peptide complexes, including complexes for two inhibitors and one activator. Our results reveal a high degree of similarity in binding geometry, affinity, and association kinetics between peptide activators and inhibitors, including peptides described previously and those identified in this work. We propose a free energy model for BAK activation that is based on the differential engagement

of BAK monomers and the BAK activation transition state that integrates our observations with previous reports of BAK binders, activators, and inhibitors.

Track: Machine Learning in Protein Science

ABS242 | Affibody Sequence Design Using Deep Learning Generative Models

Zirui Wang, Mehrsa Mardikoraem, Daniel Woldring
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Small non-immunoglobulin protein scaffolds provide unique advantages over traditional antibodies (150 kDa), offering efficient biodistribution and rapid clearance for molecular imaging and immunotherapeutics. The three-helix bundle affibody scaffold (6 kDa) has shown tremendous promise for targeting a diverse collection of disease biomarkers such as HER2, yet the enormous sequence space makes it challenging to engineer these proteins to selectively bind new targets. To overcome this challenge, generative models which learn data patterns and generate new data instances, provide a faster and more efficient route for protein sequence design by circumventing random mutagenesis. Here, we implement variational autoencoders (VAEs) and attention-based models (transformers) to generate libraries of affibody variants with improved stability and fitness. Using deep sequencing data from high-throughput yeast surface display experiments to train our models, we treat the affibody amino acid sequence as parameterized distributions or a unique language that can be contextualized for predicting beneficial mutations. The VAEs use a Bayesian probabilistic modeling approach to maximize likelihood of output sequence. The encoder encrypts inputs into means and variances. The decoder generates outputs from vectors sampled from the distributions with learned means and variances. The bidirectional autoregressive transformer processes sequences through mapping attentions among amino acids. The encoder is trained by corrupting valid affibody sequences and minimizing cross-entropy loss between the corrupted tokens and original tokens. The training of the decoder and the fine-tuning of the encoder occur jointly using high binding affinity affibodies. To measure model performance, we use order statistics of amino acids at each position and Hidden Markov Model score to analyze the similarity between training and generated sequences. We expect that a library of proteins designed using generative models will outperform a library of randomly diversified proteins in the context of engineering high-affinity proteins for clinical applications.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS243 | Structure Determination for Computationally Designed Mutants of Aspartate Aminotransferase

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Computational protein design enables the modification of natural proteins to perform new or altered functions. For example, a novel multi-state design method was recently used to create variants of the enzyme aspartate aminotransferase with tailored substrate specificity by tuning the conformational ensemble to favor either a "closed" or an "open" conformation. Previously, it was shown that a preference for either of these two conformations results in different substrate specificities. Therefore, the ability to engineer control over the enzyme conformational ensemble allows for rational tuning of function. To validate the design strategy, we used multi-temperature x-ray crystallography (5-30 °C) to determine the structures of the designed proteins. The resulting models confirmed that the intended conformational preferences were achieved. In addition to validating the computational protein design strategy for manipulation of the enzyme conformational ensemble, our structures further demonstrate the power of non-cryogenic x-ray crystallography for exploring protein conformational ensembles in atomic detail.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS244 | Exploration of Surfactant Protein D Interacting with Trimannose Using Computational Techniques

Mona Minkara
Northeastern University (United States)

Pulmonary surfactant (PS), a critical mixture of proteins and lipids found in the lungs at the air-liquid interface, plays an important role in human health. PS is essential for maintaining lung function, including the expansion and contraction of the alveoli needed for healthy breathing and acts as a first line of defense against airborne pathogens that enter our lungs as we breathe. This talk focuses on the immunoprotein surfactant protein D (SP-

D), the subject of experimental studies due to its immunological response against glycan-containing pathogens, including SARS-Cov-2 and influenza. While these studies demonstrate the critical role of SP-D in lung health, further investigations are needed to understand the interactions. We use computational techniques such as Monte Carlo and full-atomistic molecular dynamics (MD) simulations to provide molecular-level insight into SP-D's binding interactions with the glycan trimannose.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS245 | Mapping protein dynamics at high-resolution with temperature-jump x-ray crystallography

Alexander Wolff, Eriko Nango, Iris Young, Aaron Brewster, Michihiro Sugahara, Takanori Nakane, Kazutaka Ito, Raymond Sierra, Fumiaki Yumoto, Kensuke Tono, So Iwata, Nicholas Sauter, James Fraser, Michael Thompson
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Visualizing protein dynamics at an all-atom level is one of the hallmark challenges for structural biology. Time-resolved crystallography experiments offer a window into these motions, yet perturbing the intrinsic dynamics of proteins in a broadly applicable manner has remained difficult. Here we couple a solvent-based temperature-jump with time-resolved crystallography to visualize structural dynamics in lysozyme. Motions evolved over the course of the experiment, initiating as widespread atomic vibrations and transitioning over time towards localized structural fluctuations coupled to the protein's active site. Furthermore, inhibitor binding altered these dynamics by blocking key motions that allow energy to dissipate from vibrations into functional movements coupled to catalysis. Moving forward, we anticipate that this method will be broadly applicable for the investigation of protein structural dynamics.

Track: High Throughput Protein Science

ABS248 | Indigo formation capacity for predicting substrate promiscuity of large-scale P450 BM3 libraries

Jonathan Besna, Donya Valikhani
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Biocatalytic routes to chemically challenging syntheses have been significantly developed in recent years. Among these, cytochrome P450 oxidases (P450) have been shown to promote chemically challenging oxidations. The P450 from Bacillus megaterium (P450 BM3) natively hydroxylates non-activated C-H bonds of long-chain fatty acids using NADPH as an electron source and O2 as an oxidant. P450BM3 has been engineered for C−H activation of a wide variety of non-natural substrates including short-chain fatty acids, aromatic compounds, terpenes, cyclic and acyclic alkanes. Given the potency of P450s for industrial chemical synthesis, a comprehensive study on large-scale P450 libraries is needed to unlock enzymatic specificity towards promiscuity. This requires a fidelitous links between massive datasets for phenotypic and genotypic characterization. Efficient high-throughput screening methods will enable discovery of variants displaying yet broader substrate promiscuity. Colorimetric high-throughput screening at the whole-cell level to observe the conversion of indole to indigo has previously identified a correlation with increased substrate promiscuity in small number of P450 variants.

To expand the diversity of variants of P450BM3 that exhibit this trait, we screened a library of 133 functional variants of P450 BM3 for their capacity to convert indole to indigo, and for substrate promiscuity towards oxidation of anisole and naphthalene. Among 80 indigo-forming variants and 53 non-indigo producing variants identified by screening on solid media to detect indigo-forming variants (blue E. coli colonies), 70-80% of indigo-forming variants performed better than WT for the promiscuous aromatic hydroxylation reactions relative to 35-45% of the functional, non-indigo-forming variants. Notably, variants showing □ 3-fold enhancement of activity towards non-native substrates correlated with indigo formation. Overall, cost-effective, sensitive, primary screening by colony-based colorimetric observation has allowed selection of a library of indigo-forming point-mutated variants of P450BM3 displaying improved aromatic oxidation. The breadth of substrate classes that correlates indigo formation will be further investigated.

Track: High Throughput Protein Science

ABS249 | Sequence-structure-function relationships in the microbial protein universe

Julia Koehler Leman
Flatiron Institute (United States)

For the past half-century, structural biologists relied on the notion that similar protein sequences give rise to

similar structures and functions. While this assumption has driven research to explore certain parts of the protein universe, it disregards spaces that don't rely on this assumption. Here we explore areas of the protein universe where similar protein functions can be achieved by different sequences and different structures. We predict ~200,000 structures for diverse protein sequences from 1,003 representative genomes across the microbial tree of life, and annotate them functionally on a per-residue basis. Structure prediction is accomplished using the World Community Grid, a large-scale citizen science initiative. The resulting database of structural models is complementary to the AlphaFold database, with regards to domains of life as well as sequence diversity and sequence length. We identify 161 novel folds and describe examples where we map specific functions to structural motifs. We also show that the structural space is continuous and largely saturated, highlighting the need for shifting the focus from obtaining structures to putting them into context, to transform all branches of biology, including a shift from sequence-based to sequence-structure-function based meta-omics analyses.

Track: Protein Science Addressing Health Disparities

ABS251 | Cross-Seeding Controls A Fibril Populations and Resulting Functions

Henry Pan, Michael Lucas, Eric Verbeke, Gina Partipilo, Ethan Helfman, Leah Kann, Keith Keitz, David Taylor, Lauren Webb
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A number of neurodegenerative diseases are characterized by the aggregation of amyloid proteins caused by the nucleation of monomeric peptides into soluble oligomers that ultimately aggregate into insoluble fibrils. While oligomers are alleged to be the neurotoxic species, several factors have motivated research in the field into understanding amyloid fibrils and their structure. A small portion of Alzheimer's Disease (AD) cases are associated with familial mutations (FAD) in the amyloid precursor protein, which have been shown to result in significant differences in structural polymorphism of the resulting Aβ fibrils. There is also increasing evidence that these mutations lead to a diverse array of disease phenotypes. The goal of this study was to evaluate the structural and phenotypic effects of cross-seeding wild-type Aβ1-40 with various FAD mutants and isoforms. In this study, we examined both the structural and phenotypic effects of multiple generations of seeding of WT Aβ1-40 as well as cross-seeding with FAD mutants and an Aβ isoform. Specifically, we evaluated the impact of seeding wild-type (WT) Aβ1-40 with pre-formed fibrils of Aβ mutants, Arctic (E22G) and Osaka (E22Δ), as well as the isoform Aβ1-42. This was determined by kinetics measurements using thioflavin T fluorescence, fibrils structural classification and 2D class-averaging with negative-stain transmission electron microscopy, and their resulting impact on cell viability through MTT assays. We show that structure can be faithfully passed from mutant fibrils to that of WT fibrils. This cross-seeding of WT fibrils results in a toxicity profile similar to that of the parent mutant fibrils, as evidenced by cellular assays. Our findings have significant implications for neurodegenerative diseases caused by the aggregation of monomers into fibrils, demonstrating that the interaction between different forms of fibrils may be responsible for disease pathology.

Track: Protein Phase Separation in Biomolecular Condensates

ABS252 | Using Phase Separation as Mechanism to Enhance Inducibility of a Synthetic Therapeutic Pathway

Julisia Chau, Michael Westberg, Daesun Song, Michael Lin
Stanford University (United States)

The efficient identification and elimination of cancer cells without harm to normal tissues remains a challenge in cancer therapy. We recently developed the concept of Rewiring Aberrant Signaling to Effector Release (RASER), in which oncogenic protein activity, rather than being suppressed, is detected and rewired to a therapeutic output.1 Detection and therapeutic activation is accomplished by induced proximity of a protease with its substrate and the subsequent release of a cytotoxic cargo.1 RASER better differentiates between ErbB-hyperactive and normal cells compared to traditional therapies, but its output is not completely off in normal ErbB-active states. I propose to improve the inducibility of RASER by using phase separation to enhance the synthetic systems response to strong ErbB signaling while reducing background activity. Phase separation is created by fusing intrinsically disordered proteins (IDPs) to the substrate. We hypothesize that under normal ErbB activity, phase separation would spatially shield substrate from

cleavage, reducing background activity. However, in ErbB hyperactive states, co-recruitment of phase-separated substrate and protease via phosphotyrosine sites can bring the protease in close proximity to the droplets. With higher local concentration of substrate, protease cleavage is expected to be more efficient, leading to higher signal. Several IDPs have been tested and preliminary data suggests that the phase separating properties of these IDP may indeed regulate the efficiency of cleavage of cargo. Ongoing work aims to increase the inducibility of the system in order to increase RASER's therapeutic efficacy.

References:
1.Chung, Hokyung K et al. "A compact synthetic pathway rewires cancer signaling to therapeutic effector release." Science (New York, N.Y.) vol. 364,6439 (2019): eaat6982. doi:10.1126/science.aat6982

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS253 | Identifying Novel SIRT1 Regulator Compounds

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SIRT1 is from a group of Sirtuins that are found in the human body and is a lysine deacetylase that is involved in numerous cellular functions like aging, neurodegeneration, and insulin resistance. It is known that SIRT1 can be regulated by resveratrol, and the effect of resveratrol is dependent on the different peptide substrates. The goal of my project is to see if this type of substrate-dependent regulation of SIRT1 is also true for other novel small molecule regulators of SIRT1. We are testing if novel small molecules that have been developed by the Cheruzel lab at San José State University can also affect SIRT1 activity. Plate reader-based enzyme-coupled assays are used to determine the Michaelis-Menten enzymatic kinetics of SIRT1 with and without the small molecules, so we can determine if the novel small molecules can be a SIRT1 regulator for its activity against the Ac-p53W substrate. We have currently identified a few small molecules that have significantly different enzymatic activity, meaning they could potentially be a SIRT1 regulator, however, further testing is required. The importance of discovering new SIRT1 regulators is that they can lead to new therapeutics used to specifically target SIRT1 to combat diseases such as neurodegeneration and insulin resistance.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS254 | Protein adaptation in extremophiles: a story of sequence, structure and osmolytes

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Extremophiles are organisms that have optimal growth conditions in extreme temperature, pressure, acidity, salinity, or pH generally considered hostile for carbon-based life forms. Proteins have evolved to function in environments native to these different organisms. To understand how extremophiles have evolved their proteins we chose phosphoglycerate kinase (PGK), an essential enzyme in the glycolytic cycle from 6 different organisms. These were chosen to optimize for a variety of conditions across species, temperature, and pressure optimal for their growth. We monitored the tryptophan fluorescence to quantify stability of the protein under extreme pressure and temperature. The effect of cellular environment on stability was accounted by performing high pressure denaturation in the presence Trimethylamine N-oxide, (TMAO) a natural osmolyte occurring mainly in marine animals. We also use high pressure SAXS which coupled with all-atom molecular dynamics simulations provided us the conformational dynamics of different protein sequences under stress. This gave us an insight into how different organisms optimize their sequence for stability in specific conditions. In our study, while most mesophilic organisms were highly suspectible to unfolding by pressure other extremophile organisms had a more robust response to it. The amount of stabilization to osmolytes was also highly varied even amongst extremophiles. We propose that the cellular environment modulation through osmolytes helps keep the external stresses from denaturing essential enzymes.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS255 | Interrogating the Interactions between Lys48 linked Ubiquitin chains and UCH37

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Degradation of proteins by the 26S proteasome is a highly regulated process that is mediated by the type of

ubiquitin (Ub) modification present on the substrate. Ub can form polyubiquitin chains by modifying itself on any of its seven surface Lys residues and the N-terminal Met. This leads to a multitude of different chain architectures that can decorate substrate proteins. Branched Ub chains, where a single Ub is modified at two different sites, have been shown to be particularly potent signals for degradation. UCH37 is a proteasome associated deubiquitinase (DUB), which is recruited to the proteasome by its binding partner RPN13. Our lab has shown that the UCH37-RPN13 complex selectively cleaves Lys48 linked chains when they are part of a branch point, thereby promoting degradation. UCH37 selectively binds Lys48 linked chains regardless of architecture, but only displays catalytic activity when a branch point is present. However, the interactions between the enzyme and Lys48 linked branched or non-branched chains remain poorly understood. To address this problem, we used NMR spectroscopy to better understand the binding modes of Lys48 linked Ub chains. To gain a more complete picture we also employed Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS), Chemical Crosslinking as well as Small Angle X-ray Scattering (SAXS) to understand the molecular details of this interaction. Finally, we used docking and molecular dynamics (MD) simulations to generate binding models that are in good agreement with the experimental data. The combination of these methods allowed us to identify a novel Ub binding site on UCH37. The models obtained from docking and MD simulations also provide a possible explanation for the highly selective nature of UCH37.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS256 | Origins of Small Molecule Binding Site Specificity in Intrinsically Disordered Proteins

Rajesh Jaiprashad, Steven Metallo, Sachith Roch De Silva
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Intrinsically disordered proteins (IDPs) are important regulators of biological systems. These proteins lack a stable secondary or tertiary structure and, due to their conformational flexibility, often interact with multiple different binding partners. Previously, inhibition of IDPs was thought to be impractical due to their rapid fluctuation and extreme conformational heterogeneity. Using an exemplar IDP, c-Myc, we and others have shown that small molecules can bind to IDPs. We observed compounds that bind to short stretches of contiguous amino acids in the IDP sequence. Using alanine mutations within a small molecule binding site on c-Myc, we further show that seven residues of the 12-residue binding site are required for small molecule binding. We developed a model to understand the small-molecule binding specificity in this context based on the probability of finding the same arrangement of binding site residues within the proteome. Using proteome scale sequence searches, we demonstrate that the crucial residues within the IDP binding site converge to one sequence, c-Myc. We extend this model by probing the ability of the small molecule binding to tolerate non-alanine mutations (both conservative and nonconservative) at Myc positions shown to be important for binding. Understanding the sequence requirements for specific binding of IDPs points to possibility of predicting potential binding sites within IDPs which will aid the identification of additional targets in the IDP landscape.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS257 | A complex machine for lipid transport across the cell envelope of Mycobacterium tuberculosis

James Chen, Gira Bhabha
NYU School of Medicine (United States)

The complex cell envelope of Mycobacterium tuberculosis forms an impenetrable barrier around the cell, protecting it from host immune responses and the entry of harmful agents such as antibiotics. However, this barrier is a double-edged sword, as it limits the passive entry of nutrients that the bacterium requires to grow. Despite its importance, we have very little information on how small molecules are transported across the cell envelope, and how it is built and maintained. MCE transporters (originally thought to mediate Mammalian Cell Entry in M. tuberculosis) play a role in these processes, and have been implicated in virulence, but their structures and functions remain poorly characterized. Here we show that MCE transporters in M. tuberculosis form large transenvelope complexes, consisting of 10+ subunits that may be inserted in both the inner and outer membranes, simultaneously. Coupled to an ABC transporter in the inner membrane that drives transport, a ~200 Å long "needle" formed by a 6-helix coiled-coil extends across the bacterial periplasm to the outer membrane. The needle appears to create a hydrophobic tunnel for lipid transport directly between the two membranes. This remarkable machine is architecturally quite different from other transporters studied to date, and sheds light

on the role of MCE proteins in cell envelope biogenesis, transport, and virulence in an important human pathogen.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS258 | 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 molecular decoy that mimics the binding interface of the viral host receptor to attain highly specific binding to virions; 2) A solvatochromic fluorophore placed onto the host 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.

Using structural analysis, we identified the 35 amino acid long alpha-helix of human ACE2 as minimalist scaffold to recapitulate the binding interface. We then engineered its sequence maintaining the binding interface while increasing the sequence's intrinsic propensity to form alpha-helical conformation and creating a leucine zipper interface to promote its association into a coiled-coil. We then introduced the solvatochromic fluorophore 4DMN from the Imperiali lab in a key position expected to become buried in the coiled-coil and solvent exposed upon monomerization induced by RBD binding. Self-titration experiments using fluorescence, circular dichroism and size-exclusion chromatography indicate that this biosensor forms a coiled-coil complex with KD∼2.3 microM, resulting in >15-fold increase in green fluorescence, thus enabling the high-sensitivity detection of the monomer-dimer interconversion. Once prepared near the dimerization midpoint (2 microM), the sensor effectively detects the presence of RBD by turning-off its fluorescence upon binding. We also showed that under such conditions the dimer-partner tug of war operates with C50 ∼13±2 microM, and binding to surface-immobilized RBD results in detectable fluorescence signals at ∼300 nM (sensitivity limit). 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.

Track: Celebrating 100 Antibody Drugs

ABS259 | Tag-Free Specific Conjugation to Glycosylated IgG1 Antibodies Using Microbial Transglutaminase

Adem Hadjabdelhafid-Parisien, Lukas Deweid, Sebastian Bitsch, Arturo Macarrón Palacios, Harald Kolmar, Joelle Pelletier
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The synthesis of antibody-drug conjugates (ADCs) is an emerging field of study. ADCs should ideally be homogeneously modified at specific sites to carry a narrow distribution of drug molecules. Homogeneous modification of antibodies has been shown to improve their efficacy and increase their therapeutic window compared to ADCs synthesized using non-specific methods. Microbial transglutaminase (mTG) from Streptomyces mobaraense was used to selectively modify IgG heavy chain Q295. This method requires deglycosylation of the antibody prior to the conjugation reaction, which reduces the stability and solubility of the resulting ADCs. Approaches to circumvent the need for deglycosylation include the use of short peptide tags containing a reactive glutamine (Q-tags); however, these methods increase the immunogenic risk and destabilize the antibody structure. Here we present an efficient approach for label-free and site-specific conjugation of a fully glycosylated antibody. We have applied the glutamine walking strategy to create novel reactive sites for mTG-mediated conjugation of antibodies with primary amine payloads. A series of seven IgG1 trastuzumab variants were produced, each substituted with glutamine on a single surface-exposed residue. Validation of reactivity with mTG by conjugation to an amino-luorophore identified the most reactive variant, which was selected for a two-step conjugation based on mTG and click chemistry with monomethyl auristatin E. The degree of conjugation, determined by mass spectrometry and hydrophobic interaction chromatography, confirms the conjugation of one payload per antibody heavy chain. Cytotoxicity and specificity were indistinguishable from similarly conjugated trastuzumab obtained by another method. The resulting fully glycosylated ADC is unique in that it results from minimal modification of the antibody sequence and offers potential for new applications.

Track: Miniproteins: Are There Really That Many Additional Genes That We Have Been Missing?

ABS260 | Novel genes enable protein structural innovation and function in the brain

Victor Luria, Amir Karger, John W. Cain, Anne O'Donnell-Luria, Nenad Sestan, Marc Kirschner
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How genuinely new protein-coding genes originate is a central question in biology. Long thought impossible to arise from non-coding sequence, novel genes arising de novo from genomic "junk" DNA or from long non-coding RNA were recently found in eukaryotic genomes. Novel genes are taxon-restricted and may encode structurally novel proteins with new protein domains. To understand how novel genes arise, we built a mathematical model based on gene and genome parameters and dynamic factors such as mutation. We combined phylostratigraphy and proteogenomics to identify novel genes in 25 eukaryotic genomes and evaluated their predicted biophysical properties. Compared to ancient proteins, novel proteins are shorter, more fragile, disordered and promiscuous, yet less prone to aggregate or to form toxic prions. We performed biophysical experiments comparing novel and ancient proteins, showed that novel genes function in vivo in zebrafish brains, and found novel genes are expressed in human brains at multiple ages. Genomic sequence turnover generates many novel genes encoding short proteins, of which some are maintained and encode proteins with distinct structural features and expressed in the brain. Thus, genomic variation continuously generates new protein structures and new functions.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS261 | Apolipoprotein E4 has extensive conformational heterogeneity in lipid free and bound forms

Melissa Stuchell-Brereton, Maxwell Zimmerman, Justin Miller, Upasana Mallimadigula, Jasmine Cubuk, J Jeremias Incicco, Debjit Roy, Louis Smith, Berevan Baban, Greg DeKoster, Frieden Carl, Gregory Bowman, Andrea Soranno
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The ε4-allele variant of Apolipoprotein E (ApoE4) is the strongest genetic risk factor for Alzheimer's disease, though it only differs from its neutral counterpart ApoE3 by a single amino acid substitution. While ApoE4 influences the formation of plaques and neurofibrillary tangles, the structural determinants of pathogenicity remain undetermined due to limited structural information. We apply a combination of single-molecule spectroscopy and molecular dynamics simulations to construct an atomically-detailed model of monomeric ApoE4, quantify the folding the stability of its domains, and probe the effect of lipid association. Our data reveal that ApoE4 is far more disordered than previously thought and retains significant conformational heterogeneity after binding lipids. In particular, the behavior of the hinge region and C-terminal domain of ApoE4 differs substantially from that proposed in previous models and provides a crucial foundation for understanding how ApoE4 differs from non-pathogenic and protective variants of the protein.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS262 | Proteome-Wide Profiling of Protein Misfolding with Crosslinking Mass Spectrometry

Yingzi Xia, Stephen Fried
Johns Hopkins University (United States)

Many proteins are inefficient at spontaneously refolding to their native conformation following chemical denaturation, and a recent study estimated that roughly one third of E. coli proteins may fall into this category. Nevertheless, it has been challenging to structurally characterize misfolded proteins because of their heterogeneity and tendency to aggregate. On the other hand, structural maps of soluble misfolded states could elucidate the mechanisms of aggregation and indicate what structural features are used as epitopes by chaperones and the proteostasis machinery.

Here, we introduce an approach to systematically characterize the structures of misfolded conformations proteome-wide with crosslinking mass spectrometry (XL-MS). We perform global unfolding/refolding reactions on total E. coli extracts and crosslink the resulting conformations with DSBU. Furthermore we employ an isotope labeling strategy to quantitatively compare the structures of native conformations to their misfolded forms. The data point to a similar result to our recent study, not all E. coli proteins can refold from a fully denatured state. Additionally, the cross linked sites provide insight into what regions or sequence of the misfolded protein have evolved to be recognized by molecular chaperones.

This study highlights how XL-MS can complement traditional methods in structural biology to interrogate highly heterogeneous systems, such as a misfolded proteins.

Track: Machine Learning in Protein Science

ABS263 | Alternative sites for the design of chemical probes for SARS-CoV-2 protein targets

Suhasini Iyengar, Kelly Barnsley, Hoang Yen Vu
Northeastern University (United States)

Understanding the function and control of SARS-CoV-2 proteins is critically important in the fight to stop SARS-CoV-2, the viral pathogen that causes COVID-19. Our machine learning method Partial Order Optimum Likelihood (POOL) is used to predict binding sites in SARS-CoV-2 protein structures reported in the PDB. Using the 3D structure of each protein as input, POOL uses computed electrostatic and chemical properties, together with surface topology information, as input features to predict the amino acids that are biochemically active, including catalytically active residues and residues in recognition sites, allosteric sites, and exosites. Docking studies are then performed to predict ligands that bind to each of these predicted sites. For instance, for the x-ray crystal structures of the main protease (MPro), POOL predicts two sites: the known catalytic site containing the catalytic dyad His41 and Cys145 and a second nearby site on an adjacent face of the protein surface. The x-ray crystal structure of the SARS-CoV-2 2'-O-ribose RNA methyltransferase (NSP16) protein has been reported in complex with its activating partner NSP10 and with two bound ligands, S-adenosylmethionine (SAM) and β-D-fructopyranose (BDF). POOL predicts three binding sites, including the catalytic SAM-binding site, the BDF binding site on the opposite side, and a third site adjacent to the catalytic / SAM-binding site. Multiple predicted binding sites and predicted binding ligands are reported for MPro, NSP16-NSP10, RNA-dependent RNA polymerase, nucleocapsid protein, and helicase. For the currently available compounds, binding and kinetics assays to test for affinity and inhibition are in progress. Supported by NSF CHE-2030180.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS264 | The GCE4All Research Center: Unleashing the Potential of Genetic Code Expansion for Biomedical Research

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Oregon State University (United States)

Genetic Code Expansion (GCE) is a very powerful, but also highly underutilized way to site-specifically place useful chemical groups into proteins. By creating engineered tRNA and aminoacyl-tRNA synthetase (tRNA/RS) pairs specific for non-canonical amino acids (ncAAs) and a repurposed codon, GCE uses the cell's translation machinery to incorporate the ncAAs at genetically-encoded sites during protein synthesis. The GCE4ALL Research Center is a new research center funded by Oregon State University and the Biomedical Technology Development and Dissemination program of the National Institute of General Medical Sciences. The GCE4All Research Center is dedicated to the task of improving GCE tools so that biomedical researchers around the world can easily adopt and use GCE technologies in their own labs to generate specially designed forms of proteins for probing and visualizing how life works. The GCE4All Research Center mission is to improve and disseminate this powerful set of technologies so they can be more widely used for revealing mechanisms of health and disease and developing diagnostics and therapeutics.

Research efforts in the Center are organized in two Technology Development Projects focused, respectively, on making optimized GCE tools to incorporate ncAAs relevant for:

1) Bioorthogonal ligations; and

2) Biochemical probes and post-translational modifications.

The Center will "road test" developing GCE technologies through formal collaborations with selected researcher groups in the context of authentic, challenging problems in biomedical research – referred to as Driving Biomedical Projects.

Our presentation here will provide an overview of the GCE4All Research Center structure, describe our approaches to technology development and dissemination, and introduce the initial set of technologies that we have targeted for improvement and wide adoption.

Also, as you have interest, please:

- Explore our resources at https://gce4all.oregonstate.edu/
- Contact us at gce4all-center@oregonstate.edu

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS265 | Residue-level identification of backbone errors in cryo-EM models

Gabriella Reggiano, Frank DiMaio
University of Washington (United States)

Building models of large protein complexes into 3-5A cryo-electron microscopy maps is difficult, because the

data, especially sidechain details, is limited. Methods like AlphaFold can help dramatically, but local errors and conformational heterogeneity still lead to mistakes. We have developed statistical models to identify backbone and rotameric errors in protein models built into cryo-EM maps at the level of individual residues. Our model is derived from several features: expected fit-to-density for each amino acid at its local resolution, predicted lddt by Deep Accuracy Net, a machine learning model trained to predict errors from thousands of decoy structures, and scores from Rosetta's physically realistic energy function for Ramachandran angles and expected bond lengths. Two separate models were developed, one specialized for backbone errors and one for rotameric errors, on a benchmark set of pdbs that had been edited by the authors after deposition. Our backbone model was able to identify 80% of all backbone errors with a precision rate of approximately 70% in a small cross validation set of edited pdbs. The errors contained in our benchmark and cross validation sets were made by experts and are difficult to find, as they reasonably explain most of the density. The rotamer model is able to identify 50% of all rotamer errors with a precision rate of 30%. We ran our backbone model on 1100 deposited protein models with cryo-EM maps between 3-5A resolution. We identified and corrected several large scale backbone errors in multiple pdbs that made it through the deposition process. Our method will allow cryo-EM modelers to focus their attention on specific areas of the protein during the modeling process. This is critically important in a post AlphaFold world, where models with small mistakes may be docked into large, lower-resolution density maps.

Track: Protein Phase Separation in Biomolecular Condensates

ABS266 | Using Small Molecules to Dissect and Control the Energetics of Liquid-liquid Phase Separation in Intrinsically Disordered Peptide Systems

Davina Adderley, Jennifer Guo, Jessica York, Steven J Metallo
Georgetown University (United States)

Biomolecular condensates, also termed ‘membraneless compartments’, emerge as a consequence of a phase condensation reaction of biomolecules. Examples of biomolecular condensates include nuclear stress bodies that regulate gene expression, and stress granules that store translation machinery. The condensates manifest as liquid-droplets and are an example of the general phenomenon known as liquid-liquid phase separation (LLPS). LLPS is a reversible process that involves the equilibration of a uniform fluid into two distinct liquid phases: a dense phase and a dilute phase. The dense phase appears as liquid droplets in coexistence with a dilute phase.

Although LLPS is an essential process in cells, the energetics that establish such phase separating protein systems is not fully understood. We employ systematic small molecule probes to elucidate the strength of specific chemical interactions within the dense phase of LLPS protein systems. A common feature of proteins that undergo LLPS is the presence of intrinsically disordered regions: stretches of sequence that exist as an ensemble of rapidly fluctuating conformations that lack a stable secondary or tertiary structure. Here, we use the disordered Laf-1 RGG protein as a model physiological LLPS system and the partitioning of small molecule probes between the dense and dilute phase to examine the free energy of interactions. Specifically, we assess the ability of C-H/pi interactions to contribute to phase separation and the ability of an aromatic system's electron density and substitution pattern to modulate the interaction strength with a phase separated protein.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS268 | Portability of Binding Sites in Intrinsically Disordered Proteins: Sequence Specificity and Context Independence

Sachith Roch De Silva, Rajesh Jaiprasad, Steven Metallo
Georgetown University (United States)

Intrinsically disordered proteins (IDPs) lack stable secondary and tertiary structures. Because they are associated with various diseases, IDPs are desirable therapeutic targets. Despite their lack of stable structure, we and others have demonstrated that small molecules can bind and inhibit IDPs. The transcription factor c-Myc (Myc) is an IDP implicated in over 70% of cancers. Previously, we have shown that several small molecules bind to short, contiguous sequences on Myc. Through alanine scanning, we determined that a specific subset of the contiguous sequence is necessary for small molecule binding. Next, we compared the native protein binding to the isolated binding sequence. Then we ported the isolated sequence into a different IDP and observed that small molecule binding to a specific recognition site is context-independent.

We show that the binding mode differs from structured proteins, where contact residues are often noncontiguous

and scaffolded by extensive protein structure. Instead, these sequences remain unstructured when binding to small molecules, indicating a limited dependence on the overall IDP conformation. Furthermore, we investigated whether a broader context was necessary for specific binding. Our findings suggest that disordered sequences bind small molecules in a context-agnostic manner garnering implications for the prediction and broader discovery of IDP binding sites.

Track: Machine Learning in Protein Science

ABS270 | A General Method to Predict the Effect of Single Amino Acid Substitutions on Enzyme Catalytic Activity

Yu-Hsiu Lin, Cheng Lai Huang, Christina Ho, Max Shatsky, Jack Kirsch
University of Texas Health San Antonio (United States)

Over the past thirty years, site-directed mutagenesis has become established as one of the most powerful techniques to probe enzyme reaction mechanisms. However, these experiments are expensive and time-consuming to conduct. Additionally, while substitutions of active site residues are most likely to yield significant perturbations, there are many examples of profound functional changes elicited by remote mutations. Here, we sought to develop a machine-learning model to identify critical residues that do not contact the substrate directly but play important roles in enzymatic function. We collected literature data reflecting the effects of 2,804 mutations on kinetic properties for 12 enzymes. For each mutation, we extracted three features for computational modeling: 1) the distance of the introduced mutation from the active site, 2) the conservation of the residue at the mutated position based on evolutionary variations, and 3) the likelihood of amino acid substitutions based on the sequences of evolutionary divergent proteins. We trained a Random Forest classifier with these predictors and tested our model using cross-validation by leaving out one enzyme at a time from the training set. Notably, our model accurately predicted deleterious mutations in enzymes that were excluded from training with an area under the receiver operating characteristic curve (AUC) of up to 0.808, even when leaving out each of the top three enzymes in our database with the greatest number of mutagenesis data (Figure 1). In addition, compared to predictions made at random, our model showed a 2.5-fold increase in precision when identifying mutations that cause a decrease in kcat/Km value of $\geq 95\%$. Taken together, we have developed a computational model that can predict functionally significant substitutions with high accuracy and precision. This will allow experimentalists to reduce the number of mutations necessary to probe and understand reaction mechanisms in novel enzymes.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS271 | Development of porous protein nanocrystals as a delivery vector for DNA and RNA

Alec Jones, Moe Masri, Christopher Snow
Colorado State University (United States)

Oral delivery of nucleic acids is restricted by a number of limiting factors, particularly protection of guest DNA and RNA from degradation and hydrolysis within the gastrointestinal tract following ingestion. Highly-ordered, self-assembling porous protein crystals have been previously explored for enzyme immobilization, and may offer similar advantages for protection and targeted delivery of therapeutic molecules to cells. We have developed a protocol for generating sub-micrometer porous nanocrystals from CJ, a putative polyisoprenoid-binding protein from Campylobacter jejeuni, which are non-cytotoxic and capable of passively retaining DNA and RNA guest molecules of varying size . We have demonstrated that CJ micro and nanocrystals are able to adsorb RNA and DNA, as well as functional enzyme. Furthermore, while guest nucleic acids remain susceptible to hydrolysis by nucleases, we have developed a method for capping the pores of the crystals to protect guest DNA from hydrolysis by DNase. Upcoming work will investigate the pharmacokinetics of Nanoluciferase-labeled CJ nanocrystals in mice via IVIS imaging, following oral, intravenous, and intramuscular administration, along with in vitro monitoring of guest RNA hydrolysis under variable pH conditions.

Track: Machine Learning in Protein Science

ABS272 | Improved Affinity-Based Supervision on Pretrained Protein Language Models & Revealing Embedding Performance for Non-Immunoglobulins Fitness Prediction

Mehrsa Mardikoraem, Joelle Eaves, Daniel Woldring
Michigan State University (United States)

The rate of discovering novel therapeutics and disease diagnostics is directly impacted by our depth of

understanding of the causal relationship between protein sequence-structure-function. Rationally engineered proteins, with high affinity and selectivity toward overexpressed biomarkers, have proven to be an effective tool against an array of disease types. Yet, the rate of therapeutic discovery is outpaced by the need for clinical treatment. To accelerate these innovations, we propose using machine learning to guide protein engineering campaigns against aggressive disease targets. The booming trend in protein sequence databases coupled with self-supervised learning has provided opportunities that can solve previously intractable problems. Based on amino acid sequence alone, diverse features can be extracted from extensive collections of proteins. These "embeddings' of protein sequences allow us to map a protein's set of features onto a high-dimensional space. UniRep trained by next-token prediction and ESM with masked-token prediction objectives are among the revolutionary protein embeddings. These embedding methods carry meaningful patterns about the protein, including secondary structure, contact prediction, subcellular localization, etc. Despite their great promise, the self-supervised models are in their early stages in protein engineering. Still, there is an essential need for experimental and computational protein engineers' contributions. For example, the embedding methods are often analyzed in different benchmark datasets with various testing criteria. Moreover, there is still a great need for unbiased supervision from experimental data incorporation for binding affinity prediction. In this study, we analyze the performance of these embedding methods on downstream binding affinity prediction of non-immunoglobulins and resolve to alleviate sources of noise and bias in high-throughput experimental data. Non-immunoglobulins, due to their smaller structure, lower molecular weight, and better tissue penetration with respect to antibodies, have the potential to revolutionize diagnostics and immunotherapeutic medicine. We hope our improved affinity-based discriminative models help to elevate protein design.

Track: High Throughput Protein Science

ABS273 | One-step microencapsulation of cell-free lysate for improved protein synthesis and characterization

Congwang Ye
LLNL (United States)

High throughput production and screening of proteins imposes serious time and material constraints in traditional cell-based systems. Cell-free protein synthesis (CFPS) lysates containing combined transcription and translation machinery allows for the flexible and rapid production of proteins, dependent on availability of template DNA sequences. We have improved upon basic CFPS methods by developing a microfluidic system to encapsulate CFPS solutions inside 300-500 μm microcapsules for in-capsule protein synthesis and characterization. We have optimized the UV-curable polymer shell phase solution, containing polymer, photo-initiator, and surfactant, into a mixture to reduce negative effects on CFPS and facilitate one-step microencapsulation at < 1 min time scale. Once encapsulation is complete, the microcapsules were cleaned and stored in osmotically balanced fluid for cell-free lysate expression. These microcapsules, or essentially micro-bioreactors, enables increased protein production capacity compared to batch synthesis reactions and can be fabricated on the scale of 100s to 1000s per second. Combined with library screening approaches, this technique can potentially serve as an ideal platform for generating rich data sets for statistical analysis, machine learning, and computational design for rational antibody design.

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Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS274 | The Amino Acid Code to bZIP-DNA Base Recognition

Aaron Tran, Jerry Tsai, Hyun Joo
University of the Pacific (United States)

The simple DNA-binding basic leucine zipper motif (bZIP) recognizes consensus nucleotide sequences through interactions between a protein α-helices and bases in the duplex. A Knob-Socket analysis is used to characterize the multibody determinants of specificity between bZIP monomers and their respective four-base half-site. The results reveal that a conserved quadripartite pattern of amino acids recognizes individual bases from both positive and negative strands of DNA, whereas the current paradigm assigns recognition to only the positive strand. Individual half-site packing localizes around the invariant Asn and Arg on the α-helical i+4 ridge found in most bZIP families and all analyzed DNA-bZIP structures. In the four-base half-site, recognition of the DNA bases are divided by this ridge, where the first two bases

of the positive strand pack into one side of the helix, while the complementary negative strand bases of the next two positions pack into the other side. Therefore, the i+4 and i +3 ridges define quadripartite binding regions P1, P2, N3, and N4 on the bZIP α-helix. Thymine's C5 methyl group packs consistently into the N4 region and often into the P2 region. Specific recognition of dA, dG, and dC is a combination of amino acids in a region disfavoring C5 methyl packing and specifically hydrogen bonding to the respective base. This recognition also considers how the DNA duplex binds amino acids, where the DNA duplex lattices are represented as three lanes: two backbone lanes of the positive and negative strands and one base pair lane. While protein residues pack into the sockets in any three lanes, specificity is determined by residues packing into DNA sockets of the base pair lane. Abstractions from the Knob-Socket multibody packing analysis emphasize the complementary roles of the α-helical quadripartite region and DNA base lattice composition in contributing to recognition specificity.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS275 | Alternative splicing of CaMKII in the brain affects Ca2+ sensitivity via charge-dependent regulation

Bao Nguyen Viet, Noelle Dziedzic, Roman Sloutsky, Margaret Stratton
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Memory formation relies on sophisticated regulation of Ca2+ signaling, in which Ca2+/calmodulin-dependent protein kinase II (CaMKII) plays a central role as it can receive input information from Ca2+ oscillation via Ca2+/calmodulin and propagate the output on itself and downstream targets even after the initial stimuli have passed. This results in long-lasting effects from a single signaling event and is a form of molecular memory. The sensitivity of CaMKII to Ca2+/CaM thus determines the outcomes of a signaling event. Yet, the regulation for the sensitivity of CaMKII to Ca2+/CaM remains unsettling. The prevailing hypothesis is that the linker length, which connects with N-terminal kinase domain and the C-terminal hub (association) domain, as well as the hub domain play regulatory roles in Ca2+/CaM sensitivity. CaMKII is subjected to extensive alternative slicing, especially in the linker region, that will generate a diverse set of linkers with distinct lengths and sequence compositions. In this study, using biochemical assays, phosphoproteomic analyses, and mass photometry we showed that Ca2+/CaM sensitivity is not only dependent on the linker length, but it is also dependent on the overall charge of the linker. This effect was observed for both CaMKIIα and CaMKIIβ, which are the two predominantly expressed CaMKII genes in the brain. Autophosphorylation on the linker was observed in our study, yet it was not important to regulate the first Ca2+/CaM exposure. We hypothesize that the overall charges on the linker can be tuned by autophosphorylation, and will be important to regulate subsequent Ca2+/CaM exposures as in Ca2+ oscillation events. Our study suggests a more defined regulatory role of the variable linker region on Ca2+/CaM sensitivity, and that alternative splicing of CaMKII is important for its Ca2+ frequency response.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS276 | The Role of Conformational Entropy in Antigen Binding by a Single Chain Antibody Fragment

Weimin Tan, Taylor Cole, Anthony Bishop
Texas A&M University (United States)

Therapeutic antibodies make up a large portion of FDA-approved biologics but suffer from many limitations including off-target toxicity, material costs, and limited efficacy as blockades. One direct solution to this problem is to increase the affinity of the antibody for its target antigen. Numerous molecular dynamics (MD) simulation studies have shown affinity maturation and the reduction of antibody plasticity and flexibility are closely related. However, dynamic contribution in antibody-antigen interactions has remained challenging to quantity experimentally. Recent work from this lab has demonstrated that protein-ligand interactions often involve appreciable changes in conformational entropy, which contributes directly to the free energy of binding. To investigate the conformational entropy contribution in antibody binding, we are employing the well-characterized picomolar-affinity anti-fluorescein antibody (4-4-20) as a model system. The dynamic disorder of amino acid side chains of the 4-4-20 scFv in its free state and while bound to fluorescein are being determined using advanced Nuclear Magnetic Resonance (NMR) relaxation experiments. The goal is to learn the contributions of conformational entropy to the thermodynamics of antigen binding. 15N-TROSY and 1H-13C HMQC spectra of the anti-fluorescein scFv 4-4-20 in its free and bound forms have been obtained. The chemical shift difference of the free and bound spectra indicates the scFv 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. Supported by Texas A&M University and the Mathers Foundation.

Track: High Throughput Protein Science

ABS277 | Directed Evolution of Monoclonal Antibodies against Tumor Associated Carbohydrate Antigens

Sam Schmidt, Daniel Woldring, Xuefei Huang, Joelle Eaves, Theodore Belleciu, Athar Nakisa, Shivangi Chugh, Nathaniel Pascual
Michigan State Univeristy (United States)

Tumor associated carbohydrate antigens (TACAs) are a promising, yet difficult class of biomolecules to target for theragnostics. Overexpression of TACAs is a unique molecular signature that healthy cells lack. However, most are not recognized as non-self, causing low immunogenicity thereby hindering traditional antibody immunization techniques. This difficulty is increased by the massive, mostly unfunctional sequence space available. We address these challenges using a novel immunization strategy paired with large, rationally designed antibody libraries. Directed evolution of a rationally designed library based on immune-evolved antibodies decreases time and effort spent to achieve a high-affinity, high-specificity binder compared to a naïve library. Moreover, by utilizing a novel immunization technique involving the display of TACAs on multi-valent Qβ nanoparticles, a stronger immunogenic response is elicited from the animal being challenged, causing higher titers of immune-evolved antibodies. Yet even with improved titers, the resulting antibodies commonly show insufficient binding affinity. To overcome this challenge, we utilize dominant antibodies discovered from Qβ nanoparticles as a starting point for our library. In this study, we have generated immune-evolved antibodies which weakly bind NHAcGD2, a ganglioside implicated in the proliferation and invasion of multiple cancers (e.g., neuroblastoma and melanoma). We use this diverse panel of lead antibodies as starting points for cell surface display directed evolution campaigns. To navigate the mutational landscape of these promising monoclonal antibodies more efficiently, we leverage directed evolution of our rationally designed antibody libraries. This powerful approach is coupled with single cell deep sequencing for the native pairing of heavy and light chains. We also leverage in silico experiments to help determine promising candidates. From this, we can identify mutations to increase functionality and drive potent interactions with the TACA target. In this way, we provide a novel platform for discovering antibodies with clinical potential against a notoriously challenging class of biomarkers.

Track: Protein Phase Separation in Biomolecular Condensates

ABS280 | Determining the effect of stress on the nucleolar protein, Liat1

Akshaya Arva, Isabel Vega Calderon, Jasmin Manzano, Christopher Brower
Texas Woman's University (United States)

The Ligand of Ate1 (Liat1) was originally discovered through its interaction with Arginyltransferase 1 involved in protein degradation. However, its functional significance remains unknown. We recently
reported that the N-terminal half of Liat1 is intrinsically disordered and harbors conserved areas of low complexity including a poly-lysine region. We also showed that Liat1 co-localizes with Fibrillarin and
participates in nucleolar phase separation in a manner that requires its poly-lysine region. Although the nucleolus functions as the site of ribosome biogenesis, it also plays a central role in cellular stress
response and various conditions trigger distinct changes in its composition and organization. As part of our efforts to the nucleolar function of Liat1, we are characterizing its behavior in response to specific
stress conditions. So far we have found that inhibition of ribosome biogenesis using actinomycin D disrupts the Liat1-Fibrillarin co-localization. Separately, we found that Liat1 expression is upregulated
during viral infection. These ongoing studies reveal distinct behaviors of Liat1 in response to specific forms of cell stress.

Track: Protein Science Addressing Health Disparities

ABS281 | Cell-free expression and screening of Treponema pallidum outer membrane proteins for vaccine development.

Byron Vannest, Angela Evans, Melissa Caimano, Kelly Hawley, Justin Radolf, Steven Hoang-Phou, Matthew Coleman
Lawrence Livermore National Laboratory (United States)

The worldwide surge in bacterial sexually transmitted infections (STIs), including syphilis, has highlighted the need to develop effective vaccines against their etiologic agents. The syphilis spirochete Treponema pallidum (TPA) caused 7 million new cases globally in 2020 and is the world's second leading cause of stillbirth. An important recent advance for syphilis vaccine design has been the identification of TPA's repertoire of outer membrane proteins (OMPs); however, large-scale characterization has proven to be challenging. Technologies are needed to overcome the bottlenecks associated with expressing, purifying, and folding TPA OMPs in quantities sufficient for vaccine trials in animals and humans. Cell-free protein expression systems have been used in combination with nanodisc technologies to express correctly folded major outer membrane protein from Chlamydia trachomatis and is an attractive method that can also be applied to create recombinant vaccine candidates for other sexually transmitted diseases. Here, we used Escherichia coli-based cell-free lysates to express and screen selected T. pallidum OMPs for yield and solubility. Genes encoding the eight-stranded β-barrel TP0733, the FadL orthologs TP0856 and TP0858, the efflux pump outer membrane factor TP0966, and the β-barrel domains of BamA (TP0326) and T. pallidum repeat protein TprI (TP0620) were successfully expressed with and without affinity tags in Escherichia coli lysates. Co-translation with ApolipoproteinA1 and lipids was found to improve the solubility of these proteins. Expression and solubility were also compared between homemade and commercially available cell-free lysates. TrpI, TP0858 and TP0856 were expressed at >100 μg/mL and with 85-100% solubility. Functional and structural studies are on-going. These results demonstrate that cell-free systems can be used to rapidly produce and screen difficult-to-obtain antigens from T. pallidum and other STI-causing organisms.

Track: Protein Science Addressing Health Disparities

ABS282 | Characterization of the Dynamics of Interleukin-1 Receptor Antagonist by NMR Spectroscopy

Glorisé Torres-Montalvo, Anthony Bishop, Taylor R. Cole, José A. Caro, A. Joshua Wand
Department of Biochemistry & Biophysics, Texas A&M University (United States)

Dysregulation of the interleukin-1 (IL-1) family of cytokines and receptors is implicated in a vast number of autoimmune, inflammatory and neuropathological diseases. Interleukin 1- receptor antagonist (IL-1Ra), a low-molecular weight cytokine, has been used as a protein therapeutic. However, as with many protein therapeutics, IL-1Ra suffers from a short in vivo half-life time, primarily due to rapid passage through the kidney, which has limited its use in the clinic. A direct solution to this is to increase the affinity of IL-1Ra for the IL-1 receptor (IL-1R). We have recently shown that detailed knowledge of the fast-internal motion of proteins provides insight into the influence of conformational entropy in protein-ligand interactions. Advanced 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. We aim to develop a strategy for engineering enhanced affinity protein-biologics using IL-1 as a model system. Assignments of the backbone and side-chain residues of IL-1Ra have been obtained and will be presented together with advanced NMR studies of IL-1Ra in its free form.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS283 | PermaPhos: autonomous synthesis of functional, permanently phosphorylated proteins

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The large majority of eukaryotic proteins are phosphorylated on at least one serine residue, yet phosphoserine containing proteins represent a miniscule fraction of solved protein structures. In large part this can be explained by the challenged associated with making specific, homogenous phospho-proteins caused by phosphoamino acid instability, low phospho-protein yields, and off-target phosphorylation by kinases. To address these issues, a genetic code expansion (GCE) system was previously developed to translationally install non-hydrolyzable phosphoserine (nhpSer), with the g-oxygen replaced with a CH2 group, site-specifically into proteins during translation, but it has seen limited usage due to low yields and the high cost of supplemental nhpSer into the medium. Here, we achieve a 40-fold improvement in this system by engineering into Escherichia coli a

biosynthetic pathway that produces nhpSer from the central metabolite phosphoenolpyruvate. Using this "PermaPhos" system – an autonomous E. coli expression system for incorporating nhpSer into target proteins – we show production of several biologically relevant, stably phosphorylated proteins in sufficient quantity and quality for structural studies. We further show that nhpSer faithfully mimics the effects of phosphoserine in three stringent test cases: promoting 14-3-3/client complexation, disrupting 14-3-3 dimers, and activating GSK3b phosphorylation of the SARS-CoV-2 nucleocapsid protein. This facile access to milligram quantities of nhpSer-containing proteins from E. coli overcomes hydrolysis issues associated with recombinant production of pSer proteins. Simultaneously, PermaPhos opens the door to studying phosphoproteins in systems where exposure to phosphatases is expected, such as incubation in cell lysate for pulldown interaction studies, transfection into eukaryotic cells for studying phosphatase regulation by phosphorylation, and antibody development.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS284 | Engineering of genetically encoded ratiometric fluorescent calcium biosensors with broadband sensitivity and 1 ms time response

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Calcium is a universal second messenger, responsible for contraction of skeletal, smooth, and cardiac muscle, and its elevated levels are linked to heart failure and seizures. Ca+2 concentrations vary from 100 nM to sub-mM in different cellular locations and/or physiological conditions, and Ca+2 pulses occur as fast as ∼1 ms. Hence it is important to develop calcium sensors for in vivo monitoring in real-time. An attractive option are genetically encoded biosensors based on a protein conformational change in response to Ca2+, and a Förster resonance energy transfer (FRET) detection scheme between two flanking fluorescent proteins. There are many such sensors available nowadays, but they typically operate over only 2-orders of magnitude in [Ca2+], and are much too slow to resolve calcium pulses and spikes.

We are developing genetically encoded FRET biosensors that aim to break the broadband sensitivity and time-response barriers. As molecular scaffold we use the human protein calnuc, which is disordered in its apo (Ca+2 free) form, but folds upon binding two Ca2+ equivalents. Calnuc's folding makes for a large decrease in end-to-end distance, making it a most suitable transducer for FRET detection. We studied calnuc's folding coupled to Ca2+ binding using advanced single molecule FRET spectroscopy, and found that Ca2+ binding results in a large FRET change that starts at ∼100 nM and extends to mM Ca2+ with an overall time response of ∼1 ms. We have then implemented this calcium transducer as a genetically encoded biosensor flanked by mNeon-green and mScarlet-I, and demonstrated its use to detect calcium levels in HEK293 cells. We have also successfully engineered variants that tune the dynamic range and optimize the signal change. Our results confirm that calnuc's "folding coupled to binding" offers an excellent solution to develop high-performance genetically encoded calcium biosensors.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS285 | Defining the molecular code for regulating tau aggregate structure

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The tau protein forms aggregates in multiple neurodegenerative diseases termed tauopathies. In individual tauopathies, tau characteristically take on distinct aggregate conformations suggesting that the biochemical properties of the structures themselves may drive pathobiology. Understanding how specific structures arise is critical for advancing the field however, we are still struggling to obtain fundamental knowledge regarding 1) how tau's primary sequence contributes to aggregate structure and 2) how to reproducibly generate distinct structures in order to probe their pathogenic properties.

Objective: To generate a diverse set of tau aggregate structures that will decode the relationships between tau primary sequence, aggregate structure and other key properties (e.g. aggregation kinetics).

Methods: We discovered single amino acid substitutions in the wild-type tau sequence reproducibly drive the formation of distinct tau aggregate structures in vitro. We believe that an unbiased, comprehensive mutagenesis approach has the highest probability of teasing out the potentially complex sequence-aggregate structure relationships for tau. Thus, we have optimized an experimental platform for rapid, direct comparison of thousands of tau mutants via high-throughput purification and biochemical assays. Critically, we have developed a high-throughput protease digestion assay that allows us to obtain "structural fingerprints" of tau mutant aggregates.

Results: We performed an initial proof of concept with a panel of 37 disease-associated tau missense mutations. We identified 11 distinct aggregate structure profiles within this panel and were able to map the sequences involved in the tightly packed cores of individual structures. We also demonstrate how our system allows us to directly compare multiple outputs (e.g. structure and kinetics) to begin to uncover complex biochemical relationships.
Conclusions: Harnessing the simple yet powerful effects of tau mutations, we have built a platform that will generate an invaluable molecular toolkit for elucidating relationships between aggregate structure and other aggregate properties including those that predict pathogenic potential in vivo.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS286 | CASP-SAXS: Comparison of protein structure predictions and crystal structures to experimental Small Angle X-ray Scattering data.

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Machine learning algorithms have transformed protein structural biology, producing predictions that have a high probability of being accurate based on the Critical Assessment of protein Structure Prediction (CASP) 14. However, how well do these predictions truly solve the protein folding problem and match biologically-relevant protein conformations in solution? Here, we examined four CASP14 targets and collected Small Angle X-ray Scattering Data, in collaboration with the CASP14 participating crystallographers. We compared CASP14 predictions and crystal structures, with the SAXS data. We found in all cases that although AlphaFold2 indeed accurately predicted the crystal structures, both AlphaFold2 and the crystal structures did not match the solution conformations. This analysis both shows the power of structure prediction, but also its current limitations and the need for efficient, experimental validation.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS288 | CaMKII bind both substrate and activators at the active site

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Ca2+/calmodulin dependent protein kinase II (CaMKII) is a signaling protein required for long-term memory. Once activated by Ca2+/CaM, it sustains activity even after the Ca2+ dissipates. In addition to well-known autophosphorylation-mediated mechanism, interaction with specific binding partners also persistently activates CaMKII. A longstanding model invokes two distinct S- and T-sites. If an interactor binds at the T-site, it will preclude autoinhibition and allow substrates to be phosphorylated at the S-site. Here, we specifically test this model with X-ray crystallography, molecular dynamics simulations, and biochemistry. Our data are inconsistent with this model. Co-crystal structures of four different activators or substrates show that they all bind to a single continuous site across the kinase domain. We propose a mechanistic model that persistent CaMKII activity is facilitated by high affinity binding partners, which kinetically compete with autoinhibition by the regulatory segment to allow substrate phosphorylation.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS289 | TRAAK-phosphatidylserine interactions are modulated by cupric ions

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TRAAK and TREK2 are two-pore domain K+ (K2P) channels and modulated by diverse factors including temperature, membrane stretching, and lipids. In addition, copper and zinc, both of which are essential for life, are known to regulate TREK2 and a number of other ion channels. However, the role of divalent cations in the association of lipids with integral membrane proteins is poorly understood. Here, we discover cupric ions selectively modulate TRAAK binding phosphatidylserine (PS) but not TREK2. Both channels bind other divalent cations (Ca2+, Mg2+, and Zn2+) but have no impact on binding lipids. Additionally, TRAAK binds more avidly to Cu2+ and Zn2+ than TREK2. In the presence of Cu2+, PS with different acyl chains binds similarly to TRAAK, indicating a crucial role of the serine headgroup in coordinating Cu2+. High-resolution native mass spectrometry enables the determination of equilibrium binding constants for distinct Cu2+-bound stoichiometries and uncovered the highest coupling factor corresponds to a 1:1 PS-to-Cu2+ ratio. Interestingly, the next three highest coupling factors had a ~1.5:1 PS-to-Cu2+ ratio. Our findings bring forth the role of cations as an essential cofactor in selective membrane protein-lipid interactions.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS292 | A Novel Plug-and-Play Fab-Protein G Pair Platform with Multifunctional Capability

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A platform that facilitates direct delivery of a cargo to a variety of cell-surface targets with low off-target effects is highly desirable in cell biology. Affinity-based reagents such as antibodies are ideal candidates for such an approach; however, cargo attachment can be cumbersome and often not amenable to high throughput applications. We have developed a novel platform that provides for facile introduction of multiple functionalities into a class of Fab-based affinity reagents in a "plug and play" fashion. This platform exploits orthogonal pairs of different variants of a Fab scaffold and an engineered domain of an immunoglobulin binding protein, protein G. The protein G- Fab scaffold pairs are characterized by the ultra-tight binding and slow dissociation resulting in exceptionally long lifetimes of the complex. Protein G is a small protein that can be easily manipulated. This enables the attachment of various functional components with varying linker lengths, providing adjustable spacing between components. Additionally, the specificity of protein G variants towards different engineered Fab scaffolds allows for the simultaneous cargo delivery to several targets simultaneously. To demonstrate the utility of this platform, we applied it to a variety of applications, such as a detection proximity assay based on the β-lactamase (BL) split enzyme system, Bi-specific T-cell engager (BiTE) cell killing, simultaneous binding detection of two antibodies, and several more. The advantage of the system is the simplicity in how the components can be assembled and the assortment of applications that can benefit from their use.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS293 | BOS: The key enzyme in the biosynthesis of the neurotoxin b-ODAP in Grass Pea (Lathyrus sativus L.)

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The seeds of the grain legume grass pea (Lathyrus sativus L.) are highly nutritious as they are enriched in protein (28%), folic and linoleic acids, and low in fat (<1%) and unsaturated fatty acids. The crop itself is robust to environmental stresses such as drought or flood and is both cheap and easy to cultivate. However, grass pea seeds are not widely used as food and forage outside parts of Asia and Africa because they also contain a neurotoxic metabolite, b-N-oxalyl-L-a,b-diaminopropionic acid (b-ODAP), which can cause a severe neurological disorder if consumed as a primary diet component. The catalytic activity associated with b-ODAP formation was demonstrated in grass pea more than 50 years ago, yet the enzyme responsible for this activity had not been identified. By combining protein purification and enzymatic assays with transcriptomic and proteomic analyses, we were able to identify the enzyme b-ODAP synthetase (BOS) from grass pea. We then characterized its catalytic activity, 3D structure, and phylogenesis. We show that BOS belongs to the BAHD superfamily of acyltransferases and is structurally similar to hydroxycinnamoyl transferases. Employing molecular docking, we propose a mechanism for its catalytic activity, and using heterologous expression in tobacco leaves (N. benthamiana), we demonstrate that the expression of BOS in the presence of its substrates is sufficient for b-ODAP production in vivo. The identification of BOS paves the way toward engineering b-ODAP-free grass pea cultivars, safe for human and animal consumption.

Track: Protein Science Addressing Health Disparities

ABS294 | Exploring the functionally relevant intrinsically disordered variable domain (VD) of dynamin-related protein 1 (Drp1) using high resolution NMR spectroscopy and small angle X-ray scattering

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Mitochondria are dynamic organelles shaped continuously by coordinated cycles of fission and fusion. Alterations in mitochondrial dynamics lead to various neurological disorders (Alzheimer's, Parkinson's, and Huntington's) as well as cancer and cardiovascular diseases. Dynamin-related protein 1 (Drp1), a cytosolic mechanoenzymatic GTPase, is the master regulator and mediator of mitochondrial fission. In addition to various protein receptors, Drp1 binds the mitochondria-specific

lipid, cardiolipin (CL) to promote fission. However, the molecular basis of Drp1-CL interactions remained unclear, since the CL-binding, intrinsically disordered variable domain (VD) is neither present nor sufficiently resolved in any X-ray or cryo-EM structures until date. Using state-of-the-art solution NMR spectroscopy and CL-containing lipid nanodiscs (CLND), we previously identified a CL-binding motif (CBM) in the VD and provided insights into the molecular mechanisms underlying CL recognition and binding. The 15N-1H HSQC spectrum of VD in solution with spectral dispersion from ~7.6 to 8.8 ppm indicated that the isolated VD is partly folded in solution. The addition of CLND led to significant chemical shift perturbations (CSPs) and extensive line broadening of interacting residues. Intriguingly, a highly conserved WRG motif was found to be directly involved in CL binding. As conventional NMR spectroscopy only yielded partial assignments, we are currently employing 13C-detected NMR measurements to obtain a complete assignment of the VD residues. Furthermore, to gain insight into the 3-D fold and conformational landscape of the VD in isolation as well as in full-length Drp1, we have acquired SAXS measurements for both (Figure 1). Collectively, the low-resolution envelope structure of the VD from full-length Drp1, in conjunction with high-resolution 13C-detected NMR data, will help facilitate our molecular understanding of Drp1-catalyzed mitochondrial fission.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS296 | The Old Dog Still Has Some New Tricks: Non-Canonical Allosteric Regulation of SIRT1

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SIRT1, an NAD+ dependent lysine deacetylase, deacetylates many protein substrates central to transcriptional regulation and various other cellular pathways. This enigmatic enzyme has a well-studied structured catalytic core and intrinsically disordered or conformationally dynamic regions in the N- and C-terminal ends that are less explored. Many allosteric or direct regulators of SIRT1 have been studied, including native proteins as well as natural or synthetic small molecules. Our group focuses on the lesser-known mechanisms of non-canonical allosteric regulations of SIRT1. Amongst our interests are how resveratrol, a well-studied sirtuins activating compound (STAC), can also act as an inhibitor, or have no effect on SIRT1 activity at all, depending on the amino acid sequence of the enzyme substrate. And how motif A, an intrinsically disordered region within the N-terminus of SIRT1, can activate the enzyme seemingly through an intramolecular binding interaction. We use biochemical and biophysical methods to elucidate the role of protein conformational dynamics and structural propensity in these intriguing allosteric regulation mechanisms. We hope that our findings could provide further insight for the intricate web of regulation on SIRT1 activity within the cell.

Track: Protein Science Addressing Health Disparities

ABS297 | Identifying the Core Regions of Vicilin and Legumin Amyloid Fibrils

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There is growing interest in developing functional amyloid as a novel biomaterial (e.g., for use in encapsulation, gellation, and bioscaffolding) owing to the unique physical properties of amyloid fibrils. Legume seed storage proteins, mainly vicilins and legumins, could be a safe, sustainable and cost-effective input for plant-based functional amyloid. Crude extracts of legume proteins can be induced to fibrillate by heating at acidic pH, although mechanistic details are currently lacking. In this work, we sought to identify which proteins and hydrolyzed peptide fragments form the amyloid fibrils, and link information about the fibril core regions with fibrillation kinetics and morphology. Pea and soy were selected as models. Pea and soy proteins were fractionated by differential precipitation and anion exchange chromatography. Vicilins, legumins and crude protein extracts were incubated at pH 2, 80°C. The fibrillation kinetics and fibril morphologies were characterized and the fibril composition was determined using LC-MS/MS.

It was found that partially purified vicilins and legumins displayed faster fibrillation kinetics than crude proteins. Pea vicilin displayed almost no lag phase and most immense intensity increase. Fibrils formed by pea proteins were mainly straight and long, while soy fibrils were more worm-like. Mass spectrometry identified over 150 unique peptides presented in the major protein in each sample, with the highest of 49.5% pea vicilin regions capable of forming fibrils. It can be concluded that vicilins possess a higher fibril-forming capacity than legumins; among which pea vicilin showed the highest fibril forming propensity possibly due to the abundance of

fibril-forming regions. Overall, the current research found that soy and pea enriched fractions possess copious fibril-forming peptides compared to previously studied β-lactoglobulin and lysozyme, making them competent alternatives to animal protein fibrils.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS298 | Assessing the destabilizing effects of OCA8-related missense mutations in Tyrp2

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Oculocutaneous albinism Type 8 (OCA8) is a genetically inherited disease caused by biallelic mutations in the DCT gene encoding tyrosinase related protein2 (Tyrp2), also named dopachrome tautomerase (DCT). Tyrp2 is an enzyme which functions inside of melanosomes to convert dopachrome to 5,6-dihidroxyindole-2-carboxyli acid (DHICA) and regulate the production of melanin pigments. The mechanism by which these mutations alter the structure of Tyrp2 and how these changes lead to progression of OCA8 is not well understood. Considering this, we modelled the intramelanosomal domain of Tyrp2 wild-type and OCA8-related mutant structures and quantified their structural fluctuations over 50 ns of molecular dynamics simulation. These quantifications demonstrated that OCA8 related variants display varying degrees of increase structural fluctuation in relatively more stable regions of the wild-type structure. This data suggests that these mutations are likely to result in greater unfolding of the enzyme over a longer timespan which is beyond the scope of these simulations. To further investigate how mutations at these positions of Tyrp2 may lead to destabilization, we utilized our global computational mutagenesis pipeline. This pipeline is a collection of programs which allow the user to model protein structures and calculate the destabilizing effects of missense mutations. One of these programs, the Unfolding Mutation Screen (UMS), calculates unfolding propensities from free energy change (□□G) values for all possible missense variants of the structure. Unfolding propensities for all OCA8-related mutation positions in Tyrp2 suggested severe destabilization of the variant enzymes. Results from our pipeline and molecular dynamics experiments also align with experimental data from the ClinVar database and our own protein purification study of two Tyrp2 variants. Our calculations establish a mechanism by which these OCA8-related mutations alter the structure of Tyrp2 to disrupt the melanogenic pathway and cause disease.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS299 | Mimicking nature's evolutionary mechanism: design of cobalamin-binding chimeras from different protein folds

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Proteins are one of the most important biomolecules, performing key cellular functions relevant to our modern life. For this, proteins evolved by reusing stably folded fragments and repurposing functional proteins. Studies in molecular evolution have identified a number of processes such as recombination, mutation, circular permutation, and duplication as the forces that shape the abundance and diversity of protein universe. Mimicking some of these evolutionary tricks found in nature and combining them with rational design results in protein chimeragenesis as a powerful tool for the creation of new macromolecules. By swapping sequence elements among related proteins, this approach aims to build up chimeric proteins with new behaviours and functions, paving the way to the construction of new biocatalysts. Inspired by synthetic chemistry, where transition metal catalysts can be utilized for performing sophisticated reactions, we aimed to work with cobalamin, a versatile molecule used for a myriad of reactions like methyl transfer, isomerization, ribonucleotide reduction, and dehalogenation. Using a chimeragenesis-based approach, a cobalamin-binding chimera was built by replacing the flavodoxin-like domain in the hemD-like fold of Uroporphyrinogen-III-synthase from Homo sapiens with the homologous cobalamin-binding domain of Methylmalonyl-CoA-mutase from Aeropyrum pernix. The resulting chimera UShsMMap01, hereinafter called Cob01, was biophysically and structurally characterized, showing a preliminary and weak binding for cobalamin. Using this starting point, a collection of chimeras was engineered and changes implemented based on structural considerations in order to improve the binding properties, yielding the variants Cob02-Cob10. These Cob-chimeras displayed broad changes in their structural and thermodynamic properties, as well as cobalamin-binding parameters, which was proved by hydrodynamic, spectroscopic, and calorimetric methods. Furthermore, crystal structures for

some variants were obtained in order to elucidate the specific binding mode of cobalamin. This work provides crucial information towards the design of synthetic enzymes that use cobalamin, the most complex cofactor on Earth.

Track: Protein Phase Separation in Biomolecular Condensates

ABS303 | High-Throughput Screening to Identify Modulators of Raft Formation and Membrane Protein Raft Affinity in Giant Plasma Membrane Vesicles

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Lipid rafts remain an intriguing and active area of membrane biophysics research. Due to their diffraction-limited size and transient nature direct studies of rafts and their resident proteins in cells remains intractable. Giant plasma membrane vesicles (GPMVs), which spontaneously separate into ordered (raft) and disordered (non-raft) phases, have emerged as practical a tool for studying rafts in biological membranes. The inability to selectively manipulate rafts and raft-resident proteins has also stymied our ability to study raft formation and the partitioning of proteins into them. We recently developed a high-throughput screening pipeline to identify small molecule modulators of phase separation in GPMVs. Combining high-content imaging with a custom image-analysis software package, VesA, we successfully identified compounds which robustly increase or decrease phase separation in GPMVs. From this proof-of-concept study, we next sought to find compounds that alter the phase partitioning of the peripheral myelin protein 22 (PMP22). PMP22, a major component of myelin in Schwann cells of the peripheral nervous system, shows a high affinity for ordered phases of GPMVs. Disease causing mutations in PMP22 exhibit both varying degrees of misfolding and decreased raft partitioning. The identification of small molecules that selectively bind to PMP22 and alter its phase partitioning will be crucial to investigate how and why PMP22 partitions into rafts. We conducted a preliminary screen of PMP22 partitioning with a library of 1100 FDA approved drugs. A number of non-specific partitioning modulators were identified. We then conducted a screen of 20,000 small molecules from the Vanderbilt University Discover collection. Hits from these screens will be invaluable to our efforts to understand the relationship between PMP22 structure and raft affinity.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS304 | B-Myb association with DNA is mediated by its intrinsically disordered negative regulatory domain and Cdk phosphorylation

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Transcription factors (TFs) contain DNA binding domains that recognize a specific DNA sequence; however, it is critical to understand how they achieve binding specificity and subsequent function. B-Myb is a highly conserved member of the vertebrate Myb family of transcription factors that plays a critical role in cell-cycle progression and proliferation. Myb proteins activate Myb-dependent promoters by interacting specifically with Myb binding site (MBS) sequences through the DNA binding domain (DBD) of B-Myb. Transcription factors that bind to specific DNA sequences may acquire specificity and regulation through intrinsically disordered regions (IDRs). Transactivation of MBS promoters by B-Myb is repressed by its intrinsically disordered domain, named negative regulatory domain (NRD). Phosphorylation of the NRD by Cdk2-CyclinA relieves the repression to strongly activate B-Myb dependent promoters. The structural mechanisms underlying autoinhibition and activation of B-Myb through the NRD have been poorly characterized. Through ITC and CON based NMR, we determined that an IDR in the NRD B-Myb (residues 510-600) directly associates with the DBD and inhibits DBD binding to the MBS DNA sequence. Here we demonstrate that phosphorylation of the NRD at T515, T518 and T520, is sufficient to disrupt the interaction between NRD and DBD, and results in increased affinity to MBS DNA and increased Myb-dependent promoter activation. Our biochemical characterization of B-Myb auto-regulation and the activating effects of phosphorylation provides insights on how B-Myb functions as a site-specific transcription factor. Our results demonstrate how IDRs in TFs can regulate TF-DNA interactions and how this regulation can be modulated through post-translational modifications.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS307 | The Expanding World of Metabolic Enzymes Moonlighting as RNA Binding Proteins

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RNA binding proteins play critical roles throughout the lifetime of RNA in processing of new RNA transcripts, regulation of translation, and RNA stability and degradation. Within the past few years, proteomics studies have identified dozens of enzymes in intermediary metabolism that bind to RNA. The large number of these noncanonical RNA binding proteins and the conservation of their RNA binding ability across distant branches of the evolutionary tree suggest that they are involved in connections between intermediary metabolism and gene expression that comprise far more extensive regulatory networks than previously thought. Studies of a few "classic" examples such as aconitase have shown that combining catalytic and RNA binding functions in one protein can be a mechanism to sense the cell's metabolic state through availability of the enzyme's ligands and respond by regulating translation of specific transcripts. Conversely, RNA binding could regulate the enzyme's catalytic activity, through blocking the active site, allosteric effects, acting as a scaffold, or sequestering enzymes. We are studying the molecular structures and mechanisms involved, the effects of these interactions on the catalytic and RNA functions, and the cellular factors that regulate the interactions. Information gained from studying enzymes in carbohydrate, amino acid, and lipid metabolism that act as noncanonical RNA binding proteins will increase our understanding of the coordination between central metabolic pathways and RNA metabolism. This information can be applied in the future to the design and development of novel proteins that regulate RNA translation, stability, and lifetime, as well as RNAs that regulate enzyme function.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS308 | Investigation of Evolutionary Functional Origins of Promiscuity in DNA/RNA Methyltransferases

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M.EcoGII is the only known bacterial methyltransferase that catalyze non-site-specific N6-adenine methylation on both DNA and RNA. However, the evolutionary origin of this unusual promiscuity is still unknown. Here, we performed ancestral sequence reconstruction to investigate the evolutionary origin of sequence specificity in M.EcoGII. Five ancestors of M.EcoGII were selected from a maximum-likelihood phylogeny for ancestral sequence reconstruction. These ancestral enzymes were expressed in E. coli, purified, and characterized. In vitro methylation assays showed that all ancestors had a higher activity for RNA than M.EcoGII, with Anc284 showing a significant increase in selectivity for RNA. Multiple sequence alignment indicated the amino acid residues in charge of substrate specificity. The results in this study will contribute to the understanding of the molecular mechanism of selective activity between RNA or DNA methyltransferases. In addition, the RNA selective M.EcoGII ancestor would be applicable to the epigenetic tools for changing RNA methylation states.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS309 | Time Travel to the Past and Future Evolution of energy landscapes for enzymes catalysis

Dorothee Kern
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The essential role of protein dynamics for enzyme catalysis has become more generally accepted. Since evolution is driven by organismal fitness hence the function of proteins, we are asking the question of how enzymatic efficiency has evolved. First, I will address the evolution of enzyme catalysis in response to one of the most fundamental evolutionary drivers, temperature. Using Ancestral Sequence Reconstruction (ASR), we answer the question of how enzymes coped with an inherent drop in catalytic speed caused as the earth cooled down over 3.5 billion years. Tracing the evolution of enzyme activity and stability from the hot-start towards modern hyperthermophilic, mesophilic and psychrophilic organisms illustrates active pressure versus passive drift in evolution on a molecular level (1). Second, I will share a novel approach to visualize the structures of transition-state ensembles (TSEs), that has been stymied due to their fleeting nature despite their crucial role in dictating the speed of biological processes. We determined the transition-state ensemble in the enzyme adenylate kinase by a synergistic approach between experimental high-pressure NMR relaxation during catalysis and molecular dynamics simulations (2). Third, a novel general method to determine high resolution structures of high-energy states that are often the biologically reactive species will be described (3). With the ultimate goal to apply this new knowledge about energy landscapes in enzyme catalysis for designing better biocatalysts, in "forward evolution"

experiments, we discovered how directed evolution reshapes energy landscapes in enzymes to boost catalysis by nine orders of magnitude relative to the best computationally designed biocatalysts. The underlying molecular mechanisms for directed evolution, despite its success, had been illusive, and the general principles discovered here (dynamic properties) open the door for large improvements in rational enzyme design (4). Finally, visions (and success) for putting protein dynamics at the heart of drug design are discussed.

Track: Protein Phase Separation in Biomolecular Condensates

ABS310 | High resolution NMR H/D exchange of inclusion bodies reveals significant native-like structure

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Aggregation of proteins is at the nexus of molecular processes crucial to aging, disease, and employing proteins for biotechnology and medical applications. While there has been considerable recent progress in determining structural features of protein aggregates formed in cells, owing to prevalent heterogeneity in aggregation, many aspects remain obscure and often experimentally intractable to study. Here we report high resolution analysis by NMR quenched amide hydrogen/deuterium exchange of cellular aggregation of Cu, Zn superoxide dismutase (SOD1) mutants associated with amyotrophic lateral sclerosis and engineered target binding proteins known as Adnectins or monobodies. The structures of the inclusion bodies (IBs) formed by these proteins upon overexpression in E. coli were also assessed for secondary structure by ATR-FTIR and amyloid content by Congo Red binding. Strikingly, all the IBs exhibit very extensive protection against exchange throughout the proteins, beyond regions predicted as being prone to aggregation and including many amides that are protected by native structure. The IBs also have native-like secondary structure and modest amyloid signal. Remarkably, chemically and structurally diverse mutations generally have little effect on the measured IB structures, despite substantial changes in protein stability and predicted aggregation propensity. These results contrast with the observation of prominent amyloid for IBs formed by other proteins and indicate significant native-like structure may be retained through an ensemble of self-association processes contributing to IB formation.

Track: Protein Science Addressing Health Disparities

ABS311 | Co-Translational Insertion of the Sodium Leak Channel NALCN Domain I

Jose M. Acosta-Caceres, Veronica Lopez-Lopez, Maria J. Garcia-Murria
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The sodium leak channel NALCN is expressed mainly in the central nervous system and dysfunctions in this channel are associated with a variety of human diseases. In fact, mutations in NALCN are implicated in severe neurodevelopmental disorders, including congenital contractures of limbs and face, hypotonia and developmental delay (CLIFAHDD).

NALCN channel is a large multipass membrane protein lacking a cleavable signal peptide. Recent structural data confirmed four homologs repeats (domains I–IV) each composed of six transmembrane (TM) segments (S1–6) 1, many of them being poorly hydrophobic. Accordingly, these segments by themselves are not expected to stably integrate into the membrane. Interestingly, one third of the CLIFAHDD related mutations are in the domain I (DI).

We studied the biosynthesis of NALCN N-terminal DI domain in biological membranes. First, we challenged the insertion of the individual TM sequences using a glycosylation-based reporter system in which the sequences of interest were fused to the constant domain of the antibody λ light chain, CL 2,3. We found that half of the TM segments (S1, S2 and S4) are not properly recognized and inserted through the translocon. Next, we characterized the membrane integration and topology of NALCN DI using truncated C-terminal reporter tag fusions of growing lengths both in prokaryotic and eukaryotic cells 4. Our data proves that for proper insertion some of the TM segments (the less hydrophobic ones) require the presence of downstream regions. Finally, we investigated the effect of the CLIFAHDD mutations found in DI on the insertion process of this N-terminal NALCN domain.

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Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS312 | Human sperm TMEM95 facilitates membrane fusion with eggs

Shaogeng Tang, Peter Kim
Stanford University (United States)

TMEM95 encodes a sperm acrosomal membrane protein, whose knockout has a male-specific sterile phenotype in mice. How TMEM95 plays a role in gamete membrane fusion has remained elusive. Here, we show that human TMEM95 binds hamster eggs, providing evidence for a TMEM95 receptor. We determine a 1.5 Å X-ray crystal structure of TMEM95 and reveal an evolutionarily conserved, positively charged area as a putative receptor-binding surface. Amino-acid substitutions within this region of TMEM95 ablate egg-binding activity. We identify monoclonal antibodies against TMEM95 that inhibit fusion of human sperm to hamster eggs. Strikingly, these antibodies do not block binding of sperm to eggs. Taken together, these results provide strong evidence for a specific, receptor-mediated interaction of sperm TMEM95 with eggs and suggest that this interaction may have a role in facilitating membrane fusion.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS313 | Structural and Kinetic Mechanism of Substrate Specificity Change due to Enzyme Filamentation

Nancy Horton, Niloofar Ghadirian
University of Arizona (United States)

We use a model system, the sequence dependent endonuclease SgrAI, to investigate important mechanistic and biological questions regarding enzyme filamentation and enzyme regulation. SgrAI forms structured assemblies of heterogeneous stoichiometries under conditions where its DNA cleavage activity is 200-1000 fold accelerated, and surprisingly, its DNA sequence specificity is altered (Biochem. 49, 8818); SgrAI cleaves a secondary set of DNA sequences but only in the presence of its primary site, which induce the observed assemblies of SgrAI. We have shown that these assemblies are helical filaments composed of SgrAI bound to DNA (Biochem. 52, 4373, Structure 21, 1848), and that filamentation stabilizes an activated conformation of SgrAI resulting in the binding of a second divalent cation in the active site leading to rapid DNA cleavage (Structure 27, 1, JBC, 298, 101760). We have also carried out a complete kinetic investigation to create a full computational model of the entire DNA cleavage pathway including filamentation and all forward and reverse rate constants for each step (JBC 293, 14585 & 14599). This model has allowed us to predict the behavior of SgrAI within a cell, at biologically relevant concentrations, to show that the filamentation mechanism, and in particular the slow filament assembly step, allows SgrAI to target invading DNA while minimizing damage to its host genome (J. Virol. 93, e01647). We now show, with new data kinetic and global modeling data, that the highly unusual alteration of DNA sequence specificity exhibited by SgrAI when in the filamentous form is due to a 5-17-fold stabilization of the low activity (and non-filamenting) state when SgrAI is bound to its secondary site DNA sequence. Structural studies show the likely origin of this preferential stabilization in the form of sequence-dependent DNA structure, complementarity of the protein-DNA interface, and a disorder-to-order protein structural transition.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS314 | Filamentation as a New Level in Enzyme Regulation

Nancy Horton, Noura Darwish, Chad Park
University of Arizona (United States)

It is becoming clear that many enzymes, from diverse biological pathways and all branches of life, form linear, helical, or tubular polymers (or filaments) with altered function and/or activities. We present the collection of known filament-forming enzymes including their structure, function, as well as any known effects on enzyme activity resulting from filamentation. In many cases, effects on enzyme activity are not known, while in others, enzymes are activated, inhibited, show altered substrate specificity, altered cooperativity, altered response to allosteric effectors, or in some cases show completely new enzymatic or biological activity. Many filament-forming enzymes are medically important, controlling important steps in metabolic pathways and where dysfunction, including changes in filamentation, can be related to disease states. The relationship of enzyme polymeric filaments to the larger scale "filaments" (membraneless self-assemblies) seen in cells via fluorescence microscopy is also discussed.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS315 | Engineering proteins to sense specific small ligands

Birte Höcker
Universität Bayreuth (Germany)

Proteins that promiscuously bind a range of ligands provide insights into how nature mediates affinity and biological functioning. Moreover, such receptor proteins provide vantage points for the rational design of specific binding for biotechnological applications.

Here I will discuss two different classes of proteins that we engineered to bind specifically targets of interest: Periplasmic binding proteins (PBPs) and the Trp repressor (TrpR).

PBPs are a highly adaptable superfamily of proteins that undergo a large conformational change upon ligand recognition. We explored the capacity of the polyamine binding PBP PotF to recognize different ligands [1], changed its specificity [2] and engineered it into biosensors that can track binding events of spermidine or agmatine.

TrpR on the other hand is a dimeric protein that changes conformation to interact with DNA upon binding of tryptophan. We changed the specificity of TrpR to specifically bind to the plant hormone indole-3-acetic acid, also known as auxin, and constructed a biosensor for its direct visualization in plants [3]. This sensor now enables real-time monitoring of auxin concentrations at a (sub)cellular resolution and their spatial and temporal changes during the lifespan of a plant.

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Track: High Throughput Protein Science

ABS317 | Speeding up protein homology search using a neural pre-filter

Daniel Olson, Thomas Colligan, Jack Roddy, Travis Wheeler
University of Montana (United States)

Protein homology search is an integral and computationally expensive part of many protein analysis pipelines. For example, in the complete AlphaFold2 pipeline, sequences are initially searched against a large (2 TB) protein sequence database to build a multi-sequence alignment, taking up to hours of CPU time for a single input. Homology search tools like HMMER rely on pre-alignment filters to remove poor alignments before computationally expensive algorithms like Forward/Backward are employed; however current filters are both too slow and too coarse for the age of big data. Here we describe and demonstrate our neural pre-filter, which works by turning a sequence of amino acids into a reduced sequence of neural embeddings. We then cluster and hash embeddings using the FAISS library and only perform alignments when two sequences share similar embeddings. Filtering based on embedding similarity is a computationally efficient process, and using embeddings derived from modern deep learning allows for greater specificity than traditional pre-filter methods.

Track: Imaging & Tracking of Proteins in Space and Time

ABS319 | Engineered bioluminescent probes for visualizing RNA in live animals

Lila Halbers, Kevin Ng, Kyle Cole
UCI (United States)

We have a solid molecular-level understanding of gene expression and RNA turnover in cultured cells and transparent samples. However, our understanding of RNA biology in the most physiologically authentic environment—live animals—remains woefully incomplete. This is due, in part, to a lack of general and reliable methods for tracking RNA life cycles in vivo. The most used molecular tools for the tracking and localization of RNAs employ fluorescent proteins, which require exogenous and potentially tissue-damaging excitation light for visualization, resulting also in significant fluorescence background in tissues. The use of bioluminescent tools overcomes these challenges through higher signal-to-noise ratios and no excitation light, allowing for the imaging of live animals and potentially fewer molecular targets—an important quality for the visualization of low-copy transcripts. We developed bioluminescent proteins to tag and visualize RNAs in vivo. These engineered reporters bind unique RNA sequences and turn on photon production in the presence of target transcripts. Using this platform, we aim to follow the life cycle of RNA through transcription, processing, and ultimately decay in photo-sensitive locations such as the brain.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS320 | Molecular Basis of Allosteric Regulation and Isoform Specificity of Protein Kinase C Beta

Anh Cong, Taylor Witter, Elizabeth Bruinsma, Swaathi Jayaraman, Mary Kuffel, Maria Dugan, John Hawse, Matthew Goetz
Mayo Clinic (United States)

Protein Kinase C (PKC) isozymes are ubiquitous kinases that direct diverse cellular pathways, and are important drug targets for cancer chemotherapy and the treatment of neurological diseases. Key to their function is the ability to respond to second-messenger lipid and Ca2+ signals, which is conferred by a regulatory, multi-domain N-terminal region. Although PKCs are important for a wide array of signalling pathways, the underlying molecular mechanism by which PKC responds to lipid and Ca2+ second messengers has remained enigmatic due to a paucity of structural information. Using our YFP-tag system we have been able to generate recombinant PKC enzymes using a mammalian cell expression system, and we have determined a series of crystal structures of the human PKCβI and PKCβII isozymes that define the molecular basis by which PKC maintains an inactive conformation. We have identified a "lipid lever" mechanism of allosteric activation of PKC and a new step in PKC activation, which is the formation of a defined and ordered active conformation of PKC. Furthermore, we define how the isoform-specific differences between PKCβI and PKCβII alter the allosteric regulatory mechanism of PKCs to provide isoform–specific activities. Finally, we show that the allosteric regulatory mechanism of PKCβI can be altered by small molecules, providing a proof-of-concept for isoform-specific allosteric regulators of PKCs. Collectively, our data describe the full molecular basis of the second messenger–mediated regulation of PKC and defines the molecular interlocks that are targeted by post-translational modifications, mutations, or drug molecules to regulate PKC function.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS321 | A chemi-genetic ion indicator consists of a synthetic chelator and green fluorescent protein

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Appropriate concentrations of metal ions such as Ca2+, Mg2+, and others are necessary to maintain a healthy body. Take Calcium ion (Ca2+) for an example. Abnormalities in Ca2+ concentration dynamics are associated with many pathological consequences, such as cardiovascular disease, hypertension, and Alzheimer's disease. Accordingly, the investigation of the fundamental roles of metal ions is important in the fields of biology and medicine. After decades of development, fluorescent indicators that enable dynamic visualization of metal ions in biological systems are powerful tools and widely used in biological research. Generally, those fluorescent probes fall into two categories: small-molecule based synthetic indicators and protein-based biosensors. Compared with protein-based biosensor, synthetic sensors are free from the limitation of requiring a suitable, naturally occurring, ion-binding protein for the target ion of interest. Nevertheless, protein-based biosensors are highly desirable due to the fact that they are inherently biologically compatible, which allows them to be constitutively expressed in biological systems and localized to specific cellular compartments of interest.

Here, to combine the advantages of both synthetic and protein-based biosensors, I report a novel hybrid (chemi-genetic) biosensor design that is composed of a synthetic target-binding domain and a fluorescent protein. As a prototypical example, I developed a chemi-genetic Ca2+ indicator named as HaloGFP-Ca1, using a circular permutated green fluorescent protein (cpGFP), a tag protein (HaloTag), and a synthetic HaloTag-reactive chloroalkane ligand. This ligand serves to display the Ca2+ chelator, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA). After multiple rounds of optimization, the fluorescence change of HaloGFP-Ca1 was increased to over ten-fold in vitro. I also confirmed its applicability in HeLa cells and rat primary neuron cells.

In the future, we plan to change both the ion chelator ligands and the fluorescent protein domains, ultimately providing powerful new tools to accelerate biological research.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS322 | Self-Assembly And Target-Binding Properties of Centrin 1 from Toxoplasma Gondii

Luca Bombardi, Carolina Conter, Marco Pedretti, Filippo Favretto, Adele Di Matteo, Paola Dominici, Alessandra Astegno
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Centrins are conserved calcium (Ca2+)-binding proteins typically associated with centrosomes that have been

implicated in several biological processes, such as DNA repair, centrosome duplication and mRNA nuclear export. In the parasite Toxoplasma gondii, the causative agent of toxoplasmosis, three isoforms of centrin have been identified: centrin 1 (TgCEN1), centrin 2 (TgCEN2), and centrin 3 (TgCEN3). While increasing information is now available that links centrins function with defined parasite biological processes, knowledge is still limited on the metal-binding and structural properties of these proteins. Recently, using biophysical and structural approaches, including dynamic light scattering, isothermal titration calorimetry, and nuclear magnetic resonance, we have explored the macromolecular properties of TgCEN1 showing that it behaves biochemically like Ca2+ sensor and displays a Ca2+-dependent propensity to self-assemble. This process is completely abolished upon removal of the first 21 residues of the protein and is significantly reduced in the presence of a binding target peptide derived from the human XPC protein (P17-XPC). The characterization of the interaction between TgCEN1 and P17-XPC indicated that TgCEN1 possesses two binding sites with distinct affinities and Ca2+ sensitivity, a high-affinity site in the C-lobe (C-TgCEN1, 95–169 aa) which may be constitutively bound to the peptide and a low-affinity site in the N-lobe (N-TgCEN1, 1–94 aa) which is active only upon Ca2+ stimulus. These findings suggest a specific mechanism of TgCEN1 for Ca2+-modulated target binding and support a N-to-C self-assembly mode, in which the first 21 residues of one molecule likely interact with the C-lobe of the other. Overall, our results suggest that a fine interplay exists between the interaction of TgCEN1 with target proteins and its self-assembly propensity. The study of these processes at a molecular level can help to better understand the Ca2+-controlled structural changes and function of TgCEN1 in the highly dynamic structure of centrosomes.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS327 | Coevolution-derived native and non-native contacts determine the emergence of a novel fold in a universally conserved family of transcription factors

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The NusG protein family is structurally and functionally conserved in all domains of life. Its members directly bind RNA polymerases and regulate transcription processivity and termination. RfaH, a divergent subfamily in its evolutionary history, is known for displaying distinct features than those in NusG proteins, which allows them to regulate the expression of virulence factors in enterobacteria in a DNA sequence-dependent manner. A striking feature is its structural interconversion between an active fold, which is the canonical NusG three-dimensional structure, and an autoinhibited fold, which is distinctively novel. How this novel fold is encoded within RfaH sequence to encode a metamorphic protein remains elusive. In this work, we used publicly available genomic RfaH protein sequences to construct a complete multiple sequence alignment, which was further augmented with metagenomic sequences and curated by predicting their secondary structure propensities using JPred. Coevolving pairs of residues were calculated from these sequences via direct coupling analysis (DCA) using plmDCA and GREMLIN, which allowed us to detect the enrichment of key metamorphic contacts after sequence filtering. Finally, we combined our DCA predictions with molecular dynamics to demonstrate that these interactions are sufficient to predict the structures of both native folds, where coevolutionary-derived non-native contacts may play a key role in achieving the compact RfaH novel fold. All in all, emergent coevolutionary signals found within RfaH sequences encode the autoinhibited and active folds of this protein, shedding light on the key interactions responsible for the action of this metamorphic protein.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS328 | Quantitative approach to examining transcription factor-DNA binding in Drosophila melanogaster

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Homeotic genes are a group of genes that control proper body segmentation formation during early embryonic development. These genes encode for proteins called transcription factors (TFs) that turn on/off specific genes by binding to DNA. These TFs are known to bind to cis-regulatory sequences, however, the number of binding sites is unknown. TFs share a conserved DNA binding domain called the homeodomain that is responsible for mediating the interactions of TF proteins and DNA. The homeodomain is a highly conserved 60 amino acid helix-turn-helix DNA binding domain where the third helix

binds to the major groove of DNA. In this study, TF-DNA binding interactions are being examined in Drosophila melanogaster (fruit fly) to gain a better understanding of its role in cell fate in the developing embryo. By understanding the affinity TFs have for specific DNA sequences in Drosophila, we can better understand how these similar events occur in human embryo development. Previous work has examined TF-DNA binding through the use of qualitative methods and although these methods allow for fast and real time analysis, they provide little information on thermodynamic binding affinity and/or sequence specificity. Here we aim to specifically analyze the different binding affinities of TF-DNA binding interactions with the overarching goal of obtaining a deeper understanding of its role in cell fate in the developing embryo. The TF even-skipped (eve) was examined due to its important role in developing odd/even-numbered parasegments in Drosophila. Biophysical analysis of eve was performed by investigating binding affinity and specificity through the quantitative approach known as Isothermal Titration Calorimetry (ITC). This gold standard biophysical technique measures the binding affinity and thermodynamic parameters for characterizing TF-DNA interactions. This study will allow for improved understanding of the key regulatory interactions at the top of the genetic TF cascade that control neural cell development.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS330 | Conformational Dynamics of Deubiquitinase A and Functional Implications

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Deubiquitinase A (DUBA) belongs to a family of ~100 proteases in humans responsible for deconjugation of ubiquitin modification from target proteins. Deubiquitinases (DUBs) are involved in regulating protein turnover and many other biological processes. Some DUBs have been identified as therapeutic targets in human diseases, including cancer, neurodegeneration, and autoimmune diseases. Although numerous structural and biochemical studies have suggested that conformational plasticity is essential for the catalytic turnover of substrates and the regulation of DUBs, the lack of detailed characterization of dynamic processes in DUBs has hampered the understanding of the functional roles of these processes. The goal of our study was to use DUBA as a model system to understand the functional importance of conformational dynamics in the DUB family. DUBA is regulated by phosphorylation at a single residue, Ser177, which was known to induce almost no structural changes. Using solution nuclear magnetic resonance (NMR) spectroscopy, we found that the alpha1 helix of DUBA and the neighboring residues, including Ser177, adopt two conformations in the phosphorylated DUBA whereas the nonphosphorylated DUBA and mutants that have low activity do not display the same two-state conformational equilibrium. Moreover, NMR relaxation dispersion experiments revealed that most parts of the enzyme undergo coupled motions on the sub-millisecond timescale. The data also suggest that transition into a sparsely populated state on this time scale is driven by unfolding of the alpha6 helix, which harbors two positively charged residues that interact with the phosphate group attached to Ser177, according to the crystal structure. Overall, our study suggests that subtle changes in the dynamic properties of DUBA induced by phosphorylation can lead to the activation of the enzyme and the coupled motions across the entire DUBA molecule are essential for the modulation of functionally important motions of Ser177 and other key residues by phosphorylation.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS331 | A Tale of Two Agonists: How Does One Innate Immune Protein Respond to Both Soluble Protein and Small Molecule Agonists?

Lauren Chisholm, Mike Harms, Hannah Murawsky
University of Oregon (United States)

The innate immune system recognizes and responds to both external and internal danger signals. Two key signals are lipopolysaccharide (LPS) and S100A9, both of which bind to the Toll-like receptor 4 complex (TLR4) and activate inflammation. This dual activation is puzzling because these agonists have radically different structures: LPS is a small molecule derived from the outer cell membranes of gram-negative bacteria; S100A9 is a soluble protein produced by the host. The mechanism by which S100A9 activates TLR4 remains unknown. In this project, we aim to understand how the TLR4 coreceptor CD14 can recognize these diverse ligands. We also want to understand the role played by CD14 in the activation of TLR4 by S100A9. CD14 acts as a delivery protein shuttling LPS to the TLR4 complex: does it do the same for S100A9? Using extensive mutagenesis, structural modeling, in vitro biochemistry and ex vivo functional assays, we have begun to unravel the differences

between the interactions of LPS and S100A9 with CD14. We have found that S100A9 interacts with a different site on CD14 than LPS. We have also found that a membrane linker on CD14 is essential for activity with S100A9—but not LPS—suggesting a different role for CD14 in TLR4 complex formation given the two ligands. We currently hypothesize that the S100A9-CD14 complex licenses internalization of TLR4. We are testing this idea using a combination of small-molecule inhibitors and microscopy. Our work reveals that the same molecular players—TLR4 and CD14—can be differentially activated by molecules with very different structures, allowing the immune system to recognize danger—whether exogenous or endogenous.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS333 | Investigating the structure and function of the RLD domains in HERC2: a large E3 ubiquitin ligase implicated in cancer and Angelman syndrome

Kelly Waters, Marimo Oka, Donald E. Spratt
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HECT and RCC1-like-domain (RLD) containing protein 2 (HERC2) is a member of the Homologous to the E6AP C-terminus (HECT) E3 ubiquitin ligase and Regulator of Chromosome Condensation 1 (RCC1) protein families. As an E3 ligase, HERC2 uses its HECT domain to aid in the post-translational covalent attachment of ubiquitin to various substrates that typically results in targeting the protein for proteasomal degradation. However, due to its large size (528 kDa) and varied N-terminal protein-protein interaction domains, HERC2 has been a difficult enzyme to examine biochemically and biophysically. To date, HERC2 has been found to be involved in many different cellular functions due to its reported interactions with various proteins including p53, E6AP, RNF8, BRCA1, XPA, and NEURL4. These interactions implicate that HERC2 plays a significant role in double-stranded DNA break repairs, tumor suppressor activity, and cell cycle regulation. Since homolog RCC1 is a well-characterized DNA-binding protein, we postulate that HERC2 RLDs contribute to HERC2's role in the DNA damage response. RLD-containing proteins typically have only one RLD that is made up of a seven-bladed β-propeller motif, but HERC2 uniquely contains three RLDs: RLD1 (415-784), RLD2 (2949-3332), and RLD3 (3941-4324). Currently, our studies are focused on uncovering the role the HERC2 RLDs may have in neurological disorders and in cancer by regulating E6AP, another E3 ligase that is found to be involved in HPV-associated cancers and Angelman syndrome. Two missense mutations in RLD1 (P594L) and RLD3 (D4267E) have been annotated in the Human Gene Mutation Database. To investigate the secondary structural effects of these mutations, site-directed mutagenesis and circular dichroism was employed. These studies aim to uncover the HECT RLDs structure and function and will aid in understanding the role of HERC2 in the double-stranded DNA break repair response as well as in neurological disorders and cancer.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS334 | Discovering Chemical Tools to Target DnaJB6 in Polyglutamine Diseases

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Polyglutamine (polyQ) diseases are incurable neurodegenerative disorders that are characterized by the toxic accumulation of misfolded proteins containing mutations that expand repeats of glutamine. The molecular chaperone protein DnaJB6 is specialized to prevent polyQ aggregation in cells and suppress disease phenotypes by preventing polyQ aggregation and recruiting another chaperone protein, Hsp70 to assist in clearance. While DnaJB6 represents an exciting target for probing polyQ pathology, its dynamic protein-protein interactions and complex structure present significant challenges for efforts to discover and design DnaJB6 chemical ligands.
DnaJB6 associates with itself to form large, homooligomeric complexes that appear to exchange between larger and smaller oligomeric states over time. I hypothesize that binding to either polyQ clients or Hsp70 causes changes DnaJB6 oligomeric stability, and that these changes in DnaJB6 dynamics can be exploited to discover chemical probes. Thus, the goal of this project is to use computational screens and rational design to discover the first DnaJB6-targeted chemical probes that will subsequently be used to probe DnaJB6 amelioration of polyQ aggregation in vitro. Tools and insights from these studies will not only contribute to our molecular understanding of polyQ diseases (i.e. Huntington's Disease, Kennedy's Disease, Spinocerebellar Ataxias, etc.), other neurodegenerative diseases where DnaJB6 can suppress protein aggregation (i.e. Alzheimer's Disease and Parkinson's Disease), and chaperone biology more broadly.

Track: High Throughput Protein Science

ABS335 | Thermal and mechanical stability of highly-luminescent protein NanoLuc in presence and absence of chaperones

Dimitra Apostolidou, Yue Ding, Piotr Marszalek
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Despite the extensive application of NanoLuc protein as a reporter enzyme, its mechanical behavior has never been investigated. This unexplored area intrigued our interest given NanoLuc's high thermal stability in comparison to the traditionally used Firefly Luciferase. Our Atomic Force Microscopy based Single Molecule Force Spectroscopy experiments on various polyprotein constructs of NanoLuc enabled us to explore various scenarios of this protein's unfolding and possibly misfolding. Our results strongly demonstrated that despite all the constructs had similar unfolding behavior, the refolding behavior differed. The percentage of successful refolding recordings of NanoLuc was greatly decreased when the protein was linked to itself. This was a contrary result from the construct in which the NanoLuc repeats were separated by other proteins or in the construct with a single NanoLuc protein. Additionally, Steered Molecular Dynamics Simulations of NanoLuc provided valuable insight into the unfolding pathways, in which the C-terminus end of the protein was the first to break apart from the rest of the protein. Lastly, thermal denaturation experiments of poly-NanoLuc proteins showed a sudden decrease in thermal stability at the denaturation temperature of 58°C, while the monomeric NanoLuc remained mostly folded in same conditions. Addition of the E. coli DnaK/DnaJ/GrpE chaperone system to the poly-NanoLuc proteins resulted to a 70% recovery of the initial bioluminescence. The spontaneous recovery in the absence of the chaperones showed no recovery, further supporting how poly-NanoLuc proteins are robust chaperone substrates.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS336 | Allosteric Regulation of Protein Kinase C beta Isoforms

Taylor Witter, Anh Cong, Elizabeth Bruinsma, Swaathi Jayaraman, Matthew Goetz, John Hawse, Matthew Schellenberg
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Protein Kinase C (PKC) isoforms are found in a wide range of cell types and direct diverse but specific functions in cells. Because of their ubiquity, PKCs are potential targets for treatment in a plethora of diseases, however the lack of structural and functional information on the isoforms limits the ability to develop effective pharmacologic agents. PKC beta (PKCB) isoforms PKCBI and PKCBII are of particular interest as a target for chemotherapeutic agents and structurally differ by only a C-terminal segment. In our work, we examine how the variable C-terminal segment alters the allosteric regulatory mechanism of PKCBI and PKCBII to provide isoform–specific activities. We assay the differential response of the two isoforms in response to different lipid compositions and inhibitors using in vitro kinase assays. Alternatively, we use limited proteolysis to probe variations in PKCB conformation in the presence of allosteric regulators and investigate how PKCBI and PKCBII mutants perturb function. Finally, we show that the allosteric regulatory mechanism of PKCBI can be altered by endoxifen, providing a “proof of concept” for isoform-specific allosteric regulators of PKCs. Overall, our data elucidates the underlying molecular basis by which the PKCB isoforms are regulated and can inform the development of highly specific treatments that exploit isoform dissimilarities.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS339 | SPADE: A Fast and Accurate Estimation of Entropy from the Molecular Surface Properties

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NIH (United States)

Estimating entropy is crucial for understanding and modifying biological systems, such as protein-ligand binding. Current computational methods to estimate entropy are resource-intensive. Here, we present SPADE1 (Surface Properties-based Accurate Descriptor of Entropy), a new method that estimates the gas-phase entropy of small molecules purely from their surface properties. The accuracy of SPADE in estimating gas-phase entropy for the 1263 small molecules in our curated database is within 1−2% of computationally expensive quantum mechanical or molecular mechanical calculations. The small molecule gas-phase entropy can be computed in ≈ 0.01 CPU hours with an average run time of $O(\sqrt{Na})$, where Na is the number of atoms. We further show that the inclusion of the SPADE estimated entropy term for 243 ligands spanning ten protein targets improves the distance

correlation (DCOR2) between binding affinity and docking score from 0.17 to 0.60. We further used SPADE to calculate the entropy of fusion (Sfus). For a set of 304 small molecules, the DCOR between the observed and SPADE calculated Sfus is 0.96. SPADE opens up the possibility of including a rapid and direct estimation of entropy for scoring functions in virtual screening and other applications.

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2) https://doi.org/10.1214%2F009053607000000505

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Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS340 | Resolving the kinetics of transcription factor recognition of the target DNA site via advanced single-molecule FRET spectroscopy

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Transcription factors (TF) function by specifically binding to target DNA sites found in gene control elements to recruit or block the transcription machinery. Such DNA recognition process usually involves an interplay between high-affinity binding to the cognate sequence and low-affinity binding to any other DNA. The latter enables scanning of the DNA via a 1D diffusive process, which combined with 3D diffusion-collision kinetics, is believed to facilitate target location. 1D diffusion has been observed using single-molecule fluorescence tracking on many DNA binding proteins (DPBs). Powerful experimental methods are also available to determine the sequence logo recognized by any given DBP. However, there are no approaches available that resolve the kinetics of locking into the target site, which is essential to elucidate the mechanisms of cognate DNA recognition.

Here we introduce such an approach, by using single-molecule FRET spectroscopy and maximum likelihood analysis of photon arrival times. We apply it to determine the rates of lock-into and release-from the target site of the Engrailed homeodomain (EnHD). To resolve between non-specific and cognate DNA binding we exploit the recent discovery that EnHD changes conformation upon cognate binding, resulting on a large shift in transfer efficiency between a donor-acceptor pair placed at the protein ends. Our experiments on a 21-bp dsDNA containing the EnHD cognate sequence TAATTA reveal that EnHD finds its target in few milliseconds. Remarkably, the on-rate at the C50 is mostly invariant over orders of magnitude differences in ionic strength and DNA concentration. This is because the changes in affinity caused by ionic strength are mostly recapitulated on the on-rate, which is the exact opposite to diffusion-control kinetics. We hence conclude that EnHD's lock-into target kinetics are controlled by its conformational dynamics, which makes the response time for controlling transcription only dependent on site occupancy levels.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS341 | Fibrillization of lentil seed storage proteins: dependence of fibril morphology on extraction pH likely due to binding of phenolics

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Converting plant seed storage proteins into amyloid-like fibrils may improve their functionalities for applications in advanced materials in biomedicine, nanotechnology, and foods. Legumes are rich protein sources that are environmentally and economically sustainable. However, little is known about fibril formation from legume protein, especially lentil protein. Since lentils are also rich in

phenolics, alkaline protein extraction may promote complexation reactions between protein and phenolics, and thus may affect fibrilization and the resulting fibril morphology. Therefore, we investigated the effect of protein extraction conditions (i.e., alkaline or acidic pH) on lentil protein fibril morphology. Lentil proteins extracted at either pH 8.2 or pH 3.5 were incubated at 80 °C, pH 2, with stirring to form fibrils. Transmission electron microscopy (TEM) was used to examine the fibril morphology and the results showed that fibrils made from alkaline protein extract were more heterogeneous, curly, and tangled, whereas fibrils made from acidic protein extract were more homogeneous, long, and straight. UV-vis spectroscopy, fast-blue BB assay, and HPLC/QTOF analysis were used to characterize protein-phenolic interactions, and the results indicated that morphological differences between fibrils formed from pH 3.5 vs. pH 8.2 extracts are likely due to interference from phenolic compounds at pH 8.2. However, recombination of isolated protein and phenolic extracts (both from pH 3.5) did not recapitulate the effects on fibril formation observed from extracts at pH 8.2. To reconcile this paradoxical result, we propose a mechanism facilitated by polyphenol oxidase. This study demonstrated that acidic protein extraction is beneficial for producing clean, long, and straight lentil protein nanofibrils, which may have desirable functionalities and excellent potential to be developed into novel plant-based biomaterials.

Track: Protein Phase Separation in Biomolecular Condensates

ABS342 | High Rates of Refoldability and Structural Resilience of Phase Separated Proteins upon Heat Shock in Yeast

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Biomolecular condensation upon heat shock plays an integral role in maintaining cellular proteostasis in yeast. Many proteins aggregate during heat stress, and it has been proposed that, at least for endogenous yeast proteins, these aggregates are liquid-like and form adaptively. In the following, we use limited-proteolysis mass spectrometry (LiP-MS) to show that proteins that phase separate in a temperature-dependent manner display unusually high levels of refoldability, and moreover maintain their structure in the condensed form. These observations suggest that proteins which phase separate are selected for their capacity to be easily restored to their native soluble form. Furthermore, we find that 99% of the proteome retains their overall structure during heat shock and that proteins with the highest likelihood of phase separating are structurally indistinguishable from the native states upon heat shock recovery. These results support the emerging view that heat-induced aggregation in yeast reflects an adaptive rather than pathological response.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS343 | Uncovering the Selective Reactivity of bis-N-oxide Caspase-6 Inhibitors

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Currently, there are no cures for any neurodegenerative disease. Caspase-6, a cysteine aspartate protease, is present in the hallmarks of Alzheimer's Disease (AD) and Huntington's Disease (HD): amyloid-β plaques, neuropil threads, and neurofibrillary tangles. Caspase-6 is known to cause AD and HD by cleaving microtubule-associated proteins tau and tubulin, respectively. From a high-throughput screen, compound A (comp A), a bis-N-oxide, was identified as a selective caspase-6 inhibitor. Comp A covalently modifies Cys-264, a non-conserved residue unique to caspase-6. Comp A, and its analogs exhibit a rare latent reactivity where they selectively modify one Cys in a sea of ten competing Cys in caspase-6. However, the mechanism of compound A reaction and the physicochemical basis for compound A's reactivity with specific cysteines remains unknown. In caspase-6, Cys-264 is adjacent to a Lys-265, and we hypothesized that this Cys+ motif is necessary for comp A reactivity. To better develop bis-N-oxides as caspase-6 inhibitors, we investigated the physicochemical basis of compound A's selective reactivity in systems of increasing complexity from peptides to proteins. Six Cys+ containing oligopeptides in a background of varying hydrophobics and charges were incubated with compound A and no evidence of compound A modification was observed via mass spectrometry (MALDI-TOF). We conclude that compound A reactivity requires a more complex three-dimensional microenvironment likely provided in protein tertiary structure. To uncover the extent of compound A's selectivity we have incubated compound A with Cys-containing proteins as well as

caspase-6 mutants and then analyzed binding or lack of binding via LC-MS.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS344 | Restoration of the Foldability, Stability, and Function of the Underperforming WW Domain Sequences

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WW (Tryptophan-Tryptophan) domains are small proteins consisting of ~40 amino acids that recognize proline-rich peptides (1,2). They are known for their role in signaling, transcription regulation, and cytoskeletal function (1-3). Previously, our research group used WW domain sequences to explore their role in early folding steps by simulations and identified five crucial contacts that enabled the prediction of the folding probability with 81% accuracy. By introducing these five contacts on an artificial WW sequence, we restored foldability to the underperforming sequences. Surprisingly, the newly constructed variant CC16_N21 displayed lower stability but better binding to the peptide ligand compared to native ones (N21). Here, we investigated this discrepancy by rational design and computational analysis in parallel. We noticed that two tyrosine residues that contact the ligand in CC12_N21 are histidine in N21, and we swapped them to tyrosine, generating three mutants: H9Y, H19Y, and H9YH19Y. Additionally, by considering the allosteric effects of the distant residues on the binding site, our computational approach found two residues (threonine and asparagine) have the potential to affect the binding of N21. Therefore, we considered five different mutations (T10H, H9YH19Y, H9Y, H19Y, and N20D) on the N21 background to investigate folding, stability, and binding with proline-rich peptides. All variants, except N20D, showed Circular Dichroism (CD) features like their native counterpart with a positive peak at 227.4nm. Compared to the N21, thermal denaturation (Tm) of H19Y (510C), and H9YH19Y (56.40C) were higher. The binding affinity of H9YH19Y (Kd=50.0μM ± 3μM) for Group I peptide, EYPPYPPPPYPSG, was higher than N21 and CC16_N21. H9Y and T10H showed a lower affinity for the ligand compared to CC16_N21 whereas H19Y did not show any binding. In conclusion, our computational approach and rational design of the WW domain sequences observed the restoration of the folding, stability, and function.

Key Words: WW Domain, CC16_N21, N21, Rational Design, Group 1 peptide, Allosteric, thermal denaturation, Circular Dichroism, Kd

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Track: Structure and Dynamics Perspectives on Enzyme Function

ABS345 | Structural study reveals active and inactive conformations of human Protein Kinase C

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A human cell-based recombinant protein expression system is advantageous for large scale protein purification as it provides native and nearly ideal conditions for correct folding and post-translational modification of protein products. Recently, we developed an affinity tag system using llama single-domain antibodies (a.k.a nanobodies) generated against the fluorescent proteins YFP and mCherry. These nanobodies have been shown to be highly efficient, with high binding capacity while exhibiting binding affinities that are down to the sub-nanomolar Kd, serving as ideal affinity-matrices for routine protein expression and purification in a mammalian system. These mCherry-tag and YFP-tag systems have enabled us to successfully express and purify numerous recombinant human proteins with productive yields and high quality. Here, we utilize the YFP-tag system to express and purify recombinant full-length human Protein Kinase C β (PKCβ) using HEK293F cells. We have determined structures of purified PKCβ proteins using X-ray crystallography that define two distinct conformations of the enzyme,

providing structural insights behind their inhibition and activation mechanisms.

Track: Cellular Tasks

ABS346 | Characterization of a Unique Homolog of Get3 Conserved in Plants and Photosynthetic Bacteria

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The ATPase Get3 is the central targeting factor in the Guided Entry of Tail-anchored protein (GET) pathway, which is responsible for targeting tail-anchored (TA) membrane proteins to the endoplasmic reticulum. In this work, a unique homolog of Get3, termed Get3d, is identified. Get3d is found to be conserved through land plants and photosynthetic bacteria. The structure of Arabidopsis thaliana Get3d (AtGet3d) is determined using x-ray crystallography and is found to be nearly identical to that of a cyanobacterial Get3 homolog, which is refined further. Unique features of Get3d are noted including an incomplete active site, a C-terminal alpha-crystallin domain, and a hydrophobic chamber as opposed to a TA protein binding groove. To characterize Get3d further, the localization and activity of Get3d are determined. AtGet3d is found to localize to the chloroplast. ATPase assays find that Get3d is an active ATPase despite its incomplete active site. Evidence is also provided for a role of Get3d in TA protein binding. This work supports the potential role of Get3d in TA protein targeting in plant chloroplasts and photosynthetic bacteria.

Track: Machine Learning in Protein Science

ABS347 | Geometric Deep Learning for Improved Virtual Screening

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High throughput screening (HTS) is a mainstay of modern drug discovery. While undeniably effective, HTS requires extensive chemical assays of thousands to millions of unique candidate drug compounds. This process amounts to enormous costs in resources and time, ensuring that drug discovery is prohibitively expensive to all but the largest pharmaceutical companies and research institutions. Virtual screening (VS) is a growing field wherein computational methods are employed to identify promising drug candidates and eliminate dead ends, thereby paring down the number of candidates tested in the HTS process by orders of magnitude. Advances in VS are therefore vital to making drug discovery accessible to small firms and research institutions.

To this end, we have developed a software pipeline capable of identifying potential lead compounds for any provided protein target. While our pipeline is not without merit, it is far from a complete solution for virtual screening. Each stage of the pipeline represents a potential area for improvement. Chief among these is the need to develop improved measures of chemical similarity, dynamic representations of protein pockets, and improved predictions of protein-ligand binding affinity.

Recent advances in the field of geometric deep learning have introduced powerful new techniques in developing learned representations of physical systems in three-dimensional space. These techniques may be applied to bio-molecular systems, enabling the development of useful embeddings of proteins and small molecules by integrating their spatial and physico-chemical characteristics. Therefore, we have leveraged graph and attention-based deep learning methods to develop improved representations of small molecules and protein pocket pharmacophore models. These improved, spatially-informed models provide a basis for improved binding affinity prediction and early lead identification.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS348 | Engineering a Chlorogenic Acid Esterase

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Chlorogenic acid esterases are biotechnologically useful enzymes that hydrolyze chlorogenic acid into caffeic and quinic acid. They are also able to remove hydrocinnamic acid derivatives from the cell wall of plants and therefore of interest to the biomass processing and paper industries. This work presents the biochemical and structural characterization of a chlorogenic acid esterase from Lactobacillus helveticus. It is the most active chlorogenic acid esterase known to date with a Km of 0.081 mM and turnover number of 82 1/s. It is also thermostable and easily expressed in E. coli. Its structure features a typical alpha/beta hydrolase fold, but it has an unusual insertion

domain above the active site. To increase the activity of Lactobacillus helveticus chlorogenic acid esterase, the enzyme was engineered using a B-factor based strategy. We performed high-throughput mutagenesis on regions of the protein that are flexible since this is a known approach to increase turnover rate. Mutants were screened using a newly developed assay that measures chlorogenic acid hydrolysis in crude protein extracts using fluorescence and absorbance methods. Preliminary results on the first generation of engineered enzymes indicate that some mutants have activities that are equal to or larger than the wild-type enzyme. These mutants will be analyzed biochemically and structurally in greater detail to determine the features that are critical for enhanced enzymatic activity. In future work, we will use the first generation engineered chlorogenic acid esterases to further enhance activity.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS349 | Extending the HECT Domain Boundary of HERC4 Improves Solubility and Ubiquitylation Activity

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HECT and RCC1-like domain (RLD) containing protein 4 (HERC4) is one of 28 human HECT E3 ubiquitin ligases responsible for a multitude of functions pertaining to cellular homeostasis including DNA repair, apoptosis, and intracellular protein trafficking. All members of the HECT E3 ligase family, including HERC4, function through the ubiquitin-signaling pathway (USP). HERC4 has been found to influence three major physiological processes. When HERC4 is knocked out in mice, sperm maturation becomes stagnant suggesting it plays a role in spermatogenesis (male fertility). HERC4 has also been identified to regulate the Sonic hedgehog pathway during fetal development. Breast cancer tissue samples have been observed to have higher levels of HERC4 expression, suggestive that HERC4 may work alongside BRCA1 and p53 as a tumor suppressor. The focus of our studies is to better understand HERC4-dependent ubiquitylation occurs at the molecular level. Here we show that our HERC4 extended HECT domain with an extra N-terminal helix immediately before the current UniProt defined HECT domain boundary has improved solubility and ubiquitylation activity. Using circular dichroism, a stable iso-peptide E2-ubiquitin complex to mimic the short-lived thioester E2∼ubiquitin complex, and site-directed mutagenesis, we have begun to identify key residues required for E2∼ubiquitin recruitment and HERC4-dependent ubiquitylation.

Track: Miniproteins: Are There Really That Many Additional Genes That We Have Been Missing?

ABS350 | Protein Design vs Pathogen

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Nature only samples a small fraction in sequence space, yet many more amino acid combinations can fold into stable proteins. To design proteins with novel molecular functionalities, such as molecular recognition, methods to control and sample shape diversity are necessary. To explore this space, we developed and experimentally validated a computational platform that can design a wide variety of small protein folds while sampling high shape diversity. We designed and evaluated 8 different folds resulting in 6,200 stable proteins. We developed a high-accuracy classifier to predict the stability of our designed proteins. The methods and the wide range of new protein shapes provide a basis for the design of new protein function without compromising stability. Using these scaffolds, we have developed several inhibitors targeting viral surface proteins. To functionalize these scaffolds more general with developed several deep learning algorithms to design protein-protein interaction motifs from scratch. Our algorithms can recapture native interactions, detect privileged binding sites and produce new interaction motifs.

Independently of developing and functionalized miniproteins, we have been developing stabilization platforms for viral surface proteins. Viral glycoproteins mediate recognition and entry into their host cells. Protein-centric vaccine development studies over several decades have demonstrated how stabilization of these viral metastable proteins can be undertaken. However, efforts were largely performed manually and step-by-step. We have incorporated several of these molecular strategies into systematic automation protocols to stabilize viral surface proteins. We have generated a stabilized version for fusion protein of RSV, rivaling the clinical candidate in elicited immune response in mice. The structure of our design illustrates atomic accuracy of our approach. We further applied our new pipeline for the stabilization of on human metapneumovirus (hMPV) and SARS CoV-2 as model systems which both are otherwise highly instable.

Track: High Throughput Protein Science

ABS355 | Investigating KCNQ1 Mistrafficking in Long QT Syndrome via Deep Mutational Scanning

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Approximately 1 in 2500 individuals suffer from congenital long QT syndrome (LQTS), a cardiac disorder that can cause arrythmias and cardiac arrest. Loss of function mutations in the voltage gated potassium channel protein KCNQ1 cause 30-50% of cases of LQTS. Over 250 LQTS-associated mutations in KCNQ1 have been identified, and the mechanisms through which they lead to loss of function are still uncertain. We have characterized the impact of 51 mutations in the KCNQ1 voltage sensing domain (VSD) and found that the majority of the studied VSD mutants caused reduced trafficking to the plasma membrane (mistrafficking). We hypothesized that mistrafficking is a common mechanism of protein dysfunction across KCNQ1 domains. In this study, we aimed to develop a high throughput method to assess the impact of mutations across KCNQ1 on trafficking. Here, we describe a deep mutational scanning (DMS) approach to determine the impact of a library of KCNQ1 variants on KCNQ1 cell surface trafficking. Variants are stably expressed in mammalian cells, which are sorted with fluorescence-activated cell sorting (FACS) into "trafficking competent" and "trafficking deficient" populations based on their cell surface expression of KCNQ1. The proportion of each mutant in each population is determined to identify mutations that alter KCNQ1 trafficking compared to wild type. This allows us to identify residues and regions that are important for KCNQ1 trafficking. To validate the method, we have demonstrated that a small library of KCNQ1 mutants at site R231 is classified similarly with our FACS-based method and a two-color trafficking assay previously established in the lab. We have since begun characterizing trafficking phenotypes of mutations in KCNQ1 in a high-throughput fashion, providing additional information on the mechanisms of KCNQ1 loss of function in long-QT syndrome.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS358 | Multi-Scale Simulations Provide Insights into Protein Conformation under Crowding

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The function of enzymes, and proteins in general, is tightly linked with the stability of their native conformations, which can be modulated by the dense and heterogeneous environment inside living cells and biomolecular condensates. Using multi-scale molecular simulations, we inquire into the effects of macromolecular crowding on the conformations of several model proteins such as chymotrypsin inhibitor 2, superoxide dismutase 1, and citrate synthase. We consider environments of growing complexity, including crowded protein solutions mimicking the composition of a bacterial cytoplasm. Our simulations reveal a delicate balance between two factors—the entropic excluded volume and weak transient interactions—resulting in a temperature-dependent effect of crowding on protein stability. Moreover, we find that specific interactions with the surrounding crowded environment can enhance the population of partially unfolded states, which might have an impact on the processes of toxic aggregation and oligomerization. Our simulation approach will allow studies of conformational transitions associated with enzyme activity in cellular environments.

Track: High Throughput Protein Science

ABS359 | Linking genetic mutations to ageing by proteome-wide conformational metastability

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Ageing is associated with the accumulation of genetic mutations and a decline in the functional proteome. However, how genetic mutations contribute to ageing remains elusive. Here, we measured the conformational stability of the proteome in Caenorhabditis elegans (C. elegans) during ageing and in two different mutants: 1) temperature-sensitive mutant myosin and 2) aggregation-prone polyglutamine (polyQ) expressed in muscle. We found that nearly a quarter of the proteome shows age-dependent changes in conformational stability, imposing a substantial burden on protein quality control machinery. Indeed, we observed more than 30% of the metastable proteins are accompanied by significant changes in protein solubility even in early ageing. Moreover, the expression of mutant myosin or polyQ in muscle leads to distinct but age-like protein misfolding in muscle and other tissues at day 1 of adulthood. Notably, polyQ in muscle systematically accelerates age-dependent proteome instability (i.e. the

proteome of day 6 in polyQ animals highly resembles that of day 9 in wild-type animals). These results reveal an unprecedented level of complexity of proteome remodeling in response to mutations and ageing.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS362 | Establishing the Foundations for Studying Human Parkin E3 Ligase Solution Structure and Function by NMR Spectroscopy

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Parkin E3 ligase is part of the cellular ubiquitin system capable of ubiquitinating specific substrates and targeting them for degradation or cellular relocalization. Parkin plays a prominent role in eliminating damaged mitochondria by ubiquitinating outer mitochondrial membrane proteins, which initiates autophagosome assembly and mitophagy. Mutations in Parkin have been associated with several diseases stemming from mitochondrial dysfunction such as neurodegeneration, cardiac diseases, and cancer. Understanding Parkin structure, function and regulation is crucial to targeting it for therapeutic purposes.

Parkin, autoinhibited in its resting state in the cytosol, is recruited to and activated at the surface of damaged mitochondria. Recent studies have shown that Parkin undergoes large conformational changes in response to allosteric binding interactions and post-translational modifications leading to its fully active conformation. However, little is understood about how these allosteric responses are communicated across the protein structure. Moreover, the structure of full-length Parkin has not been solved. Our knowledge of Parkin domain architecture comes from biophysical studies of truncated constructs and isolated domains. The only way to fully understand Parkin's mechanism of activation is to study the full-length protein.

We are studying full-length human Parkin structure and allosteric regulation by solution nuclear magnetic resonance (NMR) spectroscopy. We have successfully purified recombinant human Parkin in biophysical quantities for the first time for NMR. We report NMR backbone chemical shift assignments for full-length Parkin and its partially active variant lacking the N-terminal autoinhibitory domain. For this, we employed a unique approach combining standard NMR triple resonance experiments with selective unlabelling strategy and in-house automated assignment tool utilizing Bayesian statistics to achieve the best assignments of our NMR data to corresponding Parkin residues. These results enable investigating Parkin solution structure and the role of protein dynamics in Parkin allosteric regulation by advanced NMR spectroscopy methods. Supported by NIH (GM129076).

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS363 | Functional expression of opioid receptors and other human GPCRs in yeast engineered to produce human sterols

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The yeast Saccharomyces cerevisiae is powerful for studying human G protein-coupled receptors as they can be coupled to its mating pathway. However, some receptors, including the mu opioid receptor, are non-functional, which may be due to the presence of the fungal sterol ergosterol instead of cholesterol. We engineer yeast to produce cholesterol and introduce diverse mu, delta, and kappa opioid receptors to create sensitive opioid biosensors that recapitulate agonist binding profiles and antagonist inhibition. Additionally, human mu opioid receptor variants, including those with clinical relevance, largely display expected phenotypes. By testing mu opioid receptor-based biosensors with systematically adjusted cholesterol biosynthetic intermediates, we relate sterol profiles to biosensor sensitivity. Finally, we apply sterol-modified backgrounds to other human receptors revealing sterol influence in SSTR5, 5-HTR4, FPR1 and NPY1R signaling. This work provides a platform for generating human G protein-coupled receptor-based biosensors, facilitating receptor deorphanization and high-throughput screening of receptors and effectors.

Track: Cellular Tasks

ABS364 | cANF Dependent Activation of the C Type Natriuretic Peptide Receptor Inhibits Endothelial Sodium Channels in Human Aortic Endothelial Cells

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A recent study suggested the C type natriuretic peptide receptor (NPRC) is a potential contributor of arterial dysfunction in patients with aortic coarctation which develop persistent hypertension despite treatment. Arterial segments from a rabbit model of CoA and human aortic endothelial cells (hAoEC) were used to demonstrate the functional relevance of altered NPRC activity as NPRC expression is reduced in CoA rabbits. Recent studies suggest activation of endothelial sodium channel (EnNaC) results in increased endothelial cell and arterial stiffness and impairment of vascular relaxation. The objective of this study was to investigate the regulation of EnNaC protein expression in rabbit aortas of CoA and its activity by the NPRC agonist cANF in hAoEC. Single-channel patch clamping was performed to measure endogenous EnNaC activity in hAoEC. Protein expression of the EnNaC alpha subunit was measured by immunohistochemistry and Western blotting. Lipids were extracted from membrane preparations of hAoEC treated with cANF or vehicle using the Bligh and Dyer method and lipid samples were analyzed using ultra-high-performance liquid chromatography mass spectrometry. Protein expression of the alpha subunit of EnNaC was augmented in CoA rabbit tissue compared to that of control rabbits. EnNaC protein expression and activity (NPo) was reduced in hAoEC treated with cANF compared to vehicle. Several forms of diacylglycerols (DAGs) increased in the cANF group compared to the vehicle treated group. Taken together these data suggest the activation of NPRC by ligand binding attenuates EnNaC protein function in hAoECs in a mechanism involving the regulation of DAGs.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS365 | Regulatory role of CAND2 in SCF ubiquitin ligases

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Through targeting specific cellular proteins for ubiquitination and degradation, cullin-RING ubiquitin ligases (CRLs) regulate a broad spectrum of biological processes in human cells. A typical Cullin1-based CRL complex, also known as SCF, is composed of a CUL1/RBX1 catalytic core, an interchangeable substrate receptor from the F-box protein (FBP) family, and the SKP1 adaptor linking FBP with CUL1. Biochemical studies have demonstrated that Cand1 promotes SCF activity by accelerating the dynamic assembly of FBPs with the common CUL1 core. In this study, we investigated how CAND2, a paralog of CAND1, regulates the assembly and activity of SCF complexes. Firstly, our HEK293 cell-based degradation assay demonstrated that CAND2 single knockout inhibits iron-induced degradation of IRP2 by SCFFBXL5, suggesting that CAND2 is a positive regulator of protein degradation in human cells. Consistently, in CAND2 knockout cells, IRP2 engaged less neddylated CUL1, the active form of CUL1, suggesting the impaired degradation of IRP2 is due to a deficiency in assembling active SCFFbxl5 in these cells. Using in vitro fluorescence resonance energy transfer (FRET) assays, we further examined the dynamic interactions among CUL1, FBP, and CAND2. Similar to CAND1, CAND2 accelerated the dissociation of a SCF complex. Reciprocally, FBP also increased the dissociation rate of the CUL1-CAND2 complex. These kinetic features suggest that like CAND1, CAND2 can also be an FBP exchange factor. This possibility was further tested by an immunoprecipitation assay coupled with stable isotope labeling by amino acids in cell culture (SILAC), to explore the effect of CAND2 on SCF dynamic assembly in human cell lysate. Our results showed that the recombinant CAND2 promoted the exchange of various FBPs associated with CUL1, demonstrating that CAND2 functions as an SCF exchange factor. Altogether, our work provides insights into the role and mechanism of CAND2 in regulating SCF ubiquitin ligases.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS366 | Predicting Mutational Impact on Membrane Protein Stability and its Implications for Long QT Syndrome

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The human potassium channel KCNQ1 plays a critical role in heartbeat regulation by producing the slow delayed rectifier current that aids repolarization during the cardiac action potential. Loss-of-function mutations in KCNQ1 predispose patients to cardiac arrhythmias, particularly Long QT syndrome (LQTS), a common mechanism for sudden arrhythmic death. While preventative treatments are available for at-risk LQTS patients, hundreds of individuals carry variants of unknown significance (VUS) for which pathogenicity has not been

determined. This precludes risk assessment and administration of potentially life-saving care. Accurate prediction of mutation pathogenicity risk is critical to properly assess and protect LQTS patients. Towards this end, previous work has been conducted to elucidate the molecular mechanisms underlying LQTS in KCNQ1, beginning with the voltage sensor domain (VSD). Protein trafficking, function, and NMR analysis of LQTS and VUS mutations in KCNQ1 revealed that VSD destabilization is a common disease mechanism, pointing to the imperative for membrane protein stability prediction as part of pathogenicity assessment. To design such a tool, the Rosetta protein modeling software suite was used to develop a free energy difference (ddG) prediction application for membrane proteins, termed flexddG. Strikingly, energy calculations of previously characterized VSD mutations using flexddG correlate in a broadly linear fashion to experimental trafficking and functional results. However, benchmarking efforts against published membrane protein ddG values revealed only a modest correlation, with prediction of proline mutations being especially erroneous. Some correction was accomplished to proline mutation predictions through the implementation of fragment insertion. To expand the dataset, the MPTherm database of membrane protein melting temperatures was employed. ddG prediction with this dataset revealed a compelling relationship between experimental structural resolution and prediction accuracy. DeepAccNet, a machine learning-based structure refinement tool, is currently being employed to refine experimental membrane protein structures prior to ddG prediction as a continuation of protocol development.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS367 | Substrate interactions and in situ chemical modification of human 2-oxoglutarate dehydrogenase enzyme complex components

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The tricarboxylic acid (TCA) cycle is at the intersection of various metabolic pathways deeply integrated within various mitochondrial functions. The 2-oxoglutarate dehydrogenase complex (OGDHc) represents a major regulator of this cycle, affecting metabolic flux and electron transport chain activity. OGDHc comprises multiple copies of 2-oxoglutarate dehydrogenase (E1), dihydrolipoamide succinyltransferase (E2), and dihydrolipoamide dehydrogenase (E3). These enzymes may carry mutations and/or acquire chemical modifications that hinder their catalytic activities. This poster summarizes our recent results in the characterization of the human (h) OGDHc components. We applied various biophysical and computational techniques to analyze the key structural effects that underpin altered/compromised catalytic activities observed for the respective components.

1.2-oxoadipate dehydrogenase (DHTKD1) can substitute for E1. Structural modeling on hE2 validated our in vitro results that not only 2-oxoadipate, but also 2-oxopimelate can serve as a substrate for this modified hOGDHc.

2.Loss in NADH dehydrogenase (ubiquinone) iron-sulfur protein 4 (Ndufs4), a mitochondrial Complex I subunit, led to a decrease in OGDHc activity in a mouse model. We propose that this activity loss is due to chemical succination at Cys178, which impact Arg358, Asp356 and the substrate binding channel, in hE2.

3.hE3 has at least 14 reported pathogenic variants. These display high structural similarity, with sometimes only minor conformational effects to explain the observed differences in activity or stability. Using hydrogen/deuterium exchange mass spectrometry (HDX-MS), we explored binding of substrates NADH and lipoic acid to hE3. This analysis pinpointed residues responsible for substrate interactions. A pathogenic variant displayed detectable structural alterations relative to hE3 by HDX-MS.

Track: Protein Science Addressing Health Disparities

ABS368 | The Amyloidogenic Propensity of the Helix-1 Region of Serum Amyloid A is Influenced by Key Charged Amino Acids

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Serum amyloid A (SAA) is an acute-phase protein that is secreted in response to inflammation. Its increased plasma levels may lead to the accumulation of amyloids in various organs which in turn can obstruct native organ functions. A previous computational study shows that the N-terminal helix, specifically the sequence containing the first 13 amino acid residues (SAA1-13), is the most amyloidogenic region of SAA. It also stresses the significant role of Arg1 and its interaction partners in the

fibrillization process. We initially confirmed the result of the said calculational analysis via single amino acid mutation of Arg1, Ser5, Glu9, and Asp12 of the truncated SAA1-13 fragment. Amyloidogenic propensity of the peptides was determined using ThT fluorescence aggregation kinetic assay, and structural changes associated with fibril formation were probed using CD and FTIR spectroscopy. Morphology of the fibrils was assessed using TEM imaging. Preliminary findings indicate that the substitution of Arg1 hindered the aggregation of SAA1-13 even in the presence of heparin which proves the importance of Arg1 in the amyloid formation process. It is also observed that the mutation of Glu9 significantly altered the behavior of the peptide while Ser5 and Asp12 mutants exhibited modest changes in aggregation propensity. Our results indicate that charged residues of SAA1-13 interact during the fibrillation process, and these interactions may aid to the understanding of the mechanism of the amyloid formation of SAA1.1 protein.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS369 | Design of fusion constructs to facilitate the production and study of viral membrane proteins and their complexes

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We study the structure, function and interaction with cellular components of viroporins that are small membrane proteins from human viruses. They oligomerize in cellular membranes affecting membrane permeability and membrane plasticity, but also form complexes with human proteins. Hence, viroporins are biomedically important to study. However, these proteins are difficult to produce, since they require eukaryotic host to be deposited in the native membrane environment. Alternative approaches for expression in E. coli is instrumental for conducting large-scale-protein-consuming in vitro studies. Here we report on our developments to design and produce fusion constructs of the viroporins Vpu, p7, p13II and E from HIV-1, HCV, HTLV, and CoV, respectively. Due to the very hydrophobic nature of these proteins, after their expression in E. coli, they are mostly found in insoluble aggregates. To increase the yield of the viroporin soluble form, we utilized protein engineering and produced them as fusion constructs with highly soluble tags. After their expression we found the fusion proteins in the cytoplasmic fraction at levels that are sufficient for structural and functional studies. Thus, we produced soluble Vpu, p7 and E protein having maltose binding protein (MBP). Thereafter, we reconstituted these proteins in lipid. The MBP tag was also beneficial for increasing the total protein size and visualizing its oligomers by electron microscopy (EM). We further produced the sigma subunit of the human AP1 complex as a fusion construct with SUMO tag to increase the protein stability; aiming to understand the structural basis of Vpu-sigma subunit complex formation. We will additionally discuss the advantages of using ubiquitin tag in the production of p13II in soluble form and its characterization. In conclusion, by utilizing stabilization and solubilization fusion tags we succeeded to express in E. coli and purify several difficult to produce viral membrane proteins and their human protein partners.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS370 | Glutamate indicators with improved activation kinetics and localization for imaging synaptic transmission

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The fluorescent glutamate indicator iGluSnFR enables imaging of neurotransmission with genetic and molecular specificity. However, existing iGluSnFR variants exhibit saturating activation kinetics and are excluded from post-synaptic densities, limiting their ability to distinguish synaptic from extrasynaptic glutamate. Using a multi-assay screen in bacteria, soluble protein, and cultured neurons, we generated novel variants with improved kinetics and signal-to-noise ratios. We also developed surface display constructs that improve iGluSnFR's nanoscopic localization to post-synapses. The resulting indicator, iGluSnFR3, exhibits rapid non-saturating activation kinetics and reports synaptic glutamate release with improved linearity and increased specificity versus extrasynaptic signals in cultured neurons. In mouse visual cortex, imaging of iGluSnFR3 at individual boutons reported single electrophysiologically-observed action potentials with high specificity versus non-synaptic transients. In vibrissal sensory cortex Layer 4, we used iGluSnFR3 to characterize distinct patterns of touch-evoked feedforward input from thalamocortical boutons and both feedforward and recurrent input onto L4 cortical neuron dendritic spines.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS372 | pyCHARMM CDOCKER Automatic Docking in Python

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The development of new drugs with traditional methods can cost anywhere in the range of 400 million to 2 billion dollars, with synthesis and testing of lead analogs being a large contributor to that sum. A successful in silico docking protocol can save a large amount of money and time, and this has been a focus of development in the field for decades. Today, multiple off-the-shelf protein-ligand docking programs, such as CDOCKER – a CHARMM input script-based docking method, are available for use. However, the scripting languages of these docking methods are relatively complicated for ones with little knowledge of docking. On the other hand, to perform a successful docking experiment, one typically would need various cheminformatics tools either in preparation or post analyzing, such as Pybel, RDKit and Scikit-learn.

Python is one of the most popular and standard scripting languages in cheminformatics. Leveraging the popularity and utility of the Python language, we decide to introduce the Python module of CHARMM (i.e., pyCHARMM). pyCHARMM provide direct access from Python to the Fortran subroutines and functions in the CHARMM API (application programming interface).

Here, we focus on the development of pyCHARMM CDOCKER (i.e., Python functionality of the CDOCKER family). One of the aims of pyCHARMM CDOCKER is to provide simple one-liner code to perform standard CDOCKER docking experiments. We demonstrate significantly improved pose prediction accuracy for rigid receptor docking. We also note this feature significantly reduces the computational cost in flexible receptor docking. To our knowledge, pyCHARMM Flexible CDOCKER is the fastest flexible receptor docking method. The Python interface also allows pyCHARMM CDOCKER more easily to integrate with other commonly used cheminformatics toolkits.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS373 | Structure/Function Analysis of Human Progranulin

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Progranulin, a secreted glycoprotein of 75-80 kDa containing 7.5 granulin modules (p,G,F,B,A,C,D and E), is encoded by the GRN gene. Mutations within the GRN gene have been linked to frontotemporal lobar degeneration (FTLD). FTLD is an early-onset dementia syndrome characterized by progressive decline in behavior or language, associated with degeneration of the frontal and temporal lobes of the brain. So far, the molecular basis for neuroprotective properties of progranulin is not well understood. Progress in elucidating the specific functions of granulins is severely impeded by challenges in production of tractable amounts of granulins with well-defined structural characteristics and unique cysteine-cysteine pairing pattern.

Our goal for this project is to purify human granulins to study the cause for variation in granulin conformation and how to control it. We cloned, expressed and tested multiple redox conditions for refolding fragments A and F of human progranulin. To study refolding processes of the purified proteins, we used circular dichroism (CD).

By establishing expression and purification protocols, we were able to increase the yield of human granulin A and F. The purified fragments were refolded and their stabilities were analyzed using CD to observe the formation of secondary structure. This work will help characterize the structure/function relationships in human granulins and explain results of human granulin expression in cells.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS374 | Binding Interface and Structure-based Design of Mutations

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Sarcomeres within striated muscle cells contain thick and thin filaments, which slide against each other to contract or relax the muscle. Thin filaments are composed of actin, tropomyosin, and other actin-binding proteins. Within each sarcomere thin filaments are of equal length, any deviation from uniformity results in muscle dysfunction. The molecular mechanisms of thin filament length modulation have yet to be explored. Our objective in this research was to develop a deeper understanding of thin filament length regulation by studying the binding affinity between leiomodin, which has been shown to nucleate filaments, and tropomyosin. We used the structure of the leiomodin/tropomyosin binding interface, which we solved by nuclear magnetic resonance, as a basis for mutation design. Five point mutations and two

truncations were chosen; two point mutations were designed to reduce affinity, three were expected to increase affinity. The truncations were performed to test if the disordered N-terminus of leiomodin has any role in strengthening the bond with tropomyosin. Unfolding experiments using circular dichroism was performed for each mutant by itself and with tropomyosin, an unmodified fragment representing the wild type was used as a control. The experiments showed that four of the point mutations reduced complex melting temperature, and one of the mutations designed to increase affinity was comparable to the unmodified fragment. For the truncated fragment, we determined that up to 11 residues may be removed from the N-terminus without weakening the complex. To reconcile the results of our experiments, we ran molecular dynamics simulations of our model with the tested mutations. Analysis of these simulations suggested that the mutations were close enough to interact with the residues we anticipated them to, but that there were additional interactions not taken into account for our design of the mutants.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS375 | Effect of Hypertrophic Scar Fibroblast-Derived Exosomes on Keratinocytes of Normal Human Skin

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Fibroblast and keratinocyte interactions form hypertrophic scars (HTSs) through mechanisms, such as abnormal proliferation, cell activation, dedifferentiation, and dysregulated apoptosis of these cells; however, the exact mechanism of HTS formation remains unknown. Here, we confirmed the effect of hypertrophic scar fibroblast (HTSF)-derived exosomes on the proliferation and apoptosis of normal human keratinocytes (NHKs) and its possible mechanism to provide a reference for clinical intervention in HTS. Fibroblasts were isolated and cultured from HTSs and fibroblast-derived exosomes were extracted via ultracentrifugation of the supernatant. NHKs were treated for 24 or 48 h with 50 or 200 μg/mL HTSF-derived exosomes. The expression of proliferation markers (keratins 5 and 14), activation markers (keratins 6, 16, and 17), differentiation markers (keratin 1, keratin 10, and involucrin), apoptosis factors (Bax, Bcl2, and caspase 14), proliferation/differentiation regulators (p21 and p27), and epithelial–mesenchymal transition (EMT) markers (E-cadherin, N-cadherin, and vimentin) was investigated. HTSF-derived exosomes altered the molecular pattern of proliferation, activation, differentiation, and apoptosis, as well as the proliferation/differentiation regulators of NHKs. E-cadherin expression decreased, whereas N-cadherin expression increased. In conclusion, our findings indicate that HTSF-derived Exos may play a role in the epidermal pathological develop of HTS.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS376 | The energy landscape reshaped by strain-specific mutations underlies the long-range epistasis in NS1 evolution of influenza A virus

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The mechanisms underlying how individual mutations affect the protein energy landscape are crucial for understanding how proteins evolve. However, predicting mutational effects remains challenging because of epistasis—the nonadditive interactions between mutations. Here, we investigate the biophysical mechanism of strain-specific epistasis in the nonstructural protein 1 (NS1) of the influenza A virus (IAV). To understand the molecular basis of epistasis, we conducted comprehensive analyses of four NS1s of IAV strains that emerged between 1918 and 2004. We find that strain-specific mutations of NS1s are near-neutral with respect to the association with the p85β subunit of PI3K. However, the individual residues on the p85β-binding interface show long-range epistatic interactions with strain-specific mutations. We reveal that strain-specific mutations reshaped the energy landscape of NS1, leading to long-range epistasis. Our findings offer a high-resolution mechanism of how near-neutral mutations silently alter the biophysical energy landscapes, resulting in diverse background effects during molecular evolution.

Track: Protein Science Addressing Health Disparities

ABS377 | Structural and functional analysis of Cavia porcellus apolipoprotein E to obtain insight into the role of human apolipoprotein E4 in Alzheimers Disease

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Apolipoprotein (apo) E4 (299 amino acids) is a major cholesterol transport protein in plasma and brain and is a genetic risk factor for Alzheimer's disease (AD). The molecular basis underlying the link between apoE4 and AD is poorly understood. Previous studies have identified apoE4 and its C-terminal (residues 201-299) in plaques composed of amyloid-beta (Aβ) and neurofibrillary tangles in the brain of AD patients, suggesting a role for apoE4 CT domain (CTD) in amyloidogenesis. The CTD is highly hydrophobic and promotes protein-protein and protein-lipid interactions. In the current study, apoE from Cavia porcellus (guinea pig, GP) was used as a non-transgenic model to further understand the role of apoE4, since it lacks residues 193-197 and 246-252 of apoE4. We hypothesize that apoE4 CTD plays a critical role in lipid binding and Aβ peptide interaction. We tested the hypothesis by generating recombinant wildtype (WT) GP apoE, insertion mutants GP apoE/Ins193-197/Ins246-252, apoE4, and the corresponding deletion mutants apoE4/Δ193-197/Δ246-252 all bearing hexa-His-tags at the N-terminal end. All variants were expressed in E. coli and purified by His-tag affinity chromatography. Western blot with anti-apoE4 antibody 1D7 revealed cross reactivity with GP apoE. Far-UV circular dichroism spectroscopy of the insertion and deletion variants revealed troughs at 208 and 222 nm indicting the variants retained the helical structure observed in WT apoE4. Preliminary lipid solubilization assay indicates that WT GP apoE solubilizes lipids more efficiently than apoE4. Interestingly, apoE4/Δ193-197/Δ246-252 displayed increased lipid solubilization, while GP apoE/Ins193- 197/Ins246-252 showed decreased lipid solubilization ability. Together, the preliminary data indicate that residues 193-197 and/or 246-252 are involved in attenuating the lipid binding behavior of apoE4. Future studies will assess the ability of these variants on cholesterol efflux and Aβ binding ability of apoE4 to gain a better understanding of apoE4's role in AD.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS378 | Structural basis for target gene binding by the pneumococcal vancomycin-resistance response regulator

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VncRS, a two-component signal transduction system (TCS) of Streptococcus pneumoniae, regulates bacterial autolysis and vancomycin resistance. Vancomycin response regulator (VncR) is a pneumococcal response regulator of the VncRS. VncR harbors two domains, the N-terminal receiver domain and C-terminal effector domain. Once phosphorylated at its N-terminal receiver domain by VncS, a histidine kinase of TCS, VncR modulates target gene expression via its C-terminal effector domain which functions as a DNA binding domain. Here we determined the crystal structure of the C-terminal DNA binding domain of VncR, VncRc, at 1.96 Å resolution. The structure of VncRc features a helix-turn-helix motif highly similar to that of the response regulator PhoB of Escherichia coli. Biochemical and biophysical analyses using size exclusion chromatography and isothermal titration calorimetry revealed that VncR binds to VncS with Kd of 49 μM. We propose a speculative model of VncRc:DNA complex. Our results will provide insight into the mechanism by how VncR recognize target genes.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS379 | Towards Sensing of Breast Cancer Biomarkers: Engineering Glucose Dehydrogenase As an Estrogenic Regulated Protein

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Glucose dehydrogenase (GDH) reacts with glucose and generates an electric signal. This oxidoreductase is used industrially for glucose blood monitoring in glucometers.. To broaden the use of GDH as a sensor towards cancer biomarkers, we seek to profiling its structure and to engineer new allosteric regulation into it. Taking a synthetic biology approach, we constructed a saturated library by inserting the ligand-binding domain of estrogen receptor (ER-LBD) - which binds therapeutics for breast cancer - at all 456 amino acids of GDH. More than 7000 colonies were screened to identify the permissible sites and allosteric insertion sites. We find that the β-propeller fold of GDH serves as a scaffold providing a relatively rigid platform. This intrinsic stability results in high insertion tolerance. The plastic loops and α-helices of GDH that bind the cofactors are also well suited for remote signal propagation. Interestingly, we also observe that the allosteric sites are mainly located at the GDH dimer interface which play an essential role in enzymatic activity. The resulting estrogenic regulated GDH can electrochemically detect a series of estrogen receptor modulators as low as 1 nM with a ≥40% dynamic range. Our successful engineering of GDH yields a self-powered antagonist sensor and establishes a platform for monitoring breast

cancer therapeutics for personalized medicine in a point of care fashion.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS380 | PKC rearranges the Kv7.4 interactions with PIP2 and calmodulin

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Kv7 voltage gated potassium channels, also known as KCNQ or M channels, are expressed in tissues throughout the body. Their primary role is to maintain the resting membrane potential. Kv7 channels generate "M-current", an outward flow of potassium that opposes the depolarizations in neurons, cardiac cells, vascular cells, auditory cells and other cell types. The Kv7 subfamily consists of 5 isoforms (Kv7.1-5) that form homo- and hetero-tetramers. Kv7 channels are regulated by calmodulin (CaM) in a calcium-dependent manner. The membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP2) is necessary for the channels to facilitate M-current. Protein kinase C (PKC) is known to reduce the sensitivity of Kv7 channels toward PIP2. These cofactors (CaM, PIP2 and PKC) are controlled by muscarinic M1 receptors (M1R). Stimulation of M1R depletes PIP2, increases intracellular calcium-bound CaM, and activates PKC. Consequently, the inhibited M-current increases neuronal excitability. Improving our understanding these mechanisms holds potential to improve targeted Kv7 therapies for pain, epilepsy, stroke and hearing loss.

CaM binds the C termini of Kv7 subunits within the A and B helices. PKC targets a conserved serine or threonine within the B helix. In Kv7.4, PKC targets Thr553. The A and B helix contain recently identified PIP2 binding sites. Whether PIP2 and CaM simultaneously bind the Kv7.4 A and B helices is unclear. Moreover, the role played by PKC in tuning this binding is obscure. In this study, I used nuclear magnetic resonance (NMR) to show that phosphorylation reduces the affinity of apoCaM for Kv7.4 by reducing the number of CaM N-lobe contacts (Figure 1). I used microscale thermophoresis to show that PIP2 interacts the complex of CaM:Kv7.4 and that phosphorylation of Thr553 inhibits this interaction. These data suggest that phosphorylation of Thr553 affects Kv7 sensitivity to PIP2 by changing the overall structure of the CaM-Kv7 complex.

Track: Protein Science Addressing Health Disparities

ABS381 | Mutations protective against prion disease redirect the folding pathway of PrP in single-molecule trajectories

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Propagation of misfolded prion protein (PrP) is associated with a wide range of prion diseases in mammals. Variations in the amino acid sequence of PrP appear to play a key role in determining the disease susceptibility for any species. Several single-point mutations in PrP have been identified as protecting against prion infections, apparently by preventing PrP misfolding and/or conversion. However, the mechanism by which these mutations act to protect against disease is unclear. Here we investigate two such protective mutants: G127V, found in humans, and N159D, found in canids. We inserted these mutations into PrP from bank voles, which are the most prion-susceptible species known, and used optical tweezers to study the unfolding and refolding of single mutant bank vole PrP molecules. We found that these mutants differed in several aspects of their unfolding and refolding behavior: G127V folded homogeneously with a narrow force range, whereas N159D showed significant heterogeneity in folding with a wide range of unfolding forces. Notably, both mutations showed no evidence of forming metastable misfolded states, as seen in wild-type bank vole PrP. However, both mutations led to a similar effect on the native folding pathway: analyzing the transition maps showing all the transitions between distinct structural states of the protein in hundreds of unfolding and refolding trajectories, we found that the protective mutations redirected the pathways away from a particular partially folded intermediate into the same new intermediate states that were not present in the folding of the wild-type protein. We propose that this change in the pathway avoids an intermediate that is particularly misfolding-prone, thereby protecting against misfolding disease.

Track: Protein Science Addressing Health Disparities

ABS382 | Investigation of the phosphorylation status of the Candida glabrata Pdr1 transcription factor

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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 and the model organism Saccharomyces cerevisiae is mediated by Pdr1, a Zn(II)2Cys6 transcription factor that initiates the transcription of drug efflux pump genes. Many Zn(II)2Cys6 transcription factors are regulated by phosphorylation, and we hypothesize that Pdr1 activity is regulated by phosphorylation as well. S. cerevisiae Pdr1 has 18 reported phosphosites, but the phosphorylation status of C. glabrata Pdr1 has not been explored. We are currently investigating the effects on drug resistance phenotypes when computationally predicted phosphorylation sites in C. glabrata Pdr1 are mutated to alanine or glutamic acid. We are also working towards experimentally determining phosphosites in C. glabrata Pdr1. Immunoprecipitation followed by western blotting visualized with a phosphoprotein-specific stain is being used to evaluate the global phosphorylation status of C. glabrata Pdr1, and mass spectrometry phosphoproteomics will be used to locate phosphosites. Results from these studies will guide future work to characterize how phosphorylation regulates Pdr1 activity.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS383 | Elucidating the Function of Motif Aan Intrinsically Disordered Region within SIRT1

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The N-terminal region of SIRT1 is involved in regulating the deacetylation activity of the enzyme. Within this region there is an intrinsically disordered region named motif A, which also potentially includes a molecular recognition feature (MoRF). Recent studies have suggested that motif A plays a role in increasing SIRT1 activity, likely through an intramolecular binding interaction. In addition, it has been shown that phosphorylation of SIRT1 at serines 27 and 47, which are within the motif A region, can increase the enzyme's activity. However, in vitro quantitative studies to characterize this phenomenon are lacking. This research aims to elucidate the detailed mechanisms behind motif A's ability to regulate SIRT1 activity through biophysical and biochemical studies. More specifically, we hypothesize that phosphorylation at S27 and S47 within motif A might affect its MoRF properties as well as binding interaction with the rest of SIRT1. Towards this goal, we have mutated S27 and S47 into aspartic acid to mimic phosphposerines. We have utilized circular dichroism to determine whether phosphomimetic mutations affect the MoRF ability of Motif A. We have also used fluorescence polarization and microscale thermophoresis to quantify the KD values for the binding interactions between different motif A constructs and the rest of SIRT1 . This research will shed light onto the mechanism behind the intramolecular regulation of SIRT1 via an intrinsically disordered region.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS384 | Mechanism of secretion of TcpF by the Vibrio cholerae Toxin Coregulated Pilus

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Many bacteria possess dynamic filaments called Type IV pili (T4P) that perform diverse functions in colonization and dissemination, including host cell adhesion, DNA uptake, and secretion of protein substrates – exoproteins – from the periplasm to the extracellular space. The Vibrio cholerae Toxin Coregulated Pilus (TCP) and the enterotoxigenic E. coli (ETEC) CFA/III pilus each mediate export of a single exoprotein, TcpF and CofJ, respectively. Here we show that the disordered N-terminal segment of mature TcpF is the export signal (ES) recognized by TCP. Deletion of ES disrupts secretion and causes TcpF to accumulate in the periplasm. The ES alone can mediate export of individual N- and C-terminal domains of TcpF, and of a heterologously expressed periplasmic protein, FbpA, from Neisseria gonorrhoeae. The ES is specific for its autologous T4P machinery, as TcpF bearing the CofJ ES is exported by ETEC but not V. cholerae, and CofJ bearing the TcpF ES is exported by V. cholerae but not ETEC. Specificity is mediated by binding of ES to TcpB, a minor pilin that primes pilus assembly and forms a trimer at the pilus tip. Finally, ES is proteolyzed from the mature TcpF protein upon secretion. Together these results provide a mechanism for delivery of TcpF across the outer membrane and release into the extracellular space.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS385 | De novo design of protein binders for peptide recognition

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The design of de novo protein-peptide interactions offers great potential for drug discovery, diagnostics, and biotechnological applications, and is an outstanding challenge for de novo protein design. Here, we developed a new computational pipeline for designing high-affinity binders for specific peptide recognition. By using existing and novel computational methods such as RIF dock, Protein Message Passing Neural Networks (ProteinMPNN), RosettaFold inpainting, and AlphaFold2 we successfully designed nanomolar affinity binders against hormone peptides such as Parathyroid hormone (PTH) and Neuropeptide Y (NY), both of which are known biomarkers of human disease. Peptide binding was initially screened through yeast display and NanoBiT assays. The best designs were subsequently characterized more extensively and showed binding affinities close to 10 nanomolar by Fluorescence Polarization. These proteins provide starting points for the development of capture reagents for the enrichment and detection of peptides with relevance to human disease. Generalizing this approach to the plethora of peptide biomarkers will bring us closer to early diagnosis and better treatment of many devastating diseases, such as Alzheimer's Disease and Diabetes Mellitus.

Track: Protein Science Addressing Health Disparities

ABS386 | The Role of Cholesterol in Regulating Huntingtin Binding Lipid Membranes

Alyssa Stonebraker, Maryssa Beasley, Justin Legleiter
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Huntington's disease (HD) is a fatal neurodegenerative disease resulting from expansion of the polyglutamine (polyQ) domain in the huntingtin protein (htt). Aggregation occurs through a complex pathway that is influenced by sequences directly adjacent to the polyQ domain, including the first 17 amino acids at the N-terminus of the protein (Nt17). Nt17 has a propensity to form an amphipathic □-helix, facilitating lipid binding and promoting aggregation through early interactions by intermolecular self-association. Htt normally functions in vesicle transport and synaptic transmission, and thus associates closely with a variety of membranes including those of the endoplasmic reticulum, mitochondria, nuclear envelope, and plasma membrane. Membrane composition influences htt aggregation and membrane affinity. A membrane component critical to HD is cholesterol. Cholesterol functions in modulating membrane physical properties, such as fluidity, permeability, organization, and overall membrane function. In HD cholesterol homeostasis is disrupted, which likely impacts toxicity. The objective of these studies is to understand the impact cholesterol content within lipid systems has on htt aggregation and lipid binding. The lipid systems used include POPC, DOPC, and POPG with varied levels of exogenously added cholesterol. Htt was exposed to the different lipid systems and their effects on aggregation and lipid binding were evaluated using colorimetric lipid binding assays, thioflavin-T assays, mass spectrometry, and atomic force microscopy. When cholesterol content was increased, aggregation increased in the presence of DOPC, but decreased in the presence of POPC and POPG. With increased cholesterol content an increase in htt lipid binding to DOPC and POPC, and a decrease in htt lipid binding to POPG were observed. Changing cholesterol content in different lipid systems results in changes to htt aggregation and lipid binding, but exact effects are dependent on overall lipid composition.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS387 | An Old Protein with New Tricks: How the Toll-like Receptor 4 Complex Repurposes Its Components to Recognize Host Danger Signal S100A9

Sophia Phillips
Non-member (United States)

The Toll-like receptor 4 (TLR4) complex is an important initiator of inflammation. It responds to molecules released by both microbes and damaged tissues. The canonical mechanism by which TLR4 recognizes microbial molecules—such as lipopolysaccharides (LPS) from gram-negative bacteria—is well characterized . Conversely, the mechanism by which damage-associated host molecules activate TLR4 is poorly understood, in part due to vast structural differences between LPS and these host molecules. One such host molecule is S100A9, a protein released by immune cells that is associated with

several cancers and inflammatory diseases such as arthritis, inflammatory bowel disease, and atherosclerosis. Although it is known that S100A9 activates TLR4, basic mechanistic questions such as the binding surface, stoichiometry, and mode of activation remain unanswered. Here, we use mutagenesis, functional characterization, in vitro biochemistry, and computational docking programs including ROSETTA and AlphaFold to propose and test several possible models describing a direct interaction between S100A9 and components of the TLR4 complex. First, we showed that A9 interacts with TLR4 on a separate site from LPS. Second, we identified mutations to TLR4 that reduced the inflammatory activity of S100A9 but not LPS. Third, we found two S100A9 mutations that completely abolished the proinflammatory activity of S100A9. Together with our computational models, these results show that S100A9 directly interacts with the TLR4 complex via a novel binding site. This also suggests that S100A9 does not directly bridge the dimers to activate the receptor complex. Based on our observations, we are exploring other mechanisms of action in which S100A9 displaces or interacts with TLR4 coreceptors MD-2 and CD14. Study of this system is revealing how the components of a single receptor complex can take on a variety of roles in order to recognize radically different danger signals and successfully activate the innate immune system.

Track: High Throughput Protein Science

ABS388 | Structure-based design and characterization of a novel pre-fusion F protein of human metapneumovirus

Antonia Stuebler, Yvonne Chan, Sukanya Sasmal, Michael Kishko, Judith Alamares-Sapuay, Sophia Mundle, Linong Zhang
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Human metapneumovirus (hMPV) is a leading cause of acute respiratory infection in infants, the elderly, and immunocompromised patients. Infection with hMPV is ubiquitous and will affect nearly every child by the age of 5, resulting in incomplete immunity and inability to prevent re-infections. Despite this widely distributed disease burden, no approved vaccine or antibody therapies exist to date. The F glycoprotein, a main protein constituent of the virus envelope, is involved in virus-cell membrane fusion and has shown to be the primary target of neutralizing antibodies, thereby making it a promising vaccine target. To develop a viable vaccine candidate, we employed structure-based design to engineer hMPV-F proteins based on the A2 strain that are stabilized in the pre-fusion conformation. Specifically, the ectodomain of hMPV-F was attached to a foldon trimerization domain and numerous mutations were introduced following strategies of cavity-filling, interface stabilization, and helix disruption. The initial down selection, based on expression in Expi293F human cells and subsequent evaluation of promising designs for their biophysical properties, identified a stable pre-fusion design of hMPV-F (A2-T160F_N46V). This lead candidate expresses at 14.4 mg/L, has a purity and trimer percentage of above 90%, and a melting temperature of 79.3°C, determined by SDS-PAGE, size-exclusion chromatography in line with multi-angle light scattering, and differential scanning fluorimetry, respectively. In addition, confirmation of various antigen sites in the ectodomain was confirmed by Bio-Layer Interferometry and a panel of conformation-specific and linear antibodies. Moreover, the lead candidate elicited higher neutralizing titers in mice as compared to other benchmarks. In summary, the stepwise screening strategy led to the discovery of a stable and immunogenic pre-fusion form of hMPV-F as a potential vaccine candidate.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS390 | Allosteric Modulation of RyR1 with Nucleotide Analogs

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Coordinated release of Ca2+ from intracellular stores is critical for excitation-contraction coupling in muscle fibres. This is facilitated by Ryanodine Receptors (RyRs), which are 2.2 Mega Dalton Ca2+ channels that are embedded in the sarcoplasmic and endoplasmic reticulum. Numerous disease-causing mutations in RyRs have been identified with the majority resulting in increased RyR conductance. Aberrant functioning of the RyR1 skeletal muscle isoform is associated with progressive muscle degeneration, especially in older individuals. In skeletal muscle, activity of the RyR1 isoform is regulated by metabolites such as ATP, which binds allosterically and increases the probability that the channel is an open state. To obtain further structural insights into the mechanism of RyR1 priming by ATP, we determined cryo-EM structures of RyR1 bound individually to ATP-γ-S, ADP, AMP, adenosine, adenine, and cAMP. We show that

these derivatives of ATP bind at the same site in the nucleotide binding pocket. Despite the ability of adenine and adenosine to bind RyR1, we find AMP is the smallest ATP derivative capable of inducing long-range (>170 Å) structural rearrangements associated with channel activation, thus establishing a structural basis for key active site interactions that are the threshold for triggering large quaternary structural changes in RyR. Our finding that cAMP is also able to induce these structural changes suggests its potential to act as an endogenous modulator of RyR1 conductance. Understanding the environment of the nucleotide binding pocket and the diversity of molecules that can fit within the site provide a foundation for the design of structure-guided therapeutic targeting of RyRs.

Track: Machine Learning in Protein Science

ABS391 | Altering the reaction specificity of the hyperthermophilic 4--glucanotransferase through a bioinformatic approach

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Glycosyl hydrolases (GH) catalyze the rupture of glycosidic bonds in starch and other related polysaccharides. Some GHs also catalyze transfer reactions. We propose a strategy to find key residues implicated in the reaction specificity in this work. This strategy is based on the analysis of contact maps of members of the GH family 13 (CAZy) enzymes, which show a preference either for hydrolysis or transglycosidation. The algorithm considers the conservation of residues in close contact in 3D structures, computed as the parameter enrichment factor (Δfaa) in hydrolases versus transglycosidases. Thus, a positive Δfaa value reflects a residue preferentially present in transglycosidases, while a negative Δfaa value indicates that the residue is more frequently found in hydrolases. Two predicted relevant positions were mutated in the 4-α-glucanotransferase from Thermotoga maritima to modify its reaction profile: Q5E (Δfaa changes from 0.45 to -0.17) and S275G (Δfaa varies from 0.005 to 0.12). Surprisingly, we found that position 275 was relevant for the reaction specificity; however, the most hydrolytic variant was a double mutant with a Trp instead of Gly residue as predicted, showing a Hydrolysis/Transfer ratio of 3.82±0.32, relative to wild type enzyme. Molecular dynamic simulations performed to explain the effect of mutations on the reaction specificity showed that the catalytic acid-base residue (Glu216) in the double mutant Q5E/S275W explores more conformations relative to wild type protein during a 500 ns simulation. Also, the average pKa values computed for this residue increased and showed a higher dispersion in this variant than in the wild type. Interestingly, a new hydrogen bond between K325 and D278 (a residue that stabilizes the transition state) was detected only in Q5E/S275W variant. Understanding interactions that play a role in determining reaction specificity will permit the design of enzymes for industrial applications with the desired activity.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS392 | Incorporating Substrate-Like Features in Amino-Acid Based Butyrylcholinesterase Inhibitors for the Potential Treatment of Alzheimers Disease

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The depletion of the neurotransmitter acetylcholine has been suggested to contribute to the reduced cognitive function observed in individuals suffering from neurodegenerative diseases such as Alzheimer's Disease (AD). For the two major cholinesterases, butyrylcholinesterase (BChE) and acetylcholinesterase (AChE), increased BChE activity observed in individuals with AD has been suggested to deplete acetylcholine levels. To reduce acetylcholine degradation and help restore the pool of the neurotransmitter, specific and potent BChE inhibitors are sought. We previously identified 9-fluorenylmethoxycarbonyl (Fmoc) amino acid-based inhibitors as effective BChE inhibitors. The amino acid-based compounds offered the opportunity to survey a range of structural features to enhance interactions with the enzyme active site. As enzymes interact with features their substrates, incorporation of substrate-like features was predicted to lead to better inhibitors. Specifically, incorporation of a trimethylammonium moiety to mimic the cationic group of acetylcholine may lead to increased potency and specificity. To test this model, a series of inhibitors bearing a cationic trimethylammonium group were synthesized, purified, and characterized. Enzyme inhibition experiments monitored by UV-Vis spectroscopy were conducted to evaluate inhibition. While the Fmoc-ester derivatives inhibited the enzyme, additional experiments showed the compounds acted as substrates and were enzymatically hydrolyzed. A more stable amide series was then synthesized and characterized. Inhibition studies for the Fmoc-amide derivatives suggested the compounds are not substrates and selectively inhibit

BChE with KI values in the 0.06-10.0 μM range. Computational docking studies support the inhibitors binding in the active site and interacting with active site features like the substrate. Overall, the results suggest that introducing substrate-like characteristics within the Fmoc-amino acid-based background increases their potency. The versatile and ready access to amino acid-based compounds offers an attractive system to further our understanding of the relative importance of protein-small molecule interactions and may help guide the development of better inhibitors.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS393 | Molecular Dynamics and Alchemical Free Energy Calculations Aid Interpretation of Mutation Effects on Regulation of Abl Kinase

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Src family tyrosine kinases are regulated by docking of their SH3 and SH2 domains. SH3-SH2 docking does not block the active site or contact the activation loop, but instead appears to modulate N-lobe orientation and dynamics as a rigid unit. Unlike other members of the Src family, Abl kinase lacks a C-terminal phosphotyrosine site to control regulatory docking through binding to its SH2 domain. Instead, Abl 1b's N-terminal myristoyl binds to the C-lobe of the kinase domain and induces a bend in the C-terminal helix, thought to enable SH2 docking. This regulation is disrupted in Bcr-/Tel-Abl fusion oncoproteins, where the domains remain, but the myristoylation is removed, forming the basis for recently-approved allosteric inhibitors which bind at this site. Recent NMR studies from the Kalodimos lab show the apo kinase domain populating active and two inactive conformations previously seen in inhibitor-bound structures. These interconvert on millisecond timescales much longer than routinely accessible by molecular dynamics (MD) simulations. We used alchemical free energy calculations, together with longer-timescale MD, to validate the previously-assumed mechanism of myristoyl-triggered assembly and regulation. We then applied relative free energy calculations to understand a drug-resistant mutation in the C-lobe, which preliminary data suggests is glycine-specific. These predict that the glycine mutation changes the thermodynamics between active and inactive states of the apo kinase domain, while an alanine mutation leads to a smaller effect. We show, using longer-timescale MD, that the mutation's effect on these three conformational states' structures and dynamics is subtle. While activating mutations can result from subtle effects on the kinetics of conformational change or binding and dissociation, we believe this to be a powerful approach for difficult-to-understand mutations that mostly repartition the kinase between existing states. In the future, we hope to revisit the connection between SH3-SH2 docking, N-lobe orientation, and activation loop conformation.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS394 | Investigating Nucleotide Binding in the T4 DNA Clamp Loading Cycle

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The DNA clamp loader, a pentameric AAA+ ATPase, uses the energy of ATP binding and hydrolysis to power conformational changes and steer the DNA sliding clamp loading cycle. Structural evidence of clamp loader complexes suggests intermediate states of clamp opening, DNA binding, and clamp closure around the DNA helix, ready for processive replication. However, precise knowledge of ATP utilization in the functional cycle is lacking.
While four ATP molecules were previously shown to bind to clamp loader complex with equal affinity at steady state, an ultrasensitive response to nucleotide concentration is observed for DNA binding. We are interested in understanding how substrate binding and conformational change are coupled to nucleotide binding. To what extent would the details of this coupling imply AAA+ATPase working largely independently, organized by their substrates, in the absence of conformational changes constrained by the closed ring of processive AAA+ motor?
In an attempt to unravel the coupling between substrate and nucleotide, we are developing a fluorescence quenching readout to directly investigate nucleotide binding, an anisotropy assay to measure clamp binding, and a FRET readout for clamp conformational change. Enhanced-sampling molecular dynamics simulations of clamp alone reveal free clamp adopting a few distinct conformations. Anisotropy measurements suggest a loader-clamp binding process independent of nucleotide conditions. However, loader-clamp binding in the presence of ATP shows enhanced anisotropy in contrast to samples incubated in ATP analogs. This enhanced anisotropy, which we tentatively interpret as clamp opening, slowly decays and

converges to the value returned by samples equilibrated with ATP analog. We investigate whether this is reproduced by kinetic models in which the inhibited hydrolysis rate of open clamp-loader complex in the absence of DNA drives the steady state population towards open clamp bound conformation. This study highlights a role of ATP hydrolysis that needs to be further characterized.

Track: Protein Science Addressing Health Disparities

ABS395 | Glycosylation limits forward trafficking of the tetraspan membrane protein PMP22

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Peripheral myelin protein 22 (PMP22) is a tetraspan integral membrane protein that comprises approximately 2-5% of the total protein content of Schwann cell myelin in the peripheral nervous system. Under normal physiological conditions, approximately 20% of expressed wild-type PMP22 properly folds and traffics to the plasma membrane of cells. Charcot-Marie-Tooth (CMT) disease is a neuropathy of the peripheral nervous system that results in progressive loss of muscle tissue as well as defects in motor and sensory perception. In CMT, an additional copy of the wild-type PMP22 allele causes overexpression of the PMP22 protein, resulting in the formation of cytotoxic aggregates. Further, various mutants of PMP22 have been shown to traffic to the cell surface with significantly lower efficiency. Here, we show that the single glycosylation site of PMP22 (N41) affects trafficking of the protein. The glycosylation deficient N41Q mutant, in fact, increases trafficking of the wild-type PMP22 approximately three-fold. Further, the trafficking of a severe disease mutant (L16P) is increased approximately ten-fold when glycosylation of PMP22 is eliminated. These results indicate how glycosylation of PMP22 affect its trafficking to the plasma membrane of cells and insights into other proteins involved in its quality control within the cell.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS396 | Progress towards a third generation near-infrared genetically encoded calcium ion indicator with improved brightness

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Introduction: Genetically encoded calcium ion (Ca2+) indicators (GECIs) are powerful tools for the noninvasive fluorescence imaging of Ca2+ dynamics in living tissues. Fluorescence imaging in the near-infrared (NIR) region (~670–900 nm) is associated with advantages such as low background autofluorescence, reduced light scattering, and minimal tissue absorption. Accordingly, all other factors being equal, NIR GECIs have advantages such as reduced phototoxicity, minimal spectral cross talk, and deeper tissue imaging.

The first and only practical NIR GECIs are the NIR-GECO series based on insertion of calmodulin (CaM)–RS20 into the monomeric infrared fluorescent protein (mIFP). However, first and second generations NIR GECOs suffer from relatively dim effective brightness in vivo, which is partly due to the low molecular brightness of mIFP and the low affinity for the cofactor biliverdin (BV). This study aims to develop a third generation NIR GECI with improved effective brightness and thereby address the problems of current NIR-GECOs.

Methods and Results

As a scaffold for the novel NIR GECI, we focus on miRFP680 which has 3-fold greater brightness than mIFP. As a Ca2+ sensing domain, CaM-RS20 was inserted into different insertion sites of miRFP680 and an NIR GECI prototype showing 15% fluorescence decrease in the presence of Ca2+ was identified (Figure). Following several rounds of directed evolution, we have now developed improved prototype NIR GECI with up to 78% fluorescence decrease upon binding to Ca2+.

Conclusion and Perspective

In conclusion, we are rapidly progressing towards development of a third generation GECI based on the bright NIR FP, miRFP680. In the future, an BV affinity-directed evolution method will be performed to improve the BV binding affinity of the indicator prototype. Moreover, we will work with collaborators to compare the effective brightness of the optimized indicator with existing NIR GECIs and apply it in zebrafish or Caenorhabditis elegans.

Track: Machine Learning in Protein Science

ABS397 | The protein organization of a red blood cell

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Red blood cells (RBCs, erythrocytes) are the simplest primary human cells, lacking nuclei and major organelles,

and instead employing about a thousand proteins to dynamically control cellular function and morphology in response to physiological cues. In this study, we defined a canonical RBC proteome and interactome using quantitative mass spectrometry and machine learning. Our data reveal an RBC interactome dominated by protein homeostasis, redox biology, cytoskeletal dynamics, and carbon metabolism. We validated protein complexes through electron microscopy and chemical crosslinking, and with these data, built 3D structural models of the ankyrin/Band 3/Band 4.2 complex that bridges the spectrin cytoskeleton to the RBC membrane. The model suggests spring-like compression of ankyrin may contribute to the characteristic RBC cell shape and flexibility. Taken together, our study provides an in-depth view of the global protein organization of human RBCs and serves as a comprehensive resource for future research.

Track: Machine Learning in Protein Science

ABS398 | Capturing idiosyncratic and higher-order epistasis in adaptive protein evolution

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The evolution of new protein functions is caused by gradual accumulation of adaptive mutations. This seemingly simple process is underpinned by intricate mechanisms that ultimately dictate the amino acid identity and chronology of the acquired mutations in a protein. One source of this complexity stems from epistasis, the non-additive functional contribution of two or more mutations. As more mutations accumulate, epistasis becomes harder to interpret due to higher-order mutational interactions and idiosyncrasies in the context-dependence of epistatic interactions themselves. In this study, we address these queries using diverse statistical approaches to quantify and capture idiosyncratic and higher-order epistasis. We analyzed 45 fitness landscapes probing 7 different enzymes and observed high levels of functional heterogeneity in mutational effects and epistasis. The mutations and epistasis also appeared idiosyncratic with respect to their average functional contribution in their fitness landscape. The idiosyncratic epistasis caused that, on average, incorporation of more mutational information in the WT-background did not enable improved prediction of genotypes with multiple mutations. We also showed two potential evolutionary implications of restrictive and permissive idiosyncratic epistasis in the adaptive evolution of two organophosphate hydrolases: phosphotriesterase (PTE) and methyl-parathion hydrolase (MPH). Ultimately, our results demonstrate that higher-order epistasis is prevalent across adaptive protein trajectories and does not merely represent the additional epistatic contribution stemming from three or more mutations, but more importantly it creates idiosyncratic epistasis by obscuring the relationship between the mean functional contribution of a mutation or epistasis from its contribution in a specific genotypic background. We hope that in the future it may be feasible to combine the predictive power of ML algorithms with biophysical analyses of idiosyncratic epistasis to further improve prediction for protein engineering efforts.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS399 | Investigating Competition between the HIV-1 Proteins Rev and Gag for Stem 1 of the Rev Response Element

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The Human Immunodeficiency Virus (HIV) persists without a cure, infecting 37 million individuals globally, and existing treatments require continual use and may cause harmful side effects. To export the HIV genome from the nucleus to the cytoplasm where it is assembled for packaging, two major viral elements are required: Rev and the Rev Response Element. The Rev Response Element (RRE) is a structured RNA that is part of the HIV genome and is found on unspliced and incompletely spliced viral transcripts. The RRE forms two significant configurations, a 4 and 5 stem loop structure. The HIV-1 genome also codes for Rev, an RNA-binding protein translated in the early phases of viral replication. Later, Rev re-enters the nucleus via its nuclear localization sequence and has been shown to bind to the RRE on two stem II binding sites, and one stem I binding site. The RRE also interacts with the structural viral protein Gag –primarily on stem I– which is involved in genome and virion packaging. Given that both Gag and Rev bind stem I, this project seeks to characterize the interactions between stem I of the RRE and Rev and Gag through competitive EMSA studies, ITC, and NMR. We use a peptide containing the RNA-binding, arginine-rich motif of Rev, a protein containing the nucleocapsid domain of Gag, and truncated RRE stem I fragments. Using these techniques and constructs, we determine binding affinities and interactions to explore the competition of these two proteins when binding to the RRE. Greater binding affinity by Gag may suggest that Gag binds the RRE in the cytoplasm, displacing Rev from the

stem I binding site. This interaction would be biologically relevant and represent a link between nuclear export of the genome and subsequent genome packaging.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS400 | Structure-Function Rationale for Enhanced Viral Fitness in SARS-CoV-2 Variants of Concern

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Following limited genetic variation in 2020, several SARS-CoV-2 variants have since emerged, many of which have become globally dominant. A major focus of SARS-CoV-2 variant characterization has been on the spike glycoprotein – a protein crucial for viral attachment and subsequent entry into human cells – which is hypermutated relative to other viral proteins and is the major protein immunogen in most vaccine formulations. For every variant of concern spike protein (Alpha, Beta, Gamma, Delta, Omicron) we have solved high-resolution cryo-EM structures to provide a structural basis for enhanced aspects of viral fitness. We specifically analyzed human cell receptor (ACE2) engagement and immune escape in SARS-CoV-2 convalescent patients and vaccinated individuals as two important markers of variant advantage. Further, we studied variant of concern mutations both in isolation and in multiple combinations to develop predictive insights into future variants that may emerge. The structural and biochemical information gleaned from these studies provides a molecular basis to rationalize the emergence of present and future SARS-CoV-2 variants.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS401 | Exploring Nucleotide Dependence of DNA Binding in the T4 Bacteriophage Clamp Loader

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Clamp loaders are ATPases that exist across all domains of life. They hydrolyze ATP to load a circular clamp around DNA which the polymerase then binds, enabling processive replication. This molecular machine utilizes several conserved motifs present across ATPases, one of which is the Walker B motif. Previous deep mutagenesis of the T4 bacteriophage clamp loader revealed that the last aspartate of this motif can only be replaced with a glutamate or cysteine. To characterize the role of this residue, a crystal structure of the Walker B mutant bound to clamp and DNA was obtained, and a fluorescence anisotropy-based assay was designed to characterize its DNA binding ability. Wildtype clamp loader can bind DNA with both ADP-Beryllium Fluoride and ADP-Aluminum Fluoride, which mimic the ground and transition state of the ATP hydrolysis cycle respectively. However, the Walker B mutant binds DNA only with the transition state analogue. Further biochemical characterization of nearby mutations provided some insight on its role in DNA mediated ATP hydrolysis. Upon DNA binding, via a series of identified interactions including the Walker B motif, the sugar phosphate backbone is recognized, and the clamp loader can transition to a catalytically active state seen in crystal structures. Utilizing a transition state analogue might promote conversion to this active state, regardless of disrupted communication, explaining why some mutants can't bind DNA with a ground state analog. The sequence and location of the Walker B motif is similar across several ATPases suggesting that its role in substrate mediated ATP hydrolysis is conserved, which in the T4 clamp loader involves the transition from a catalytically inactive to active state.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS402 | Self-complementing split fluorescent protein-based biosensors for calcium ion

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Calcium ion (Ca2+) plays critical roles in many biological and physiological activities in living organisms. Genetically encoded Ca2+ indicators (GECIs) have been developed from fluorescent proteins (FPs) to realise non-invasive Ca2+ monitoring. Existing GECIs have mainly focused on the investigation of Ca2+ dynamics at the single cellular compartment such as cytosol, mitochondria and endoplasmic reticulum (ER). However, few of them can be applicable to track Ca2+ shuttling at organelle contact sites (OCSs), which a number of recent studies suggest is important to regulate many cellular processes ranging

from energy production to apoptosis. To provide suitable biosensors that can monitor Ca2+ dynamics at OCSs, we developed a new type of self-complementing split GECIs.
To engineer split GECIs, we first developed new self-complementing split FPs, scpGFP1 and scpmApple1. We split circularly permutated (cp) versions of EGFP and mApple RFP between β8 and β9 strand. We demonstrated that these new split FPs can self-complement with each other without any associating proteins in vitro and in living cells. We then engineered split GECIs by genetically fusing Ca2+ sensing proteins (calmodulin and RS20). Several rounds of linker optimisation and directed protein evolution on the prototypes dramatically increased brightness and sensitivity to Ca2+, and we finally established sG-GECO1 and sR-GECO1, which displayed Ca2+-dependent fluorescence increases of 11 and 20, respectively. We have also developed Ca2+-affinity tuned variants of sG-GECO1 with apparent dissociation constants ranging from 3 to 600 μM to make our biosensors applicable in wider range of Ca2+ concentration. We then have confirmed that our sGECO biosensors, expressed in cytosol in living cells, could complement with each other and monitor histamine-induced cytosolic Ca2+ oscillation.
In future work, we will target our sGECO biosensors at the OCS between mitochondria and ER in living cells to investigate their suitability for monitoring Ca2+ dynamics at OCSs.

Track: Miniproteins: Are There Really That Many Additional Genes That We Have Been Missing?

ABS403 | Identification of Proteins Profiles on Vietnam Bacteria Causing Tomato Wilt

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The major sources of food is the crops. However, the yield and quality of harvest depends on the plant diseases and insect pests mainly. Therefore, identifying the plant diseases and insect pests are important in prevention.
The tomato, Solanum lycopersicum, distributes in tropical and subtropics region and is serves as the vegetable or/and the fruit. However, some diseases result in the plat wilt from the pathogens, Ralstonia solanacearum. It secrets the polysarccharides to block the vascular bundle in wilt and death of plant. The characteristics of R. solanacearum vary with the geographical distribution.
Recently, a new microorganism of R. solanacearum was isolated under Koch's postulates in Vietnam. The bacteria was identified with 282 bp PCR products amplified with R. solanacearum specific primers. The sequence of 282bp DNA fragment was also obtained and aligned with the database. Then, its proteins were extracted and analyzed on the proteomics technique. And the profiles of the proteins were collected. From the results, the prevention strategy can be designed in future.

Track: Machine Learning in Protein Science

ABS404 | Functional proteomics-directed artificial intelligence to identify messenger molecules between mammalian cells and Gram-positive bacteria

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It has been understood that during Gram-positive bacterial infection, the bacterial cells interact with host mammalian cells via the virulence factors whose anchorage onto the bacterial surface are mainly catalysed by Sortase A. The activity of Sortase A and the resulting bacterial infection are mediated by its equilibrium of dimerization in vitro and in vivo. We hypothesize that the host mammalian cells can interfere with the bacterial infection by interacting with Sortase A dimer or monomer via mammalian protein(s) (messengers). In order to identify the messenger molecules, we devised an artificial intelligence using machine learning and deep learning algorithm coupled with statistical principal component analysis based on data sets generated from functional proteomics studies to accurately and precisely predict the sensing molecule(s) that can affect the bacterial infection. Herein, we show the AI predicted result of a messenger molecule, NPY receptor and its cognate ligands are biologically significant in the process of bacterial infection, whilst maintaining consistency with human manual search thus suggesting that the algorithm is capable of substituting manual experimental efforts that would drastically improve research time.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS405 | Structural Basis for Regulation and Higher Order Assembly of Human Phosphofructokinase

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University of Washington (United States)

Glycolysis is an ancient and conserved pathway for extracting energy from glucose. This process is vital to human health, as mutations in pathway enzymes lead to metabolic diseases and many cancers rely on glycolysis for proliferation. Phosphofructokinase-1 (PFK1) catalyzes the rate-limiting step going from fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (F16BP). As a major regulator of the glycolytic pathway, PFK1 is tightly regulated to fine-tune flux in response to changing energetic demands. Human PFK has a regulatory and a catalytic domain and assembles into a tetramer. In humans, three genes encode PFK1 liver (PFKL), muscle (PFKM), and platelet (PFKP) isozymes. Each isoform has unique kinetics and sensitivities to allosteric regulators. We have shown in previous work that recombinant PFKL assembles filaments that are connected by regulatory domains, indicating filaments may have a role in regulation. Allosteric effectors bind the regulatory domain to influence the equilibrium between active and inactive states; in particular, at high concentrations of ATP, binding in allosteric sites inhibits PFK1.The molecular mechanisms underlying functional differences among PFK1 isoforms have not been determined. This is due in large part to a lack of structural data surrounding the human isoforms. In this work we employ cryo-EM, which is ideal for studying conformational changes in response to allosteric regulators, to understand the structural basis of regulation for the PFK1 isozymes. We present high resolution structures of PFKL and PFKM in multiple functionally important ligand states. These initial structures suggest both isoforms can form filaments, and that PFKL C-terminus positioning assists in stabilizing the inactive state. Additionally, the inactive PFKL structure revealed multiple inhibitory ATP binding sites. This work will provide fundamental insights into the role of PFK1 filament formation and tissue-specific regulation of glycolysis.

Track: Protein Phase Separation in Biomolecular Condensates

ABS406 | Evolutionary Conservation of Systemic and Reversible Amyloid Aggregation

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In response to environmental stress, human cells have been shown to form reversible amyloid aggregates within the nucleus, termed amyloid bodies (A-bodies). These structures share many of the biophysical characteristics of the pathological amyloid aggregates observed in neurodegenerative diseases, such as Alzheimer's and Parkinson's. However, unlike pathological amyloid aggregates, A-bodies are rapidly disassembled after the stressor is removed. Functionally, A-body formation induces a state of cellular dormancy by sequestering proteins away from their downstream effectors, in order to conserve energy during periods of cell stress. Here, we show that A-bodies are evolutionarily conserved across the eukaryotic domain, with their detection in Drosophila melanogaster and Saccharomyces cerevisiae marking the first examples of these functional amyloids being induced outside of a cultured cell setting. The conditions triggering amyloidogenesis varied significantly among the species tested, with results indicating that A-body formation is a severe, but sublethal, stress response pathway that is tailored to the environmental norms of an organism. RNA-sequencing analyses demonstrate that the regulatory low-complexity long non-coding RNAs that drive A-body aggregation are both conserved and essential in human, mouse and chicken cells. Thus, the identification of these natural and reversible functional amyloids in a variety of evolutionarily diverse species highlights the physiological significance of this protein conformation, and will be informative in advancing our understanding of both functional and pathological amyloid aggregation events.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS407 | Structure and dynamics on human somatostatin receptor 2/angonist/G-protein complex

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Somatostatin is a peptide hormone that regulates endocrine systems by binding to G protein-coupled somatostatin receptors. Human somatostatin receptor 2 (SSTR2) is highly implicated in hormone disorders, cancers and neurological diseases. Here, we report the high-resolution structure by cryo-EM, which shows full-length human SSTR2 bound to the agonist somatostatin (SST-14) in complex with inhibitory G (Gi) proteins. Our data show that seven transmembrane helices form

a deep pocket for ligand binding and that SSTR2 recognizes the highly conserved binding motif of its ligand at the bottom of the pocket. To validate our structure, we mutagenized critical residues involved in the interaction and performed a functional assay in HEK293 cells. We measured the degree of inhibition of cAMP generation upon forskolin stimulation by homogeneous time-resolved fluorescence resonance energy transfer. This work provides the first glimpse into the molecular recognition mechanism of somatostatin receptors, serving as a crucial resource to develop therapeutics targeting somatostatin receptors.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS408 | The Dimensions of an Ensemble of Collapsed Disordered Protein Molecules Under Folding Conditions

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In order to detect the earliest formed intramolecular contacts in the refolding ensembles by detection of the intramolecular distances, we must first determine the dimensions of the transient collapsed ensemble. Does an ensemble of disordered molecules of foldable globular protein under folding conditions follow Flory's theory of collapsed polymers? Using E. coli Adenylate kinase (AK) as a model protein and microfluidic mixing device combined with time resolved FRET measurements ("the double kinetics" method) we tested the hypothesis that at the initiation of refolding, the dependence of the mean of the segmental end-to-end distance on the segment length, Δ n, is weak (Δn>∼30).We measured the transient distributions of segmental end to end distances of seven segments of the AK molecule, with Δn from 45 to 196 residues at 50 μs after initiation of mixing. The means of these distributions range from 45±2 Å, for the short segment, to70±2 Å for the long segment. The Flory exponent for the Δn dependence of the mean segmental end to end distance is 0.32±0.01. Thus, this transient ensemble is indeed a collection of disordered molecules. We now have a benchmark for the segmental end to end distance distribution of unfolded AK molecules under folding conditions (poor solvent). Any intramolecular mean distance that would be significantly shorter can be considered as an indication of an early formed intramolecular contact. This experiment enables systematic detection of the time sequence of formation of non local contacts in the refolding protein molecules. A step which is essential for deciphering the mechanism of folding of globular proteins.

Track: High Throughput Protein Science

ABS409 | High-throughput discovery of sequences that promote proteolysis in bacteria

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All bacteria possess multiple ATP-dependent proteases that degrade cytosolic proteins. These enzymes help maintain protein homeostasis and regulate discrete pathways, including the expression of virulence phenotypes in pathogenic bacteria, and have emerged as attractive antibacterial targets. ATP-dependent proteases are able to selectively recognize substrate proteins and ignore non-substrate proteins, minimizing harmful or wasteful off-target proteolysis. Many substrates are recognized directly by short, unstructured terminal sequences, termed degrons. While a small number of degrons have been identified, there is little known about the overarching rules that allow proteases to effectively discriminate between valid degrons and the millions of other possible terminal sequences.

To address this gap in our knowledge, we have developed a cell-based screening platform that will allow us to interrogate global degron specificity and define the sequence-based rules that govern recognition of protein substrates by ATP-dependent proteases. Our method incorporates a novel selection-based screen, in which a library of protein toxin bearing a randomized terminal tag is expressed in host bacteria. Accumulation of toxin in host cells causes cell death. However, toxins bearing bona fide degrons are proteolyzed by endogenous proteases, allowing cell survival. Bacteria expressing valid degron sequences are enriched over time, and identified by Next-Generation Sequencing. Here we describe the efficacy of our method in E. coli, supported by NGS data from screening experiments performed in multiple proteolytic deletion strains. In addition to our toxin-based approach, we demonstrate a complementary method that utilizes a fluorescent protein substrate as a reporter for proteolysis, coupled with fluorescence-activated cell sorting (FACS) to isolate cells expressing valid degrons. The information gathered from these methods will ultimately help us understand the roles that ATP-

dependent proteases play in individual pathogenic bacteria.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS410 | Rational design and optimisation of Beclin 1-targeting stapled peptides to promote autophagy and inhibit cancer cell proliferation

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Autophagy is an evolutionarily conserved process that degrades and recycles cytosolic content in lysosome-dependent manner. Beclin 1 is an essential autophagy gene and fulfils this role by being a scaffolding member of the Class III phosphatidylinositol-3-kinase complex (PI3KC3). Beclin 1 recruits either Atg14L or UVRAG to form biochemically and functionally distinct Atg14L-containing PI3KC3 Complex I or UVRAG-containing Complex II with significantly elevated lipid kinase activity. The Beclin 1-Atg14L/UVRAG interaction relies critically on their respective coiled coil domains.

Our lab determined the crystal structure of the Beclin 1 coiled coil domain that forms an anti-parallel homodimer and the Beclin 1-UVRAG coiled coil complex that forms a parallel heterodimer. Guided by these structures, we designed Beclin 1-targeting stapled peptides that mimic the N-terminal half of the Beclin 1 coiled coil domain so that they bind to the C-terminal half to reduce self-association and to promote the Beclin 1-Atg14L/UVRAG interaction. We used the Beclin 1 protein sequence at initial template to build a peptide library and conducted iterative rounds of computational screening. All designed peptides were virtually modified with a hydrocarbon staple to stabilize their helical structure. Location of the hydrocarbon staple was optimized by scanning through all possible positions. Interestingly, our data shows that placing the hydrocarbon staple closer to the Beclin 1-peptide interface enhanced their binding affinity by ~10- to 30-fold in vitro.

Our designed peptides readily induced autophagy in vivo and enhanced endolysosomal trafficking by promoting the degradation of cell-surface receptors like EGFR and HER2. These peptides also inhibited the proliferation of cancer cells that overexpressed EGFR and HER2 by inducing necrotic cell death but not apoptosis. Given that Beclin 1 is a haplo-insufficient tumour suppressor and frequently deleted in human cancer, our designed peptides may serve as potential anti-cancer therapeutic by targeting this Beclin 1 vulnerability.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS411 | Engineering switchable binding activity into a T-cell engaging bispecific antibody

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The convergence of protein engineering and cancer immunotherapy has generated innovative therapeutics to treat disease by engaging a patient's own immune system to destroy cancer cells. T-cell engaging bispecific antibodies (T-bsAbs) are one example of these new therapeutics. T-bsAbs simultaneously bind tumor cells and T-cells, thus stimulating T-cells to directly attack the tumor. Despite FDA approval of the T-bsAb Blincyto in 2018 for treatment of acute lymphoblastic leukemia, toxicity has hindered the clinical value of T-bsAbs for other malignancies. One major toxicity risk of T-bsAbs is cytokine release syndrome (CRS). When CRS occurs, the immune system becomes hyperactivated, leading to pro-inflammatory activity and a multitude of toxicities which can be lethal for patients. While these harmful side effects of CRS can be clinically managed by limiting doses, the need to develop safer T-bsAbs remains. The long-term goal of this project is to engineer a T-bsAb with built-in control over T-cell-induced cytokine release to ameliorate the toxicity of CRS. My central hypothesis is that controlled tumor-specific cytokine release can be achieved by designing a masking domain into a T-bsAb that is removable in response to tumor-specific stimuli. The masking domain is made to switch from an inert autoinhibited conformation (no T-cell cytotoxicity) to an active conformation (stimulation of T-cell cytotoxicity) in the presence of a tumor-targeted activator molecule. This design allows T-cell binding to be dependent on the activator domain binding kinetics and half-life, enabling switchable control over cytokine release and preventing CRS.

I demonstrate the design of switchable masking domains incorporated into T-bsAb design to control T-cell cytotoxicity. To achieve these results, I am leveraging computational protein design using Rosetta in conjunction with quantitative binding assays and reporter T-cell activation assays. These findings will demonstrate an approach that addresses the need for safer T-bsAb therapeutics to advance disease treatment.

Track: Protein Science Addressing Health Disparities

ABS412 | Identification and Characterization of Phosphoarginine Modifications in Mycobacteria

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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. 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. Based on strong sequence and structural homology between BsuClpC and MtbClpC1 NTDs, we predicted that pArg modifications exist in Mtb and serve a similar function. Phosphoproteomics of Mycolicibacterium smegmatis, a non-infectious surrogate of Mtb, reveals six proteins with high confidence pArg modifications. Additionally, we find that pArg binds to ClpC2, a small stress-associated protein with homology to the ClpC1 NTD. Crystal structures of ClpC2 with and without bound pArg suggest that pArg binding alters the oligomeric state of ClpC2, providing a possible mechanism for pArg binding to perturb protein function. Our work provides the first direct evidence of pArg in mycobacteria, and point towards a role for these modifications in stress pathways.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS413 | Enrichment of elusive ciliary complexes for proteomics

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The cilium, or eukaryotic flagellum, is a conserved membrane-bound eukaryotic organelle in many cell types such as sperm cells, olfactory neurons, and respiratory epithelial cells. There are two distinct primary functions of cilia: transduce and regulate signaling pathways (primary cilia), and movement (motile cilia). Ciliary defects, known as ciliopathies, can lead to different congenital abnormalities such as anencephaly, skeletal defects, and exencephaly. Inside cilia, intraflagellar transport (IFT) trains carry cargo, such as dynein arms, to the tip and back. IFT trains are composed of two protein complexes: IFT-A and IFT-B. Currently there are low resolution tomography maps of the IFT trains and high-resolution crystal structures of some IFT-B proteins, but there are no high-resolution structures of full complexes due to their structural heterogeneity. We enriched IFT-A from Tetrahymena thermophila, a highly ciliated protozoan. We grew Tetrahymena thermophila and induced shedding of their cilia using an anesthetic agent. We break open the ciliary membrane to separate the axonemes and the ciliary matrix. With the matrix, we use a size exclusion column to isolate and concentrate IFT-A. The final concentrated sample was analyzed by mass spectrometry to calculate the percentage of IFT-A in the sample. With our workflow, we are able to enrich IFT-A in a near native environment.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS414 | Virulent Factor Regulatory Proteins Could Inform Alternative Medical Treatment

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Globally, Enterotoxigenic Escherichia coli (ETEC) is the leading cause of travelers' diarrhea (TD), indiscriminately affecting persons abroad and of particular concern for volunteer and military organizations. Children under five in lower to middle income countries affected by ETEC die at staggering rates and survivors can be left with irreversible growth defects due to severe diarrheal cases. With the increased prevalence of resistance, antibiotics are now considered as last resort treatment options for diarrheal diseases, and the need for novel alternatives is growing. ETEC virulence requires two factors: the toxin and pili/adherence proteins. Regulation of adherence factors in ETEC depends on Rns, a transcription

factor that belongs to the AraC protein family. Without the ability to adhere and colonize, ETEC would be non-pathogenic. Previously, fatty acid ligands have been shown to bind Rns and other AraC homologs, leading to increased protein stability, locking them in a conformation that prevents DNA binding domain and transcription of the genes required for ETEC fimbriae. To determine how FA stabilizes Rns and blocks DNA binding, we have used the crystal structure of Rns to design a series of Rns variants. These include binding pocket mutants: H20A, R75A, H20A/R75A. Fatty acid coordination by an arginine side chain (R75) and potential participation from a histidine side chain (H20) found in the native Rns structure is absent in the R75A mutant structure and subsequent mutants. To clarify our understanding, we have crystallized each mutant to approximately 2.3-2.8 Å and are in the process of solving the structures. These, and other mutants, are shedding light on other key ligand binding residues. Structures like Rns, and its mutants, are bringing about insightful information regarding the nature of what can bind in the pocket and how to design mimetics for novel antivirulence drug alternatives.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS416 | Direct observation of prion-like propagation of misfolding in single protein molecules

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Templated conversion of natively folded protein molecule into misfolded isoform, by another misfolded protein molecule, is characteristic of many neurodegenerative diseases. However, such prion-like propagation of misfolding has never been directly observed in experiments. mainly due to lack of suitable method, hindering efforts to decipher conversion mechanisms and develop therapeutic countermeasures. Here, we have devised a unique method for direct observation of templated conversion at the single-molecule level. We explore the prion-like conversion in single molecules of superoxide dismutase-1 (SOD1), which misfolds in amyotrophic lateral sclerosis (ALS), by tethering misfolded mutants associated with familial ALS to wild-type molecules held in an optical tweezer. Tethered mutants vastly increased the misfolding of the wt-domain. Furthermore, different mutants induced different misfolded conformations, reflecting the templating expected in prion-like conversion. This work demonstrates a new approach for directly probing conversion mechanisms, with applications for studying potential therapeutics.

Track: Protein Science Addressing Health Disparities

ABS417 | Towards Improved Cell Culture Models of Type 1 Charcot-Marie-Tooth Disease

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Myelin biogenesis requires tightly controlled trafficking of structural proteins from the endoplasmic reticulum to the plasma membrane. Dysregulation of membrane protein trafficking can therefore have dire consequences for myelin formation and integrity, resulting in assorted neuropathies. Peripheral myelin protein 22 (PMP22), which is present in the plasma membrane of myelinating Schwann cells of the peripheral nervous system, is one such mistrafficking-prone structural protein. Mutations in the PMP22 gene can exacerbate mistrafficking and cause dysmyelination or demyelination of peripheral nerves, resulting in the class of neuropathies known as Type 1 Charcot-Marie-Tooth Disease (CMT1). PMP22's mistrafficking severity was recently shown to scale with its expression level, providing some insight into the etiology of disease phenotypes associated with PMP22 gene duplications and deletions. However, current cell culture models used to study PMP22 mistrafficking overexpress the protein constitutively, making further investigation into expression level dependencies of CMT1A pathophysiology difficult. Enabling tighter control of PMP22 expression in cell culture models would ease recapitulation of CMT1 phenotypes at physiologically relevant expression levels. To this end, we are developing HEK 293 cell and rat Schwann cell (RSC) populations with genomically integrated single copies of the PMP22 gene under control of a Tet-On system. So far, doxycycline inducible PMP22 expression has been demonstrated in both HEK 293 and RSC lines by Western blot and immunofluorescence microscopy. Furthermore, a tunable range of expression levels has been demonstrated in clonal isolates of the HEK 293 cells by varying the inducer concentration. Further refinement of these systems should ease studying CMT1 at physiologically relevant levels of PMP22 expression.

Track: High Throughput Protein Science

ABS419 | A Directed Evolution Platform in Bacteriophage T4

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Directed evolution has resulted in biomolecules with enhanced properties but is time consuming and labor intensive to carryout, with even one iteration of gene diversification and screening often taking multiple days. Here, we report the development of a bacteriophage T4 platform for performing directed evolution that takes under 30 minutes – the duration of phage lifecycle – for each iteration. DNA replication relies on a plasmid-borne copy of the T4 DNA polymerase or its variants, which can be chosen for each iteration to have mutation rates between 10-3 to 10-8 per base. We discuss the results from applying this platform to improve the function of artificial constructs of the T4 DNA polymerase clamp loader complex. We expect this platform to be a powerful tool in experimentally investigating the effect of mutation rate and population size on exploration of the adaptive landscape.

Track: Protein Phase Separation in Biomolecular Condensates

ABS420 | Quantitative Kinetic Analysis of the Role of Phase Separation within the Wnt Signaling Pathway

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Within the Wnt signaling network, the scaffold protein Axin facilitates the phosphorylation of β-catenin by glycogen synthase kinase 3β (GSK3β). This scaffolded kinase-substrate pair is known to undergo liquid-liquid phase separation (LLPS) in-vivo, producing a liquid phase with high concentrations of all three pertinent proteins. While phase separation plays a pivotal role in wnt signaling, the underlying mechanism by which LLPS facilitates pathway activity is poorly understood. To this end, we aim to assess the role that LLPS plays in Wnt signal propagation through quantitative kinetic analysis. Using a minimal, biochemically reconstituted system, we measured GSK3β reaction rates in the presence and absence of phase separation. We observed a modest rate increase in the phosphorylation of β-catenin under phase separation conditions, which was further enhanced by the presence of an auxiliary Wnt scaffold, APC. Additionally, phase separation increases the rate of β-catenin phosphorylation by casein kinase 1α at a phosphosite that significantly increases GSK3β activity. While phase separation has been demonstrated to coordinate multistep reactions and increase signaling specificity in other pathways, in the context of Wnt signaling, it is capable of regulating kinase activity and enhancing phosphorylation rates for Wnt-specific substrates.

Track: Protein Phase Separation in Biomolecular Condensates

ABS421 | Can -wrapin AS69 efficiently inhibit -Synuclein fibril formation under the conditions of phase-separation?

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In Parkinson's disease, neuronal dysfunction is associated with the aggregation of the protein α-Synuclein (αS), which leads to the formation of insoluble amyloid fibrils that accumulate intracellularly in the brain as protein inclusions. Once the formation of amyloid fibrils has begun in a specific part of the brain, the number of aggregates increases rapidly. However, the early events involved in fibril formation remain unclear. Recently, a new trend in explaining the cause of aggregation led to the field of liquid-liquid phase separation (LLPS), which is a thermodynamic process resulting in formation of liquid droplets. The observed phenomenon is that the high local protein concentration in the droplets accelerates the aggregation. Lately, it has been shown that the aggregation of αS in droplets consists of two steps: first, the droplet formation itself, and second, the transition into β-sheet forming fibrils.

A recently developed inhibitor of αS aggregation is the engineered β-wrapin protein AS69. Upon binding, it induces a β-hairpin formation in the monomeric αS, thus, mediating inhibition through structural changes. It has already been shown that AS69 inhibits the aggregation of αS under non-LLPS conditions. In this study, the effect of the inhibitor AS69 on liquid-liquid phase separated αS was investigated. The core question we address is whether AS69 is still able to inhibit the αS fibrillation or to prevent the formation of the droplets. In this work, we are aiming to identify which step AS69 is interacting with. Interestingly, the data suggest that AS69 can separately inhibit the formation of fibrils. In contrast, it shows

no inhibitory effect on αS droplet formation, moreover, AS69 may favour it. The transition from liquid to solid droplets is suppressed in presence of AS69 excess, indicating the prevention of αS fibrillation.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS422 | SWiCAM (sliding window comparative alignment metrics): An open-source program for visualizing differential amino acid enrichments across subsets of homologous protein families

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There are many bioinformatic tools for assessing site-specific identity in protein sequence alignments, but sequence elements that underly global or positionally diffuse traits—such as flexibility or substrate charge steering—are more challenging to discover using site-specific conservation analyses. When selective pressure acts in three-dimensional rather than sequential space, and is rooted in a broad property (such as size) rather than strict identity, traditional conservation-detection tools are only partially effective. Here, we present SWiCAM (sliding window comparative alignment metrics), an open-source Python program that allows users to visualize and quantify the differential enrichment of user-specified amino acid sets across partitioned protein sequence alignments. By dividing alignments into subfamilies, users can compare, for example, clusters of oppositely charged residues, or patterns of amino acids that promote flexibility vs. rigidity, using a sliding window protocol that accounts for positionally diffuse conservation. We have tested our protocol on two families of bacterial enzymes: methyltransferases and topoisomerases. SWiCAM is able to correctly identify known specificity-determining charged residues in methyltransferase subfamilies while also strongly suggesting previously unidentified regions that may contribute to substrate selectivity. In topoisomerase sequences partitioned by extremophilicity, SWiCAM suggests that the commonly observed overall enrichments of glycines in psychrophiles and prolines in thermophiles swap places in regions associated with DNA binding and bending. By allowing the selection of multiple amino acids and visualizing how enrichments change across subfamilies, SWiCAM provides a tool for understanding how subtle shifts in amino acid enrichment can lead to global changes in protein stability and ligand engagement.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS423 | Mutations in Human IMP Dehydrogenase 2 Disrupt Allosteric Regulation and Cause Neurodevelopmental Disorders

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Inosine 5'-monophosphate dehydrogenase (IMPDH) is a highly conserved enzyme that catalyzes the rate-limiting step of de novo guanine nucleotide biosynthesis, converting inosine 5'-monophosphate (IMP) to xanthosine 5'-monophosphate (XMP) and reducing nicotinamide adenine dinucleotide. Because IMP is also a precursor in the de novo synthesis of adenine nucleotides, IMPDH controls a metabolic branchpoint in de novo purine nucleotide biosynthesis and is regulated allosterically by the binding of purine nucleotides and through reversible assembly into filaments. An IMPDH monomer consists of a catalytic domain and a regulatory Bateman domain with three allosteric sites where purine nucleotides bind. These monomers associate through their catalytic domains to form tetramers, and occupancy of the three allosteric sites induces dimerization of tetramers into octamers. GTP binding stabilizes a compressed, catalytically inhibited octamer conformation, while ATP binding stabilizes an extended, catalytically active conformation. In vertebrates, octamers stack to form filaments.

Recently, five mutations in human IMPDH2 were linked to early-onset neurodevelopmental disorders, including dystonia (Zech et al., The Lancet Neurology, 2020). Two additional mutations in the regulatory domain have since been identified by other collaborators—Orly Elpeleg at Hebrew University Medical Center and Alyssa Ritter at Children's Hospital of Philadelphia. All mutations are located near allosteric sites, or in the flexible loop connecting catalytic and regulatory domains. Using electron microscopy (EM) and spectrophotometric assays, we are characterizing the effects of each mutation on the structure and function of IMPDH2 filaments. We have found that six mutations disrupt allosteric inhibition by GTP. However, the structural phenotypes of the mutants vary. We are solving cryo-EM structures of these mutant filaments to describe structural differences at near-atomic resolution. These mutations in IMPDH2 provide new tools to better understand the relationship between allosteric regulation and filament structure. Additionally, characterizing these mutations will allow us to define molecular mechanisms of IMPDH2 dysregulation.

Track: Cellular Tasks

ABS424 | Investigating Allostery and Electron Transfer in Human DNA Primase

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Redox-active [4Fe4S] clusters have been identified in an increasing number of enzymes involved in DNA replication and repair, though the specific roles they fulfill remains ill-defined. In human DNA primase, the redox state of the [4Fe4S] cofactor serves as an on/off "switch" for DNA binding. Because the DNA binding region in primase is located ~25Å from the [4Fe4S] cluster, an allosteric mechanism must exist to relay redox-state information between the two sites. We first investigate how the structure of the cluster changes in response to DNA binding using Fe-K-edge EXAFS spectroscopy and Nuclear Resonance Vibrational Spectroscopy (NRVS). Our EXAFS results show that the average Fe-Fe and Fe-S bond distances do not change in response to DNA binding, despite clear frequency shifts in the complementary NRVS spectra. Together, these observations suggest the substrate-dependent redox behavior of the [4Fe4S] cluster may be attributed to changes in the immediate protein environment rather than a change in cluster geometry. Moreover, the 25Å distance between the [4Fe4S] cluster and DNA binding region precludes direct electron transfer from DNA; therefore, a distinct electron transfer pathway must exist within the protein. Three conserved tyrosines, whose mutation to phenylalanine disrupts primase's electron transport and enzymatic capabilities, have been proposed to serve as the charge transport pathway; however, only direct observation of a tyrosyl radical by EPR spectroscopy can confirm their involvement. Here, we provide a description of our methodology, including our unique instrumentation, and present our current progress towards elucidating the electron transport pathway in human DNA primase.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS425 | Mechanical Unfolding and Refolding of Ankyrin Repeats Studied with Magnetic Tweezers

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Many proteins contain ankyrin repeat domains consisting of tandem repeats of the ankyrin repeat motif of 33 amino acids arranged into a pair of antiparallel helices. The helices from one repeat are connected to the next repeat by a characteristic loop that protrudes perpendicular to the solenoid-like structure assembled by contiguous repeats stacked on top of each other. Ankyrin domains typically mediate protein-protein interactions where they are subjected to mechanical forces. The mechanical and folding properties of native and consensus ankyrin repeat proteins were studied by AFM-based force spectroscopy and were found to exhibit interesting sequential unfolding and sequential refolding against applied forces of approximately 20 pN. This mechanical unfolding and refolding behavior of individual proteins studied under vectorial stress conditions was very different as compared to a simple two-state unfolding/refolding behavior found for ankyrin repeats in traditional bulk biochemical assays. However, the nanomechanical behavior of ankyrin repeats under physiologically relevant forces that are expected to be lower than 10 pN have not been characterized so far. Here, using high-resolution single molecule magnetic tweezer as a force spectroscopy technique together with hetero-covalent attachment chemistry used to form mechanically stable tethers of individual proteins, we examine the nanomechanical behavior of consensus ankyrin repeat protein, NI6C composed of eight ankyrin repeats. Our preliminary results obtained from these measurements show that NI6C may unfold in a single step or in multiple steps under a force-ramp or force clamp conditions. When measured at physiological forces, we find that ankyrin repeats within NI6C exhibit an equilibrium unfolding and refolding reflecting a complex behavior with equilibrium occurring at various forces between 4-9 pN, which is rather an atypical behavior when compared to other proteins, which commonly display unfolding/refolding equilibria in a narrow range of forces, e.g. within 0.5-1 pN.

Track: Protein Science Addressing Health Disparities

ABS426 | Engineering two-in-one antibody of CD19 and CD20 to mitigate tumor antigen escape in CAR-T therapy

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Engineering "two-in-one" antibody of CD19 and CD20 to mitigate tumor antigen escape in CAR-T therapy

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CD19 or CD20 chimeric antigen receptor (CAR) T therapies have produced unprecedented achievements to treat relapsed B cell leukemia and lymphoma. However, a significant number of patients still have therapy-resistant relapses caused by CD19 or CD20 antigen loss. One strategy to mitigate this relapse is to develop dual targeting CARs. While dual targeting CARs with tandem scFv antibody recognition domain have been developed, the reduction of cytolytic efficiency is found related to tandem scFv features. Thus, to develop a recognition domain for dual targeting CAR, we propose to design a "Two-in-One" scFv that can bind to both the CD19 and CD20 receptors for a dual specific CAR recognition domain.

To develop the "two-in-one" antibody for structurally unrelated antigens CD19 and CD20, we design to distribute two paratopes on complementarity-determining regions (CDRs) loops in a single scFv. We are using CDR libraries designed by computational and rational antibody design methods based on Rosetta, rather than using the randomized CDR library. The designed library will then be screened with yeast cell surface display (YSD) to find the most promising antibody candidates. the successful design of "two-in-one" scFvs that efficiently recognize CD19 and CD20 antigens will be applied to CAR-T studies. This "two-in-one" scFv antibody fragment can generate more reliable CD19 and CD20 dual targeting CAR-T therapy and improve treatment of B cell malignancies with antigen loss.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS427 | Structure/function studies of the putative Type 4 pilus tip-associated protein PilY1

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Type 4 pili (T4P) are long thin retractile filaments on many Gram-negative bacteria including the human pathogens Acinetobacter baumannii (Ab) and Neisseria gonorrhoeae (Ng). T4P are critical for cell adhesion, microcolony formation, exoprotein secretion, twitching, and natural transformation. T4P are comprised of many copies of the major pilin protein, plus a small number of minor pilins that prime pilus assembly and are thought to form a complex on the pilus tip. Some T4P have an additional large (~110 kDa) non-pilin protein, PilY1, which is involved in pilus assembly and host cell adhesion. PilY1 family proteins contain two domains: a variable N-terminal domain (NTD) that is critical for cell adhesion, and a conserved beta-propeller fold C-terminal domain (CTD). Cryo-electron tomography imaging suggests that PilY1 is located on the pilus distal to the minor pilin priming complex, held in place by the CTD. To explore PilY1 structure, various constructs were expressed, including the NTD, CTD and full-length PilY1 from Ab and Ng, resulting in only one soluble protein, Ab PilY1-NTD. Crystals were obtained for both the native and selenomethionine-substituted Ab PilY1-NTD, which diffract in the 5-7 Å range. Protein construct and crystal optimization experiments are underway. To explore the localization and function of PilY1, I introduced AviTags into loops in Ng PilY1, guided by an AlphaFold model. These tags will be used to visualize PilY1 within the pilus by immunofluorescence microscopy and immunogold electron microscopy.

Track: Protein Science Addressing Health Disparities

ABS428 | Designed bifunctional proteins for induced degradation of androgen receptor in prostate cancer

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Prostate cancer is the most frequently diagnosed cancer among European men and the third most common cause of cancer-associated deaths in Europe. A hallmark of tumour progression is persistent androgen receptor (AR) signalling, hence the development of AR inhibitors is of profound clinical importance. Most available AR inhibitors compete with endogenous ligands by binding to the ligand-binding domain. Although treatment is initially effective in most patients, prostate cancer inevitably progresses to its therapy-resistant, lethal form known as castration-resistant prostate cancer following AR mutation and constitutive activation. Consequently, novel therapeutic compounds with alternative mechanisms of action are highly sought after.

To target therapy-resistant AR mutants, we design hetero-bifunctional molecules to degrade AR by targeting it to the ubiquitin-proteasome pathway. We graft AR- and E3 ligase-binding moieties onto artificial modular

protein scaffolds to induce AR ubiquitination and degradation. As these degrader molecules bind AR through protein-protein interactions (PPIs), we can effectively target sites distinct from the ligand-binding pocket that are not disrupted by cancer-associated mutations of the latter. The larger binding interfaces of PPIs enable us to target previously untapped domains that cannot be bound with high enough affinity and specificity by small molecules. For the design of the modular binding proteins, we harness known protein-protein interactions and use computational saturation mutagenesis to screen for optimal interaction residues that do not abolish scaffold stability or solubility.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS429 | On the Role of Hydration in DNA Recognition by the ETS Transcription Factor PU.1

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DNA-binding proteins recognize targets by direct readout of bases in the DNA groove as well as indirect readout of sequence-dependent DNA shape. In co-crystallographic structures, water is often observed to participate in both binding modes, but it is unclear whether and how hydration is essential to high-affinity binding. To better understand the structural origins of hydration effects in DNA recognition, we study as model system the ETS family of transcription factors. ETS proteins share a structurally conserved DNA-binding motif while differing in amino acid sequence. High-affinity binding by ETS members such as PU.1 is highly sensitive to osmotic pressure, implying strong involvement of water in DNA recognition, but not other members such as Ets-1, despite comparable affinities for optimal sites under normo-osmotic conditions. To dissect the role of hydration in direct and indirect readout by PU.1, we examined osmotically dependent binding by PU.1/Ets-1 chimeras, in which elements of the DNA-contacting interface of PU.1 were replaced with Ets-1 sequences, and using chemically substituted DNA. PU.1/Ets1 chimeras exhibited variably decreased osmotic sensitivity of binding. The recognition helix H3, which mediates direct readout in the DNA major groove, was strongly sensitive in both affinity (~100-fold) and osmotic dependence to substitution. Similar observations were produced by substituting an H3-contacting guanine with 2-aminopurine or iso-guanine, after controlling for the base-paired pyrimidine. However, substitution with inosine, without chemical perturbation of the major groove, yielded little change in hydration. Elsewhere, chimeric substitution of some adjacent elements resulted in a disproportionate loss of hydration, similar to low-affinity binding, but with minimal change in binding affinity to high-affinity DNA. Thus, hydration is not essential for high-affinity DNA binding by PU.1, nor does the incorporation of hydration water guarantee high binding affinity. We propose that transcription factors such as PU.1 primarily utilize hydration as a mechanism for DNA site selection.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS430 | De Novo Design of Conformationally Dynamic Proteins

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Computational design of novel proteins normally focuses on identifying an amino acid sequence which stabilizes a single conformation. While the resulting stability of proteins designed in this manner is useful for many applications, the structural rigidity of such proteins precludes any functionality facilitated by conformational dynamics often seen in natural proteins.

Here, we are designing "hinging" proteins that can adopt multiple well-defined conformational states. Starting from existing designed helical repeat proteins, we construct potential alternative conformations by splitting the protein and sampling rigid-body movements of the individual domains. We then design sequences that are compatible with both the alternative and the original conformations of the input protein. Finally, we use ab initio structure prediction to assess the fitness of the resulting sequence for the designed conformations and to check for potential off-target states.

With this procedure, we have designed hinge proteins in which the alternative state can bind to another target molecule, such as a helical peptide, a helical bundle, or another hinge protein. By an array of biophysical characterizations including size-exclusion chromatography, fluorescence polarization, Förster resonance energy transfer (FRET), and double electron-electron resonance, we have shown that these proteins directly couple binding and conformational change. This represents a step toward designing de novo conformationally dynamic proteins, which we envision could be used to build

switchable protein assemblies, proteins with allosterically modulated function, or even motor proteins.

Track: Protein Science Addressing Health Disparities

ABS431 | A triheme CcoP in cbb3 oxidase directs electron flow to support O2 or nitrite reduction in Neisseria gonorrhoeae

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Dartmouth College (United States)

Neisseria gonorrhoeae (Ng) infection, when left untreated, can cause a wide array of long-term health effects which are of greater concern in the face of the increasing number of treatment-resistant strains. A better understanding of the mechanisms of virulence is needed to identify new antibiotic targets against this pathogen and allow for treatment innovations. Ng is known to thrive in both aerobic and anaerobic environments by diverting electron flow from the terminal oxidase cbb3 to nitrite reductase under microoxic conditions. The triheme subunit CcoP of cbb3 oxidase has been implicated as a critical electron distribution hub as it serves to deliver electrons to support enzymatic reactions in both aerobic and anaerobic electron-transfer pathways. The mechanisms which partition CcoP-mediated electron load between these two pathways are largely unknown. We are investigating CcoP interactions with its redox partners monoheme cytochrome c2 and diheme cytochrome c4 to develop greater understanding redox properties of these proteins and their interactions. Modeling studies of CcoP, cytochrome c2 and cytochrome c4 have provided insight about their general fold and spectroscopic analyses of these proteins have revealed Met-ligation to the heme iron. Crystals of cytochrome c4 have been obtained to enable further structural characterization of this protein. Electrochemistry studies, together with spectroscopic and structural analyses of protein complexes, will be used to determine which of the three hemes in CcoP interacts with cytochrome c2 and cytochrome c4, to ultimately guide the design of specific inhibitors.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS432 | Defining Split Intein Splice Junction

Carla Madrid, Thuy Nguyen, Ryan Otto, Kimberly Reynolds, Kendra King Frederick
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Split inteins are an intervening polypeptide sequence that excises from a precursor protein, also known as, an extein. Split intein undergoe protein splicing which is an autocatalytic process and considered to be a post-translational
modification. Protein splicing consist of a series of chemical reactions that may be dependent on the amino acids sequence of the splice junction. The splice junction is found at the intein-extein interception and refers to the sequence
of the extein. We have set out to determine what amino acid sequence combination leads to successful/unsuccessful protein splicing . We are developing a sequence library for the N and C terminal splice junction sequences for a
series of split inteins to design new NMR protein labeling techniques.

Track: Protein Science Addressing Health Disparities

ABS433 | Evaluating Sheep Hemoglobins with MD simulations as Animal Models for Sickle Cell Disease

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Wake Forest School of Medicine (United States)

Sickle Cell Disease (SCD) is a widely characterized human genetic disorder arising from a single β2-Glu6Val substitution in tetrameric (α1β1α2β2) hemoglobin that promotes polymerization and subsequent disease, but there still remains limited therapeutic options. Recent mammalian cloning and CRISPR gene editing advances have made possible the precise introduction of mutations in animals, such as sheep (Ovis aries), to create translationally relevant human disease models. To compare human and sheep hemoglobin (87% sequence similarity), we generated high quality SWISS-MODEL sheep hemoglobin structures using the x-ray crystallographic structure of a dimer two deoxyhemoglobin S, the building block for polymerization, for MD simulations. In our 2 μs MD simulations of human normal and sickled Hb (huHbA and huHbS) and its sheep variants (shHbB and shHbS), we observe both huHbS and shHbS to remain stable with low RMSD while huHbA and shHbB had markedly higher and fluctuating RMSD. The center of mass distances between β2-Val6/Val5 and their partner

hemoglobin hydrophobic pockets in huHbS and shHbS remained low, and their interface interaction energies were nearly identical (R2 = 0.97) while their corresponding interactions in huHbA and shHbB were very different (R2 = 0.13). We reproduced previously reported β2-Glu6 solvent interactions in huHbA that were absent in β2-Val6 huHbS, and we observed analogous results for shHbB and shHbS, respectively. Recent works suggest that solvent interactions with β1-Asp73 found in huHbA but not huHbS plays a critical role in the aggregation. Even though shHbB and shHbS contain β1-Asn72 at that position, this role is apparently conserved. All of our MD simulation data is not only consistent with huHbS aggregation and huHbA non-aggregation, it further supports a consistent molecular-level similarity between huHbS and shHbS that suggests that Ovis aries could be a viable SCD animal model candidate.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS434 | Identification of a secondary binding site for acyldepsipeptide fragments within the bacterial Clp protease

Monika Prorok, Karl Schmitz
The University of Delaware (United States)

The increasing prevalence of drug-resistant bacterial infections is widely recognized as an impending public health crisis. As a consequence, there is an urgent clinical need for the development of new antibacterial therapeutics, and a related need to characterize novel drug targets. The Clp proteases, a widely distributed class of ATP-fueled proteolytic machines, have emerged as promising targets in a cross section of important bacterial pathogens. These enzymes degrade cytosolic proteins through the cooperation of an ATP-dependent unfoldase (e.g., ClpX) and the barrel-shaped ClpP peptidase. Multiple classes of compounds that kill bacteria by specifically inhibiting or dysregulating ClpP have been identified. The most widely studied class of these are the acyldepsipeptides (ADEPs), which are derivatives of naturally occurring non-ribosomally encoded peptides produced by some Streptomyces. Structural and biochemical studies have shown that ADEPs bind to the surface of ClpP and dysregulate Clp protease assembly and function. ADEPs typically consist of a complex peptide macrocycle linked to an N-acylphenylalanine substituent. Here, we examine the activity of ADEP fragments lacking the full macrocycle. We find that fragments with partial macrocycles retain considerable ability to bind and dysregulate ClpP. Curiously, some fragments appear to inhibit proteolysis at high concentrations, suggesting an alternate binding site at the peptidase active sites. Further, we report crystal structures of ADEP fragments bound to ClpP, which provide additional evidence for secondary inhibitory effects. Our findings open new possibilities for future ADEP development and provide a new area for ADEP structure optimization targeting an alternate location within the Clp protease.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS435 | The Oligomerization of E.coli and Human Glutamine Synthetase Reveals class specific filament assembly

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university of washington (United States)

Nitrogen fixation and mobilization is controlled through inter-conversion of glutamate to glutamine by glutamine synthetase (GS). GS is highly conserved and converts glutamate and ammonium into glutamine in an ATP-dependent reaction. GS assembles into filaments whose formation is conserved among E. coli, yeast and plants. Studies have shown that enzyme oligomerization into superstructures modulates activity suggesting GS filaments also regulate activity. This study aims to resolve at an atomic level the principles governing GS filament assembly and test the allosteric control of filament formation on GS activity. E. coli and human GS monomers assemble into a dodecamer and decamer ring, respectively, and here we find that these rings stack to form filaments with different assembly contacts. E. coli filaments were observed in the presence of CoCl2. Treatment with EDTA, to sequester divalent metal ions in E. coli GS, disrupts filament formation and quaternary structure, while reintroduction of CoCl2 rescues filament formation. Using cryo-EM, we resolved the filamentous structure of E. coli GS to 2.7 A. This model showed the filament interface is coordinated by cobalt ions and the N-terminal helix (H1) of each monomer making contact with another H1 in the adjacent dodecamer. Human GS filaments readily formed in 50 mM HEPES ph 7, 150 mM NaCl and 10 mM MgCl2, and addition of various combinations of substrates (ATP, Glutamate, NH4Cl) and products (ADP, Glutamine) causes filaments to collapse into decamers, while individual substrate addition did not. Cryo-EM reconstruction of apo human GS filaments produced a model at 2.3 A and suggest filament formation is

coordinated by five disulfide bonds between cysteine residues at position 52 of each monomer. Structural analysis has shown E.coli and human GS filament assembly is coordinated very differently and the structures now provide targeted studies to understand the functional consequences of polymerization.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS436 | Unravelling the fold switching pathway of a metamorphic protein, KaiB using NMR and molecular dynamics simulation

Damini Sood, Adam Antoszewski, Supratim Dey, Tobin Sosnick, Aaron ``` Dinner, Andy LiWang
University of California, Merced (United States)

Metamorphic proteins are a rare class of proteins that are known to switch between multiple folds reversibly under native conditions. KaiB is a unique protein that metamorphizes from an inactive fold (***βαββααβ***) during the day to an active fold (***βαβαββα***) during night. The remarkable change in its structure is facilitated by the interactions of other clock proteins. This unique phenomenon in KaiB is a slow process and it is integral to the functioning of the circadian clock of cyanobacteria. We probed the KaiB fold switching intermediates using native state hydrogen exchange using NMR and molecular dynamics simulation to gain insight into the mechanism of metamorphism. The spectroscopic and computational studies were well corelated and the integrated approach suggested a common pathway where the isomerization of proline residues plays a significant role. We will present our findings on the potential fold switching pathways for KaiB.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS437 | Binding of Hydroxy Steroids to Human Aromatase in Lipid Nanodiscs

Nirupama Sumangala, Sang-Choul Im
Department of Biophysics (United States)

Human Aromatase (CYP19A1) is a membrane-bound enzyme and a target for endocrine therapeutic drugs that are used to treat estrogen-dependent cancers. CYP19A1 catalyzes an irreversible conversion of androgens to estrogens by three sequential steps in a distributive manner – The first two steps are hydroxylation of the 19-methyl group of the androgens generating 19-hydroxy and 19-aldehyde intermediates. The final step is the aromatization of the steroid A-ring with loss of carbon-19 as formic acid. While it is known that the 19-hydroxy steroids are stable and active metabolites that dissociate from the CYP19A1 enzyme, a more complete understanding of hydroxy steroids binding to CYP19A1 in lipid bilayers might inform the development of superior inhibitors. We used phospholipid nanodiscs to stabilize human CYP19A1 in membrane-like environments. Our results suggests that the binding of 19-hydroxyandrostenedione to CYP19A1 in lipid nanodiscs is comparable to liposomes. The titration of 19-hydroxyandrostenedione displayed a relatively limited low spin to high spin shift compared to androstenedione. These experiments demonstrate the feasibility of using versatile and stable phospholipid bilayers to study the intermediate steps of CYP19A1 catalysis, which facilitate further mechanistic studies and discovery of improved inhibitors to treat cancer.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS438 | Targeting conformationally distinct amyloid polymorphs via symmetric de novo protein design

Hailey Wallace
UCSF (United States)

Amyloid fibrils are composed of stacked, misfolded proteins forming polymorphic, insoluble filaments. With the recent advancements in electron microscopy and NMR, we can now view high-resolution structures of amyloid fibrils and their conformational polymorphs, which are notoriously difficult to study due to their identical sequences and insolubility. Amyloid formation is not rare; it is hypothesized that nearly all native proteins can form amyloid fibrils. Because of this, polymorph-specific tools need to be developed to parse these different amyloid conformations and their corresponding functions more efficiently.

Here, we present a computational approach that finds peptide sequences that will bind to distinct amyloid polymorphs. After parsing distinguishable regions from high-resolution structures, we use the DeGrado lab's Qbits algorithm to find backbones that are statistically likely to interact with the distinguishable regions. A translational symmetry technique is applied to these backbones and cross-referenced with the software MASTER. These

backbones are finally designed with flexible-backbone design tools from Rosetta. I will be presenting preliminary results on the computational approach and in vitro validation of our amyloid binding peptides.

Track: Machine Learning in Protein Science

ABS439 | Deep Learning Receptor-Bound Ligand Structures

Nicolas Aziere, Sinisa Todorovic
Oregon State University (United States)

Interactions between molecules are fundamental to all of life's processes, providing a mechanism by which a biomolecule's function can be regulated. Small molecule ligands (endogenous and exogenous) have been shown to be able to activate, inhibit, or modulate the activity of a specific protein receptor to which it binds. Because these ligands can function as probes to study protein function and can also be potential drug candidates if bound to therapeutically interesting target receptors, great efforts have been expended by many research groups toward rational strategies for ligand discovery, particularly through structure-based virtual screening methods. In the present study we use machine and deep learning algorithms to recast the intrinsically three-dimensional (3D) receptor–ligand binding prediction problem.
In our work, we use unbound ligand conformations as input data and predict 3D atom displacements representative of their receptor-bound structures, independent of any receptor information. The ligand is represented as a data structure in which each atom is composed of a set of computed features, and the prediction of atom displacements is accomplished using a Transformer architecture with learned attention weights. Our results not only allow for accurately predicting bound ligand conformations (which can be used to improve subsequent receptor–ligand docking simulations), but can also yield insights into the structural and non-structural determinants of biomolecular recognition and binding.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS441 | Hidden potential of the supporting scaffold as a structural module for plant cystatin engineering

Jonathan Tremblay, Marie-Claire Goulet, Charles Goulet
Universite Laval (Canada)

Different approaches have been proposed to improve the inhibitory properties of plant cystatins towards herbivorous pest digestive Cys proteases, typically involving sequence alterations in the inhibitory loops and/or N-terminal trunk of the protein interacting with specific amino acid residues of the target protease. Here, we assessed whether the loops-supporting frame, or "scaffold", would represent a valuable structural module for cystatin function improvement. Twenty hybrid cystatins were designed in silico, consisting of the N-terminal trunk and two inhibitory loops of a given donor cystatin grafted onto the scaffold of an alternative, recipient cystatin. Synthetic genes for the hybrids were expressed in E. coli, and the resulting proteins assessed for their potency to inhibit model Cys protease papain and the digestive Cys proteases of Colorado potato beetle (Leptinotarsa decemlineata) used as an insect pest model. In line with the occurrence of positively selected amino acids presumably influencing inhibitory activity in the scaffold region of plant cystatins, grafting the N-terminal trunk and inhibitory loops of a given cystatin onto the scaffold of an alternative cystatin generally had an effect on the inhibitory potency of these function-related elements against Cys proteases. For instance, hybrid cystatins including the three structural elements of model tomato cystatin SlCYS8 grafted on the scaffold of cystatins from other plant families showed Ki values altered by up to 3-fold for papain, and inhibitory efficiencies increased by up to 8-fold against L. decemlineata cathepsin L-like proteases, compared to wild-type SlCYS8 bearing the original scaffold. Our data point to a significant influence of the cystatin scaffold on the inhibitory activity of the N-terminal trunk and protease inhibitory loops. They also suggest the potential of this structural element as a module for plant cystatin design to generate functional variability against Cys proteases, including the digestive proteases of herbivorous pests.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS443 | Key structural features in bacterial sialoglycan-binding serine rich repeat adhesins affect breadth of selectivity

Haley Stubbs
Vanderbilt University (United States)

Protein recognition of glycans plays a vital role in mammalian, viral, and bacterial life. For example, bacteria can use glycan binding proteins to adhere to host glycosylations on saliva, platelets, blood plasma, or other host

components. The proteins that facilitate this process are surface expressed proteins called adhesins. Many strains of streptococci contain serine rich repeat adhesins. These proteins have two long portions where serine makes up ~50% of the amino acid content. Within certain serine rich repeat adhesins, a sialic acid binding region (SLBR) is positioned between the two serine repeat regions. The SLBR, so named for its ability to recognize and bind sialylated glycans, is made up of two domains, the Siglec and Unique domains. The Siglec domain directly binds sialylated glycans. Surrounding the binding pocket are three loops that differ significantly in length and sequence. These SLBRs are structurally homologous, but different SLBRs may prefer different glycans. Furthermore, SLBRs vary substantially in their breadth of selectivity. Selectivity profiles of many SLBRs have been characterized by glycan array analysis, but the structural determinants of selectivity for glycan modifications remains unknown. Here, I present a structure of apo SLBRSK1, an adhesin from S. sanguinis strain SK1 that contains two Siglec and Unique domains in tandem. Additionally, I provide structural analysis of SLBRSK1, SLBRHsa from Streptococcus gordonii strain Challis, SLBRSrpA from Streptococcus sanguinis strain SK36, and SLBRGspB from Streptococcus gordonii strain M99 with and without ligand bound. From the available structures I describe a conserved sialic acid recognition motif. I also detail the contributions of each loop surrounding the binding site and show that these are responsible for recognition of specific glycan elaborations.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS444 | Linking Structure and Function Use of PDB data as baseline for in-solution characterization

Emil G.P. Stender, Henrik Jensen
Fida Biosystems (Denmark)

In biophysics, the detection of molecular size changes is a strong, quantitative source of information about the structure of a protein and its complexes. However, correlating a protein's structure to size measures, like Hydrodynamic radius (Rh), requires consideration of structural features of the protein and its assemblies based on either experimental structures or predicted ones by e.g., AlphaFold.
To establish a strong and reliable size baseline, we have developed the Fidabio PDB (Protein Data Bank) Correlator. In the presented work, we use the Fidabio PDB Correlator to predict the in-solution size, Rh, of bovine β-lactoglobulin, human serum albumin, bovine serum albumin and chicken lysozyme. Subsequently, using Fida 1 equipped with a UV fluorescence detector, the Rh of label-free proteins is experimentally determined and compared with the predicted Rh values.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS445 | Capturing The Interplay Of Membrane Lipids And Structural Transitions In Human ABCA7

Le Thi My Le, Le Thi My Le, James R. Thompson, Sepehr Dehghani-Ghahnaviyeh, Shashank Pant, Phuoc Xuan Dang, Takahisa Kanikeyo, Emad Tajkhorshid, Amer Alam
University of Minnesota (United States)

ABCA7, a member of the ABC transporter superfamily, plays an important role in cellular lipid homeostasis, a particularly crucial function in the central nervous system. Dysfunction of ABCA7 is associated with Alzheimer's Disease, although the specifics of substrate interactions and transport mechanism remain poorly understood at the molecular level. Here we present cryo-EM structures of human ABCA7 in 3 distinct conformations at 3.6 – 4.0 Å resolution in lipids or detergent environments. Lipid embedded human ABCA7 in an open state reveals an ordered patch of bilayer lipids traversing the transmembrane domain (TMD), while the nucleotide bound closed state reveals a lipid-free, closed TMD with a small extracellular opening. These structures offer a structural basis for both substrate entry and exit from the ABCA7 TMD and emphasize conserved rigid body movements that determine the associated conformational transitions. Combined with functional analysis and molecular dynamics (MD) simulations, our data provide insights into ABCA7 TMD lipid partitioning, membrane perturbations, and lipid extrusion.

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS446 | Expanding the antibody recognition-based toolkit to map native cell surface protein-protein interactions

Kelly M. O'Leary, Tomasz Slezak, Anthony A. Kossiakoff
University of Chicago (United States)

Intracellular proteins that are translocated to the outer surface of the plasma membrane in a disease-dependent manner represent a novel class of tumor-associated antigen (TAA) with potential to be targeted by anti-cancer therapies. However, the mechanism of translocation, nanometer-scale organization, and molecular relationships formed with "inside-out" TAAs on the cell surface remain poorly understood. Therefore, we aim to develop broadly applicable technology enhancements that will lower the barrier for native protein-protein interaction profiling of the tumor surfaceome. In doing so, high throughput approaches will be established in two main categories. The first category is discovery-based proteomics of cell surface protein-protein interactions without genetic manipulation. We show that an engineered protein G variant (GA1) can serve as a portable module to bridge enzymatic cargo with TAA-targeting synthetic antigen-binding fragments (FabLRT). The "plug and play" GA1-FabLRT platform enables interchangeable coupling of a proximity labeling enzyme to various cellular targets leading to more flexibility and greater throughput than current approaches. The second category is building new tools to enable rapid quantification of nanometer-scale spatial proximity between two cell surface proteins. A synthetic Fab (FabRapC) was engineered to specifically recognize the tripartite complex of rapamycin, FK506-binding protein (FKBP12), and the FKBP12-rapamycin-binding (FRB) domain of mTOR. Here, FabRapC is harnessed as a molecular sensor to create a novel AND-gated recognition-based proximity assay. The concept of the assay is based on coupling FKBP12 to one Fab that targets a cell surface protein and coupling FRB to another Fab that targets a distinct cell surface protein. If the targeted cell surface proteins are located close enough, the spatial proximity of each Fab carrying FKBP12 and FRB, respectively, will enable ternary complex formation and recognition by the reporter FabRapC for fluorescent or colorimetric readout. Together, the methods described here will open new opportunities for characterizing important molecular relationships inhabiting the tumor surfaceome due to their high throughput capability in native cellular environments.

Track: Protein Phase Separation in Biomolecular Condensates

ABS447 | How phase separation is encoded in intrinsically disordered low-complexity domains

Tanja Mittag
St. Jude Children's Research Hospital (United States)

Phase transitions underlie cellular compartmentalization and mediate fundamental biological processes. How they are encoded in the protein sequence is therefore an important question. Here, we use biophysical experiments, theory, and simulations to generate a conceptual stickers-and-spacers framework to understand phase behavior of intrinsically disordered prion-like low-complexity domains (PLCDs) of RNA-binding proteins. Stickers form non-covalent inter- and intramolecular crosslinks, whereas spacers enable or suppress the formation of these crosslinks. We have previously shown that aromatic residues are the stickers in the PLCD of hnRNPA1. Here, we demonstrate that tyrosine is a stronger sticker than phenylalanine and account for interactions of charged residues. Negatively charged residues are solubilizing spacers. Arginines act as stickers through pairwise interactions with aromatic residues, while lysines weaken sticker-sticker interactions. Low or high values of net charge per residue weaken phase separation via mean-field electrostatic effects, while a net charge per residue close to zero minimizes solubility and is optimal for phase separation. We further characterize the function of spacer residues, particularly that of the two most frequent spacer types glycine and serine, to ask whether serine acts as a weak sticker via its side chain. Instead, we find that increasing serine contents decreases the driving force for phase separation in agreement with the higher effective solvation volume of serine vs glycine. Our analytical and coarse-grained models accurately predict PLCD phase behavior. I will also discuss how the stickers-and-spacers framework can be sued to understand material properties of sequences.

Track: Protein Phase Separation in Biomolecular Condensates

ABS448 | The coding potential of pseudogenes: between relics and new beginnings

Marie Brunet
University of Sherbrooke (Canada)

Recent technological advances have revealed pervasive translation throughout the genome. These novel sequences are found in "non-coding" regions or overlapping canonical CDS in a different reading frame.
We developed the first proteogenomics resource endorsing a multi-coding annotation of eukaryotic genomes, OpenProt. OpenProt cumulates experimental evidence for all predicted CDS from an exhaustive transcriptome. Large-scale mining of Ribo-seq and proteomics

(MS) datasets highlighted 4,478 non-canonical CDS in the human genome detected in at least 3 independent Ribo-seq datasets or with at least 2 unique peptides within at least 2 independent MS datasets. About 40% of these novel CDS are from mRNAs, whilst the remaining come from ncRNAs and pseudogenes.

Pseudogenes are widely accepted to be defective copies of protein-coding genes. We identified unique peptides for 9 proteins encoded by different pseudogenes from the GAPDH family within datasets of GAPDH affinity purification. Identification of each protein by MS was validated using synthetic peptides and folding and docking simulations further supported the interaction of each pseudogene-encoded protein with the parental protein GAPDH. Pseudogenes are usually identified as any genomic sequence similar to another gene but that is defective and often without introns. However, these criteria are ill-advised to predict the functionality of a gene or absence thereof. Using data cumulated with OpenProt, we developed CoP3E, a machine learning algorithm to predict the coding nature of pseudogenes. With an accuracy of 90%, our model largely outperforms existing models for ncRNAs. Out of 15,152 pseudogenes annotated in human, 1,717 presented strong experimental evidence in OpenProt (training dataset) and 3,892 were identified with high confidence by CoP3E.

As pseudogenes are annotated as non-coding and non-functional, they are discarded from analyses and absent from protein databases. Our results call for reconsideration of the coding nature of pseudogenes, and their role in the evolution of genomes.

Track: Protein Phase Separation in Biomolecular Condensates

ABS449 | Dissecting biomolecular condensates across stratified epithelial tissues

Felipe G. Quiroz
Georgia Institute of Technology and Emory University (United States)

Intrinsically disordered proteins (IDPs) recently emerged as major drives of intracellular self-assembly. Specifically, through their liquid-liquid phase separation (LLPS), IDPs form biomolecular condensates that appear to orchestrate wide-ranging cellular mechanisms. For example, at the crux of skin barrier formation are enigmatic biomolecular condensates that suddenly disappear as stratifying keratinocytes (KCs) initiate terminal differentiation. Absence of these condensates has long been linked to skin barrier disorders. Using biomolecular engineering approaches and novel LLPS-sensors, my team recently showed that epidermal biomolecular condensates drive the process of skin barrier formation. Dwelling in this differentiation program, in this talk I will demonstrate that filaggrin (FLG) assembles epidermal biomolecular condensates through a canonical process of LLPS. I will show that the LLPS of FLG is governed by its repeat architecture and that disease-associated mutations, which truncate it, potently abolish LLPS. Probing these LLPS dynamics directly in mammalian skin, I will show how endogenous FLG synthesis crowds the intracellular spaces with condensates that are liquid-like, but whose coalescence is restricted by a specialized keratin network. Crucially, I will show evidence that pH-regulated condensate disassembly actuates terminal epidermal differentiation. These findings demonstrate a crucial role for biomolecular condensates in a mammalian tissue and provide unprecedented insights into the poorly understood process of skin barrier formation. The discovery of environmentally sensitive biomolecular condensates in the skin points to new avenues for dissecting the skin barrier and for addressing skin barrier disorders. Finally, focusing on abundant FLG-like IDPs in the hair and tongue, I will end by exploring enigmatic biomolecular condensates across stratified epithelial tissues. Overall, this talk will present skin and its appendages as outstanding models to uncover LLPS-driven mechanisms in tissue biology.

Track: Protein Science Addressing Health Disparities

ABS450 | The Science Behind Protein Cancer Health Disparities

Renee Reams
Florida A&M University (United States)

Blacks are second only to Native Americans in terms of having the highest incidence of renal cancer. Despite this statistics, very few studies have examined whether there are racial/ethnic differences in gene and protein expression. Since higher stage/grade ccRCC is uniformly lethal, it is imperative that candidate genes as well as corresponding protein products be found that can identify patients across various demographics who are at greater risk of progressing to advanced stage/grade, drug-resistant clear cell renal carcinoma as well as that can point to the drivers of ccRCC progression in different demographic groups. Using KIRC (clear cell renal carcinoma) TCGA dataset, we have observed that ARFGAP1,

a GTPase-activating protein, is significantly overexpressed in ccRCC (p<1E-12). Moreover, ARFGAP1 transcripts were significantly elevated in higher stages and grades (p<.001). Higher ARFGAP1 transcripts levels were also correlated with metastasis (p<0.01) and were associated with the more aggressive ccB phenotype (p<0.001). Overexpression was also associated with more unsatisfactory overall patient survival (p<0.0001; HR = 2.1370 (1.7775 to 2.5691) with a mean survival of 2079.677 days for the high expression group compared to 3422.299 days for the low expression, lower Disease Specific Survival [p<0.0001 HR= 2.409 (1.9466 to 2.9813)] and shorter progression free disease time [p<0.0001, HR= 1.8322 (1.4924 to 2.2493). Higher levels of ARFGAP1 transcripts were also associated with the need for additional pharmaceutical therapy (p = 0.022 (t = -2.301)). In addition, we observed that Blacks exhibited higher expression of ARFGAP1 transcripts relative to Whites (p=1.98E-04). Analysis of the CPTAC database revealed ARFGAP1 protein levels are indeed higher in ccRCC patients (p= 5.9 E-10). However, we were unable to ascertain whether ARFGAP1 protein levels were higher in Blacks because CPTAC data only had one sample derived from a Black patient with ccRCC. Taken collectively, our data suggests that higher levels of ARFGAP1 is associated with cancer progression and more aggressive ccRCC subtypes. Moreover, ARFGAP1 behaves in a manner that suggest it may be a possible oncogene and is a ccRCC biomarker. Moreover elevated levels of ARFGAP1 may be associated with lower treatment success. Lastly, our data underscores the need for proteomics studies that analyze protein expression differences of putative oncogenes or tumor suppressor genes and proteins across racial and ethnic demographic groups.

Track: Protein Science Addressing Health Disparities

ABS451 | Probing Human Milk Glycoproteins

Steve Townsend
Vanderbilt University (United States)

Breastfeeding protects the infant against pathogen infection. Major protective macromolecules include human milk oligosaccharides and related glycoproteins that serve as soluble receptor decoys that inhibit pathogen binding to the mucosal cell surface, prebiotic stimulation of gut colonization by favorable microbiota, immunomodulation, and serving as substrates for fermentation in the gut. This discussion will focus on ocmplex human milk glycoproteins that serve as protective agents in infant health and wellness.

Track: Protein Science Addressing Health Disparities

ABS452 | Role of Ancestry in Tumor Biology

Melissa Davis
Weill Cornell Medicine (United States)

The International Center for the Study of Breast Cancer Subtypes (ICSBCS) has produced global oncology work that is the result of nearly two decades of clinical service and research partnerships in Ghana, recently expanding to additional African nations including Uganda, Kenya, Ethiopia, and Nigeria. Pivotal findings of our work from this global "Oncologic Anthropology" approach have uncovered that certain genetic pathways have distinct or altered function in genetically admixed patients of African descent, across the diaspora. Specifically, the Davis lab has produced findings proving that unique genetic signatures and epigenetic mechanisms are associated with African ancestry in both breast and prostate tumors of African and African American (AA) patients, are enriched for mechanisms associated with aggressive tumor progression, including cell signaling and immunological responses, compared to European Americans (EA). Dr. Davis will present findings emerging from the ICSBCS's African-enriched cohort and the New York Genome Center's Polyethnic 1000 projects, which overview several pivotal findings; including: 1- distinct epigenetic gene regulation from altered miRNA, exosome and chromatin remodelling networks2; 2- variation in steroid receptor networks that have different functionality, such as Androgen Receptor3,4 in the Quadruple Negative Breast Cancer subtype; and 3- intrinsically more aggressive TNBC subtypes have distinct immunological composition in AA compared to EA, contributing to survival disparities.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS453 | Structural insights into the recognition of transcription terminators by ProQ/FinO RNA chaperones

Mark Glover
University of Alberta (Canada)

The ProQ/FinO family of RNA binding proteins mediate sRNA-directed gene regulation throughout gram-negative bacteria. To understand the structural basis for RNA recognition by ProQ/FinO proteins, we determined the crystal structure of the ProQ/FinO domain of the Legionella pneumophila DNA uptake regulator, RocC, bound to its primary partner, the transcriptional terminator of the sRNA RocR. The structure reveals specific recognition of the 3' nucleotide of the terminator by a conserved pocket involving a □-turn-□-helix motif, while the hairpin portion of the terminator is recognized by a conserved □-helical N-cap motif. Structure-guided mutagenesis reveals key RNA contact residues that are critical for RocC/RocR to repress the uptake of environmental DNA in L. pneumophila. Structural analysis and RNA binding studies reveal that other ProQ/FinO domains also recognize related transcriptional terminators with different specificities for the length of the 3' ssRNA tail.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS454 | Solving 3D puzzles of biomolecular interactions by integrative modelling.

Alexandre Bonvin
Universiteit Utrecht (Netherlands)

The prediction of the quaternary structure of biomolecular macromolecules is of paramount importance for fundamental understanding of cellular processes and drug design. In the era of integrative structural biology, one way of increasing the accuracy of modelling methods used to predict the structure of biomolecular complexes is to include as much experimental or predictive information as possible in the process.

We have developed for this purpose a versatile information-driven docking approach HADDOCK (https://www.bonvinlab.org/software) available as a web portal from https://wenmr.science.uu.nl . HADDOCK can integrate information derived from biochemical, biophysical or bioinformatics methods to enhance sampling, scoring, or both. The information that can be integrated is quite diverse: Interface restraints from e.g. NMR, mutagenesis experiments, or bioinformatics predictions; shape data from small-angle X-ray scattering and cryo-electron microscopy experiments.

In my talk, I will introduce HADDOCK and illustrate its capabilities with various examples including among others recent work on the inclusion of shape information to drive the modelling process.

Track: Machine Learning in Protein Science

ABS455 | Programming biology for acceleration of biotherapeutics and sustainability

Deborah Marks
Harvard University (United States)

Design and prediction of biotherapeutics with neural machines

There's now an amazing opportunity to develop machine learning methods that can exploit both enormous natural sequence diversity and our ability to synthesize large libraries to design biological sequences for therapeutics and biotechnology. This includes predicting the effects of human genetic variation on disease and drug response, viral evolution and protein design for antibodies and sustainable biomaterials. In this talk I will describe new methods in probabilistic deep learning that are enabling huge acceleration in discovery in these fields. I will focus here on the challenges for designing specific protein functions, biased library design, probabilistic models to generate completely novel functional biotherapeutics, viral forecasting and vaccine design

https://marks.hms.harvard.edu/

https://github.com/debbiemarkslab

Track: Synthetic Biology & Biosensing: Engineering Protein Components

ABS456 | Divide et impera

Barbara Di Ventura
University of Freiburg (Germany)

To build or dissect complex pathways in bacteria and mammalian cells, it is often necessary to resort to at least two plasmids, for instance harboring orthogonal inducible promoters. To allow for the selection of cells carrying two plasmids with a single antibiotic we developed a method based on rationally designed split enzymes and split intein-mediated protein trans-splicing, which we call SiMPl. In the first part of the talk, I will show that SiMPl can be employed to boost the production of valuable compounds by bacteria and to obtain highly pure populations of mammalian cells double positive for two constructs of interest. SiMPl relies on the natural split intein gp41-1. Sometimes, it is convenient to use an intein split at a different position (for instance to have two intein fragments of different sizes or with low binding affinity for each other). To facilitate the engineering of novel split inteins, we developed a web server, Int&in, that users can employ to predict functional splice sites in any intein of choice. In the second part of the

talk, I will explain the pipeline we used to develop the algorithm and will show its performance.

Track: Imaging and Tracking of Proteins in Space and Time

ABS457 | How do oncogenic mutations affect receptor tyrosine kinase dimerization and function?

Adam Smith
University of Akron (United States)

Receptor tyrosine kinases (RTKs) influence many cellular functions including proliferation, migration, and cell-cell contacts. These same functions are misregulated in cancer and thus RTKs are targeted in cancer therapy. One aspect of RTK structure that has been challenging to resolve is how direct, molecular contacts in the plasma membrane regulate their phosphorylation state and enzymatic activity. Measuring these interactions can be especially challenging in the live cell environment, where there is confounding spatial and compositional heterogeneity. In my lab we use pulsed interleaved excitation fluorescence cross-correlation spectroscopy (PIE-FCCS) to measure RTK interactions in live cells because it is sensitive to protein mobility, concentration, and monomer/dimer/oligomer distributions. We have recently identified several interfaces in the EphA2 RTK that regulate its multimerization state and have a direct effect on protein function and cellular behavior. By resolving this structure-function relationship at the level of protein multimerization, we were able to determine how EphA2 can act as an oncogene in some contexts, but a tumor suppressor in other contexts. I will also present recent data about homomeric and heteromeric interactions between ErbB/HER family RTKs and the effect of novel oncogenic mutations.

Track: Imaging and Tracking of Proteins in Space and Time

ABS458 | A PPR protein-based FRET sensor for RNA

Charlie Bond
Lorne Exchange Speaker (Australia)

Pentratricopeptide repeat (PPR) proteins are modular single-stranded RNA-binding proteins. They consist of an alpha-solenoid structure composed of repeating 35 amino acid alpha hairpins, which form an extensive superhelix. Modification of just two amino acids per repeat, according to a derived code, can alter the specificity of a protein to a different target RNA sequence. Studies of wild-type, and consensus PPR proteins demonstrate a conformational change on RNA binding. For example, our crystallographic studies of idealised consensus ("designer") PPR proteins in the presence and absence of RNA show that they have a superhelical structure of 9, or 10 repeats per superhelical turn in the presence or absence of RNA, respectively which results in a contraction of the superhelical pitch from 85 Å to 43 Å. As this change is compatible with the Foerster distance of commonly used FRET fluorophores, we built a protein-based RNA FRET sensor by introducing two cysteine residues at appropriate spacing in the structure, and chemically labelled them with Cy3 and Alexafluor647 fluorophores. Having established a plate-based FRET ssRNA-binding assay which yields comparable dissociation constant to alternative methods, we then built a biotinylated version of the protein which can be immobilised suitably for single molecule FRET measurements. In these experiments we explore the conformational repertoire of populations of individual PPR proteins in the presence of variant RNA sequences thus providing insights into the mechanism and kinetics of RNA-binding by this class of biotechnologically useful proteins.

Track: High Throughput Protein Science

ABS459 | Characterizing virus-host interactions using Multi-omics approaches

Andreas Pichlmair
Technical University of Munich (Germany)

Virus replication and spread depends on intimate interactions between the pathogen and the host. We commonly use affinity proteomics to identify cellular host and restriction factors that physically interact with viral proteins or viral nucleic acids. These findings are complemented with an "effectome" analysis, which encompasses the effects of individual viral protein expression on the cell's proteome and which enables us to functionalize the observed interactions. To gain maximal insights into the cellular processes that are perturbed by virus infections we employ a network-based diffusion approach, which connects the observed interactions to downstream effects through pre-annotated signaling pathways. This analysis not only enables us to better interpret proteomics data but it is also suited to link datasets of diverse natures. As an example I will present a multi-proteomic study of the Varicella Zoster virus (VZV), which is best known for its fulminant but mostly uncomplicated infection in children (chicken pox), but which can also cause

crippling shingles and neuronal disorders in individual patients. This dataset, further enriched with data from an arrayed loss-of-function screen, was integrated with whole exome sequencing data of patients suffering from VZV-associated encephalitis or meningitis. In sum our approach provides in-depth insights in virus-induced perturbation events and to pinpoint host factors that are linked to virus-induced pathology in patients.

Track: Celebrating 100 Antibody Drugs

ABS460 | Antibodies for the prevention and treatment of infectious disease

Peter Kim
Stanford University (United States)

We have introduced a strategy for creating vaccines to elicit antibodies targeting a specific epitope (1). A spike-functionalized ferritin nanoparticle vaccine (2) has been shown to elicit a durable antibody response in mice with neutralizing activity against known variants of concern. Non-neutralizing SARS-CoV-2 antibodies targeting conserved epitopes have been converted into potent broad-spectrum inhibitors (ReconnAbs) through receptor blockade (3). The high-affinity IgG receptor Fc□RI has been shown to potentiate HIV-1 neutralization via antibodies that target the pre-hairpin membrane-fusion intermediate (4, 5).

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Track: Miniproteins: Are There Really That Many Additional Genes That We Have Been Missing?

ABS461 | Discovery and Characterization of Novel Microproteins

Alan Saghatelian
Salk Institute for Biological (United States)

Determining the number and understanding the function of protein-coding genes in the human genome is one of the most important challenges in biology. Using a combination of cutting-edge proteomics and genomics tools we have found thousands of new protein-coding genes in the human genome. These protein-coding genes were initially missed because they encode proteins of less than hundred amino acids (microproteins), revealing a blind spot in gene finding algorithms for small ORFs (smORFs). The functional characterization of several smORFs has led to the discovery of new pathways that regulate diverse cellular processes. These results highlight the existence of a large new class of understudied protein-coding genes that should contain many additional bioactive microproteins.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS462 | An Open Source Workflow for Virtually Screening Billions of Molecules for Binding Affinity to Protein Targets

Travis Wheeler
University of Arizona (United States)

The SARS-CoV2 pandemic has highlighted the importance of efficient and effective methods for identification of therapeutic drugs, and in particular has laid bare the need for methods that allow exploration of the full diversity of synthesizable small molecules. While classical high-throughput screening methods may consider thousands to millions of molecules, virtual screening methods hold the promise of enabling appraisal of billions of candidate molecules, thus expanding the search space while concurrently reducing costs and speeding discovery. Here, I will describe a new screening pipeline, called drugsniffer, that is capable of rapidly exploring drug candidates from a library of billions of molecules, and is designed to support distributed computation on cluster and cloud resources. As an example of performance, our pipeline required ∼40,000 total compute hours to screen for potential drugs targeting three SARS-CoV2 proteins among a library of ∼3.7 billion candidate molecules. I will also discuss directions for improving over the accuracy of current methods at various stages of this analysis pipeline.

Track: Integrating Techniques to Address Challenges in Protein Structural Biology

ABS468 | Structural principles regulating the ubiquitination machinery

Sonja Lorenz
Max Planck Institute (Germany)

The astounding precision by which ubiquitin governs countless cellular processes requires the regulated formation of specific ubiquitin modifications on specific substrates, catalyzed by a cascade of protein-protein interactions. It is thus critical that the protein interfaces formed throughout this cascade are tightly controlled. My talk will focus on the structural principles regulating two classes of ubiquitination enzymes: ubiquitin-conjugating enzymes (E2s) and HECT-type ubiquitin ligases (E3s). Both classes of enzymes transfer ubiquitin to substrates via a thioester-linked intermediate and can thus control specificity for modification sites or linkage types during ubiquitin chain formation.

Track: Celebrating 100 Antibody Drugs

ABS469 | Antibody Drug Lead Isolation and Optimization by Cell Surface Display and Flow Cytometric Screening

K. Dane Wittrup
Massachusetts Institute of Technology (United States)

Cell surface display plays a significant role in in vitro antibody drug discovery, due to several advantages of this form of phenotype-genotype linkage. Some of the central concepts of optimal cell surface display will be reviewed, with a particular emphasis on simple quantitative kinetic, thermodynamic, and statistical analyses.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS470 | Protein plasticity underlies phosphoryl-transfer efficiency and flow directionality in bacterial signaling

Alejandro Buschiazzo
LAPS (Brasil)

Bacteria use two-component systems (TCS) to sense environmental/intra-cellular signals and respond adaptively. TCS proteins act as switching devices, not only as on/off toggles, but also as modulators, shaping disparate output effects according to input information. Almost ubiquitous to all prokaryotes, TCSs catalyze a rather unique type of chemistry: the same phosphoryl group is transferred sequentially, in tandem, first auto-catalytically from ATP to a histidine on the HK itself, and then from the P∼HK to an aspartate on the RR partner component1. It is well known that most HKs, when inactive as kinases, catalyze the specific dephosphorylation of their cognate P∼RR, a paradoxical activity with a relevant physiological role2. However, the molecular means by which TCSs ensure proper efficiency, minimizing phosphorylation/dephosphorylation futile cycles, is not known. Structural studies of the DesK:DesR TCS, which senses cold shock in B. subtilis, uncovered a malleable catalytic site that assembles with concerted contributions from both proteins, blurring the distinction between enzyme and substrate3. By combining X-ray crystallography, QM/MM calculations and integrative kinetic modeling, we have recently uncovered the molecular workings that modulate phosphoryl-flow directionality, why HKs cannot act simultaneously as phosphotransferase and phosphatase, and how the HK's intrinsic conformational equilibrium makes the phosphotransferase state unlikely in the absence of histidine phosphorylation, minimizing back-transfer in irreversible systems4. These mechanisms showcase the key role that protein plasticity and structure-encoded allostery play in signaling proteins, to store and transmit information efficiently.

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Track :

ABS471 | Dynamics of Proteins discovered by time-resolved studies with X-ray Free Electron Lasers

Petra Fromme
Arizona State University (United States)

X-ray Free Electron Lasers (XFELs) have opened a new avenue for structural discovery of the function and dynamics of biomolecules. Processes in biology are highly dynamic and the study of their dynamics is one of the grand challenges of Structural Biology as most structures determined so far only provide a static picture of the molecule. Serial Femtosecond Crystallography (SFX) provides a novel concept for structure determination, where X-ray diffraction "snapshots" are collected from a fully hydrated stream of nanocrystals, using femtosecond pulses from high energy X-ray free-electron lasers (XFELs) [1-4]. The XFEL pulses are so strong that they destroy any solid material, but a femtosecond is so short (1 fs =10 -15s) that X-ray damage is diminished and diffraction from the crystals is observed before destruction takes effect [3]. Structural Biology with X-ray Free

electron lasers allows data collection at near physiological conditions at room temperature [5-13] thereby opening new avenues for the study of light-driven systems in pump probe experiments [7-12] as well as the study of medical important proteins that could enhance structure-based drug design with SFX studies [5,13]. The talk will give an overview of XFEL studies on medical important proteins, including the first XFEL studies on an important enzyme from SARS-CoV2 as well as reporting on our most recent time-resolved studies on light-driven systems including Photosystem I and II.

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Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS474 | From Genes to Lead Molecules: a workflow tuned for drugging challenging therapeutic targets using Fragment based Drug Discovery

Dipen Shah, Masakazu Kobayashi, Willem-Jan Waterreus, Marta Carneiro, Johan Veerman , Gregg Siegal
ZoBio BV (Netherlands)

There are many increasingly popular pharmaceutical targets with many indications. With several different target families with different specificities, it can be difficult to develop small molecule inhibitors. At ZoBio we have developed a package of biophysical technologies designed to enhance the robustness of Fragment-Based Drug Discovery (FBDD). This has guided hit-to lead campaigns across multiple target families and led to the discovery ligand efficient, non-covalent inhibitors. Our technologies go from primary hit identification, confirmation, and validation of binding site -> high resolution 3D structures -> Medicinal chemistry. FBDD uses small (< 300 Da) "drug fragments" as starting points and has been used to develop several ligand efficient, potent and specific inhibitors. We have applied our FBDD approach to multiple target families involved in many therapeutic areas, including neurodegeneration, oncology, viral targets to name a few. The challenges of preparing high quality recombinant proteins have been overcome using creative protein engineering ideas and a workflow capable of screening up to many protein variants to select the most amenable constructs with optimal solution conditions for biophysical studies. Primary fragment hits against a target are discovered using a combination of our proprietary NMR based-TINS® and SPR fragment screening technologies that are also able to rapidly assess the ligandability. By incorporating isotopic labels in the target protein, we can efficiently validate primary hits from fragment screens and rapidly determine the binding site of the ligand using a package of NMR techniques. We use both NMR and X-ray crystallography to rapidly determine high resolution structures of the complex which guides medicinal chemistry and structure-based drug design efforts. The synergy provided by orthogonal techniques, has allowed access to a greater understanding of the protein target and protein-inhibitor interactions accelerating drug discovery campaigns.

Track: Seeing 3D Structures in Cells: Cryo-electron Tomography Blazes the Trail

ABS475 | Structural studies of viruses by correlative cryo-microscopy

Elizabeth Wright
University of Wisconsin-Madison (United States)

Respiratory Syncytial Virus (RSV) is a pleomorphic, enveloped, negative-sense, single-stranded RNA virus. The RSV genome contains 10 open reading frames, encoding nine structural proteins and two non-structural proteins. The surface exposed attachment glycoprotein (G), fusion glycoprotein (F), and small hydrophobic protein (SH) are embedded in the viral membrane. The matrix protein (M), which drives virion formation and elongation, lines the interior of the viral membrane. The genomic RNA is encapsidated in the ribonucleoprotein complex (RNP) that is present in the interior of virions.

Cryo-ET studies have shown that the morphology of individual virions does vary, with the majority of virions having an average diameter of ~130 nm and a length that ranges from ~500 nm to over 10 μm [1, 2]. The general arrangement of structural proteins within the virion is known, however, the molecular organization of M and other structural proteins has remained elusive. We will discuss developments with correlative microscopy, whole-cell cryo-electron tomography (cryo-ET), multi-shot montage tomography [3], and sub-tomogram averaging and their application to structural studies of RSV. We will show that RSV M is arranged in a packed helical-like lattice of M-dimers ordered preferentially to the viral long axis. Sub-tomogram averages including F and M suggest that the position of F is correlated with the underlying M lattice.

Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS476 | In Search of New Antimicrobial Classes Against Drug-Resistant Pathogens

Sergio Hassan
NIH (United States)

A systematic approach to searching for novel antimicrobial classes will be described. The core of the modular workflow is a structural-biology processing unit that takes data at the organism and network levels (omics) and produces pharmacophores for complex molecules expected to have high affinity, site-specificity, and protein and organism selectivity. The intended applications are macrocyclic/branched compounds, ultrasmall nanoparticles, and nanobodies. These emerging agents bridge the gap between traditional small molecules and antibodies while combining the best attributes of both. The low-throughput algorithm integrates recent advances in data science, forcefield developments, and adaptive multiscale simulations, speeding up computation while avoiding the use of empirical scoring functions. Central to the method is the definition of a reduced set of physicochemical features (metrics) known to determine protein function and already exploited as therapeutic strategies. A basic hypothesis is that perturbations of one or more of these features in a protein will alter its function, and simultaneous perturbations of multiple proteins in one or more networks will be detrimental to the microorganism's survival. This multidrug, multitarget approach could enable low dosages of each drug, minimizing the chances of resistance and toxicity. I will illustrate the pipeline from networks to pharmacophores in a few serious-threat pathogens, including viruses, bacteria, and fungi and show how cyclic peptides targeting non-druggable protein surfaces can be inverse-designed in practice.

Track: Synthetic Biology & Biosensing: Engineering Protein Components for Cellular Tasks

ABS477 | Towards the next generation of genetically encoded fluorescent indicators for neuromodulators

R. R. Dalangin, A. Pal, J. Roshgadol, J. Sun, J. A. Chouinard, S. Kulhavy, Y. Hua, J. R. Wickens, L. Tian
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Serotonin (5-HT) and dopamine (DA) are two of the most important neurotransmitters, modulating almost every neuropsychological function including mood reglation, learning and memory, reward seeking, and motor function.1-4 Consequently, the serotonergic and dopaminergic systems are major targets for treating neurological disorders, such as anxiety and depression.5-7 Despite advances in our understanding of the anatomic pathways and functional significance of 5-HT and DA signalling, there are still significant gaps in our knowledge regarding the mechanisms of these systems and how aberrations in 5-HT and DA signalling contribute to these disorders. To address these gaps in knowledge, we have developed genetically encoded fluorescent protein sensors, based on either bacterial periplasmic binding proteins (PBPs) or G protein-coupled receptors (GPCRs), which have emerged as powerful tools for studying neurotransmitter dynamics due to their specificity and high spatiotemporal resolution.8-11 GPCRs as a scaffold are attractive for sensor design because they bind a large diversity of molecules and lead to high affinity indicators.12 On the other hand, PBP-based sensors are orthogonal to neurons, offer large changes in fluorescence in response to ligand binding and can be targeted to arbitrary cellular compartments.12 Here, we present our efforts to engineer the next generations of dLight, a GPCR-based indicator for DA, and iSeroSnFR, a PBP-based indicator for 5-HT. Our new dLight sensors maintain the high affinities found in the dLight1 series but show improved brightness and dynamic range, enabling robust detection of sparse DA transients in awake animals. We also present iSeroSnFR2.0, which has higher affinity and shows more sensitivity at detecting 5-HT transients with single-trial resolution in various brain regions in mice. We anticipate that these optimized indicators will be valuable additions

to the toolbox of genetically encoded indicators for studying neuromodulator dynamics.

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ABS478 | What do we understand about how enzymes work, and why don't we understand the rest?

Daniel Hershlag
Stanford University (United States)

Structure-function studies have been the backbone of biochemistry for decades, with extraordinary success. Now, with over 105 protein structures, we can predict the reaction and reaction mechanism in nearly all active sites, and, most recently, we can predict the structure of nearly all proteins from sequence alone. Nevertheless, we cannot predict function, in terms of reaction rates, and we cannot design new enzymes that rival those from nature. These limitations arise from experimental and conceptual limitations of the "structure–function" paradigm, limitations that can now be overcome through a shift to an "ensemble–function" paradigm.

Structure is of great value, but all molecules, and especially proteins, exist as an ensemble of states defined by the molecule's energy landscape. As different positions on this landscape provide different ligand affinities and reaction probabilities, knowledge of the ensemble of conformational states is needed to relate structure to function, to understand catalysis and specificity, and to design highly-proficient enzymes.

Function involves the entire protein, yet traditional site-directed mutagenesis only quantifies effects at a small subset of positions, and deep-mutational scans return qualitative and incomplete information. HT-MEK (High-throughput Microfluidic Enhanced Kinetics) was developed in collaboration with Polly Fordyce (Stanford) to overcome these limitations, bringing the rigor of traditional biochemistry to 1000s of enzymes in parallel and in days rather than years.

I will describe early examples of ensemble–function studies that have allowed us to test models that cannot be distinguished from static structures alone and to uncover new mechanisms of catalysis that appear to common across many enzymes. Finally, I will address why limitations tend to persist in scientific fields and how individuals can more effectively identify and address these needs and make novel contributions to science.