PROTEIN SCIENCE

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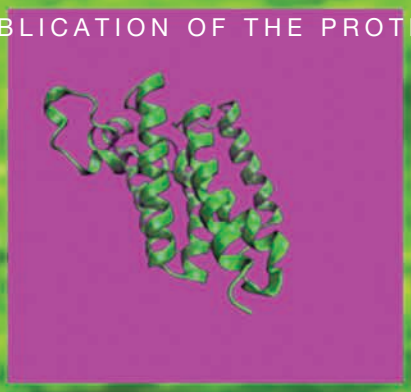
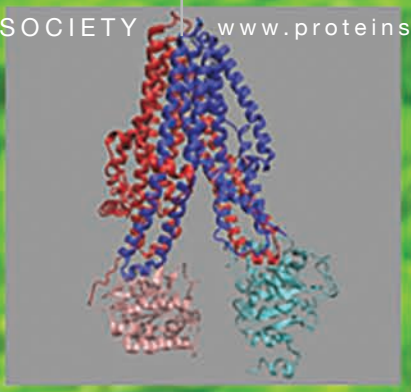

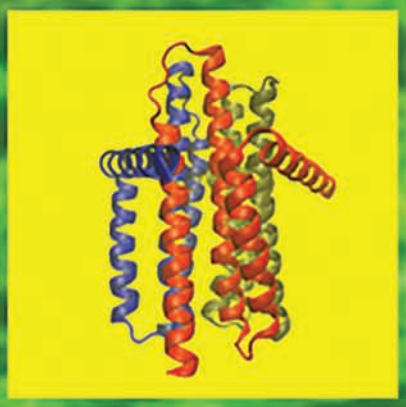






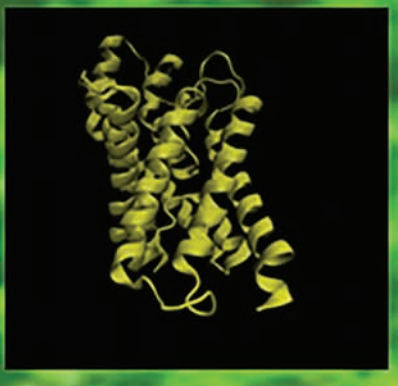


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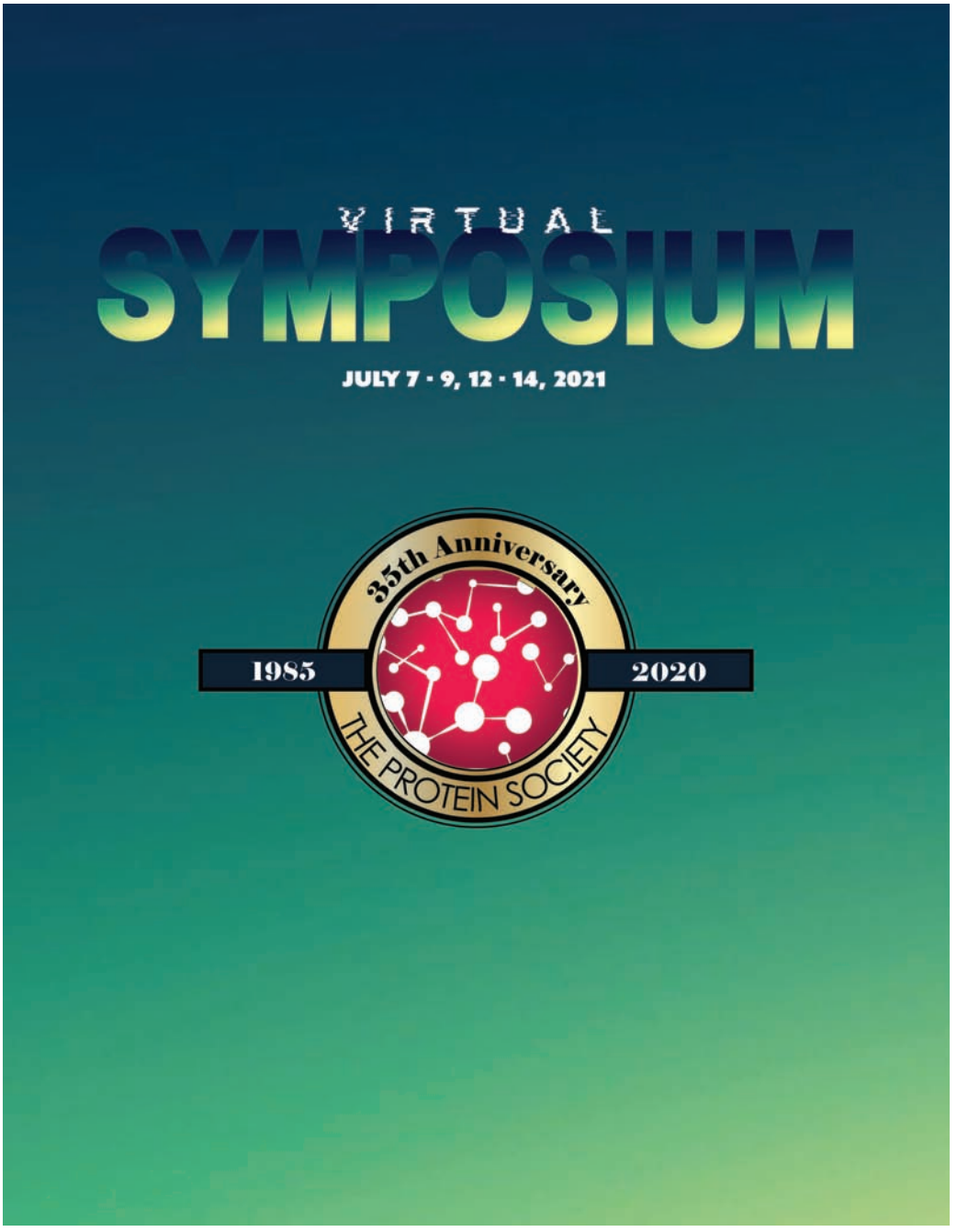

Award Winners and Abstracts of the 35th Anniversary Symposium of The Protein Society, July 7-14, 2021 | Virtual

President's Note to The 35th Anniversary Symposium of The Protein Society

Dear Colleagues and Readers of *Protein Science*,

The 35th Anniversary Symposium of The Protein Society was held in virtual format from July 7-14, 2021. The Society's mission was to provide an affordable meeting that delivered exceptional science, and connected protein scientists all over the world. I laud the leaders, sponsors, staff and partners of the Society who did a wonderful job figuring out how to successfully configure 6 days of programming in an on-line format. Many good choices were made. Particular credit goes to Society staff members Raluca Cadar, Charney Robinson-Williams, and Shannan Cunniffe! Also awesome was the work of the energetic and dedicated Program Planning Committee—Jeanne Hardy (chair), Gabe Lander (co-chair), Charlotte Deane, and Woody Sherman. A sure sign of a great meeting is one's sense of frustration that interesting talks are being missed because they are double-booked with great talks in a parallel session. There were lots of these! A number of TPS committees also made major contributions to the programming of the meeting—Diversity, Equity, and Inclusion (chaired by Bil Clemons), Networking and Career Discussion Tables (Mary Munson), Awards (Giovanna Ghirlanda), Education (Matt Gage), and Abstracts (Chris Snow). Thanks to all who contributed to the meeting. The Society remains committed to providing grants in support of participation in the annual meeting, and we made more than 150 awards this year, including almost 100 Anniversary Award winners, 37 Diversity Awards and the 30 Poster Awards winners. Finally, thanks also goes to the Protein Society Award Winners, speakers (including 30+ contributed talks), and to the over 700 who attended the meeting.

In this special issue of *Protein Science* it is our pleasure to present the over 400 abstracts for the posters and other presentations made at this meeting. This body of abstracts is a tribute to the fact that a great many labs were able (and continue) to safely conduct world-class research in the midst of a global pandemic. It has, of course, not escaped our notice that protein science has made some pretty useful contributions to society in combatting SARS coronavirus-2.

The current plan is for us to convene in San Francisco next summer, July 7-10, 2022. We look forward to your abstract submissions and award applications in 2022. We would love to see you there and hope you will pencil this into your calendars!

With Warm and Respectful Regards,

Charles R. Sanders

Chuck Sanders, Vanderbilt University School of Medicine-Basic Sciences
President of The Protein Society

PROGRAM PLANNING COMMITTEE

With diverse expertise and a breadth of experience that spans the full spectrum of disciplines, methodologies, technologies, and fields involved in the study of protein structure, function and design, the all-volunteer 35th Anniversary Symposium Program Planning Committee members are:

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Gabriel Lander, Ph.D. (Co-Chair)
Scripps Research

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Giovanna Ghirlanda, Ph.D. (Chair)
Arizona State University

CAREER DEVELOPMENT COMMITTEE

The mission of the Career Development Committee is to enhance networking between early-career scientists and established protein researchers in academia, government and industry. One of the most significant contributions to the meeting has been the introduction of Young Investigator talks to the sessions (selected from submitted abstracts), which provide early-career researchers the chance to give

talks as part of the scientific program. Each year, the Career Development Committee hosts career panel discussions on various topics, open to all attendees at the Annual Symposium.

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University of Waterloo

Heather Pinkett, Ph.D.
Northwestern University

Charney Robinson-Williams, Ph.D.
The Protein Society

EDUCATION COMMITTEE

The Education Committee strives to facilitate discussions about education at the undergraduate and graduate levels in protein science. They believe protein science is a vital component of curricula in chemistry, biology, medicine, and the disciplines that bridge these fields.

The aim of the Committee is to provide forums for discussion and debate about protein science through integral programmed elements at Society meetings, both national and international, and through web-based media. In addition to discussions around curricula, they also hope to provide opportunities for conversation and mentorship of practitioners of protein science through the development and training of their careers. The Education Committee hosts an Educator's Workshop and Undergraduate Research Session, at the Annual Symposium.

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Ohio State University

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The Protein Society

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The Membership Committee is involved with developing plans to attract new members and retain existing members. At the Annual Symposium, the Membership Committee hosts the New Member Welcome Breakfast/Business Meeting, giving members an opportunity to speak with Society leadership.

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Grant Walkup, Ph.D.
Agios Pharmaceuticals

Margaret Stratton, Ph.D.
UMass Amherst

Robert Fairman, Ph.D.
Haverford College

James Fraser, Ph.D.
University of California San Francisco

Cesar Ramirez Sarmiento, Ph.D.
Pontificia Universidad Catolica de Chile

Raluca Cadar
The Protein Society

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Lisa Jones, Ph.D.
University of Maryland

Ho Leung Ng, Ph.D.
Kansas State University

Francesca Massi, Ph.D.
University of Massachusetts Medical Center

Joanna Slusky, Ph.D.
University of Kansas

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PRESENTS...

Diversify Protein Science

*My favorite scientific meetings, committees, and events are the ones where I meet new people with **novel ideas** and perspectives. We all find it hard to include people whom we don't already know, but now **The Protein Society** can help. **Diversify Protein Science** is a resource to identify individuals from **diverse** backgrounds who can **enrich** our understanding as scientists and as human beings. Look up people on our list. Invite them, interview them, listen to them, and **engage** with them. Make some new friends! We all have much to learn.*

- Amy Keating, President-Past
The Protein Society

www.diversifyproteinscience.org

2021 PROTEIN SOCIETY DIVERSITY AWARD WINNERS

Attendance at our symposia presents a valuable opportunity for undergraduate students, post-baccalaureate students/research assistants, graduate students, postdocs, and junior faculty from minority-serving institutions. The Protein Society is committed to supporting the participation of junior scientists from diverse backgrounds and individuals who work to promote diversity, equity, and inclusion in our field. The newly introduced Diversity Awards include 2 years of society membership, a 2021 symposium registration waiver, and abstract submission fee reimbursement or waiver. The Diversity Awards are partially sponsored by Malvern Panalytical, MiTeGen, and Refeyn. Award selections are made by the Diversity, Equity, and Inclusion Committee.

Sara Aljoudi, University of Windsor
Paul Ikujuni Ayotunde, University of Kansas
Ana Chang-Gonzalez, Texas A&M University
Charnice Hoegnifioh, Yale University
Clarissa Durie, University of Michigan
Patterson Dayna, The Pennsylvania State University
Harmeen Deol, University of Waterloo
Rodrigo Alonso Ochoa Deossa, University of Antioquia
Sunanda Dey, Michigan State University
Josephine Esposto, Trent University
Gabriel Fuente, The Scripps Research Institute
Jose Guerra, Stony Brook University
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Valeria Guzman Luna, University of Wisconsin
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Rachel Hutchinson, University of Wisconsin-Madison
Jean Badroos, Caltech
Sebastian Kenny, Purdue University
Hannah Kurka Margolis, NIH and MIT
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Meranda Masse, University of Wisconsin-Madison
Nina Moore, The Scripps Research Institute
Nipuna Weerasinghe, University of Arizona
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Alhadid Yazan, University of California, Los Angeles
Perry Zion, Yale University

2021 PROTEIN SOCIETY ANNIVERSARY AWARD WINNERS

Under the strong belief that attendance at our Symposia presents an invaluable opportunity for future protein scientists, TPS is committed to making it possible for young scientists to participate and benefit from our Annual Meeting. The Anniversary Award is sponsored by The Protein Society in honor of Dr. Wold. The Protein Science Anniversary Award is sponsored by Wiley-Blackwell. Award selections are made by the Education Committee.

Soumya Aggarwal, Jawaharlal Nehru University
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Francesca Anson, UMASS Amherst
John Bedford, Old Dominion University
Ankan Bhadra, Washington University School of Medicine in Saint Louis
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Stephanie Breunig, California Institute of Technology
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2021 PROTEIN SOCIETY AWARD WINNERS

2021 Carl Brändén Award Winners: Sheila Jaswal, Ph.D., Amherst College

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

2021 Christian B. Anfinsen Award Winner: Petra Fromme, Ph.D., Arizona State University

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

2021 Dorothy Crowfoot Hodgkin Award Winner: Janet Smith, Ph.D., University of Michigan

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

2021 Emil Thomas Kaiser Award Winner: Lei Wang, Ph.D., University of California, San Francisco

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

2021 Hans Neurath Award Winner: Toshiya Endo, Ph.D., Kyoto Sangyo University, and Amy Rosenzweig, Ph.D., Northwestern University

Hans Neurath played an integral role in the early life of the Society, as a founding member and later—at age 81—as founding editor of Protein Science. His contributions to the early success of the Society were surpassed only by his larger contributions to the field of biochemistry and our early understanding of proteins. Reflective of his prolific contributions to the understanding of the physical chemistry of proteins, The Hans Neurath Award, sponsored by the Hans Neurath Foundation, seeks to honor individuals who have made a recent contribution of exceptional merit to basic protein research.

2021 Stein & Moore Award Winner: David Agard, Ph.D., University of California, San Francisco

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

2021 *Protein Science* Young Investigator Award Winner: Bruno Correia, Ph.D., EPFL

The *Protein Science* Young Investigator Award, formerly known as The Irving Sigal Young Investigator Award, recognizes a scientist in the first 8 years of an independent career who has made an important contribution to the study of proteins.

ABSTRACTS

Abstract Topics

1 – Amyloid and Aggregation
2 – Bioinformatics
3 – Chaperones
4 – Chemical Biology
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7 – Dynamics and Allostery
8 – Enzymology
9 – Evolution
10 – Folding
11 – Intrinsically Disordered Proteins
12 – Membrane Proteins
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14 – Motors & Machines
15 – Peptides
16 – Protein Interactions and Assemblies
17 – Proteins in Cells
18 – Proteomics
19 – Proteostasis and Quality Control
20 – Single Molecule Studies
21 – Structure (X-Ray/NMR/EM)
22 – Synthetic Biology
23 – Systems Biology
24 – Therapeutics and Antibodies
25 – Transcription/Translation/Post-Translational Modifications

DOI: 10.1002/pro.4191

THE PROTEIN SOCIETY WILEY

Track: Structure (X-Ray/NMR/EM)
Session: Protein Evolution, Design and Selection

ABS# 008 | A second specificity-determining loop in Class A sortases: Biochemical characterization of natural sequence variation in chimeric SrtA enzymes

Jeanine Amacher[1], Izzi Piper[1], Sarah Struyvenberg[1], Alex Johnson[1], Melody Gao[1], Hanna Kodama[1], Justin Svendsen[1], Jordan Valgardson[2], Kelli Hvorecny[3], John Antos[1]
[1]*Western Washington University,* [2]*Stanford University,* [3]*University of Washington (WA, United States)*

Sortases are cysteine transpeptidases that gram-positive bacteria use to covalently attach proteins to their cell wall in order to assemble pili, display virulence factors, or for other uses. The ability to cleave a signal sequence and subsequently attach two peptides via a covalent bond make sortases an attractive tool for protein engineering efforts. However, sortase A from Staphylococcus aureus (SaSrtA), the gold standard for in vitro sortase-mediated ligation experiments, is incredibly selective, recognizing the pentapeptide LPXTG motif. In contrast, SrtA from Streptococcus pneumoniae (SpSrtA) is more promiscuous, recognizing 10 of the 20 amino acids at the P1' position (or the Gly in LPXTG), but its activity is approximately one-third that of SaSrtA, measured using a FRET-based assay. Therefore, we sought to use natural sequence variation, biochemistry, and structural biology to investigate this difference. Principal component analysis revealed that the largest degree of sequence variation amongst sortase class A enzymes is in 3 conserved loops near the protein active site. Previous work revealed that the beta6-beta7 loop affects selectivity at the N-terminal residues of the target sequence; therefore, we focused our work on the beta7-beta8 loop, which directly interacts with the P1' position. We engineered over 20 variant enzymes, using SpSrtA and Class A sortases from other Streptococcus species (S. pyogenes and S. agalactiae) as our scaffolds. Our biochemical data in combination with a number of crystal structures of peptide-bound and loop-swapped variants and mutagenesis studies, reveal conserved selectivity determinants mediated by beta7-beta8 loop residues in these enzymes. Critically, we find that while some of our variants are enzymatically dead, others are as active as SaSrtA while maintaining the promiscuity of SpSrtA. Taken together, we greatly expand our understanding of Class A sortase target recognition, providing exciting new tools for use in sortase-mediated protein engineering.

Track: Chemical Biology
Session: Designer Proteins Through Genetic Code Expansion

ABS# 012 | Chemical Epigenetics Approaches to Probe Molecular Recognition Events in Transcription: Progress Towards BPTF Inhibitor Development

William Pomerantz[1]
[1]*University of Minnesota (MN, United States)*

Inhibitor discovery for protein-protein interactions has proven difficult due to the large protein surface areas and dynamic interfaces. To address this challenge, structural biology approaches for characterizing native protein-protein interactions (PPIs) and ligand discovery have had a significant impact. Inspired by the protein-observed NMR approach using 1H-15N-HSQC NMR, we have applied a complementary protein-observed 19F NMR (PrOF NMR) approach using 19F-labeled side-chains that are enriched at protein-protein-interaction interfaces. Here we describe our efforts targeting the Bromodomain PHD Finger Transcription Factor, BPTF, an emerging protumorigenic factor. Using a PrOF NMR screen, we reported the first selective inhibitor of the BPTF bromodomain, AU1. This molecule demonstrated the importance of the bromodomain for mediating transcription as well as serving as a mechanism for reducing c-Myc occupancy on chromatin. Given the challenges of further improving affinity and metabolic stability of AU1 for in vivo studies, we now report several new inhibitors with increased potency for BPTF. We further describe our medicinal chemistry efforts using structure-based design to improve affinity and selectivity, and highlight the first small molecule cocrystal structures to help explain the origins of selectivity. Finally,

preliminary cellular data in a breast cancer model system demonstrating synergistic effects of our inhibitors with chemotherapeutic drugs is shown. These new inhibitors are envisioned to serve as useful chemical probes of BPTF function in normal and pathophysiology.

Track: Protein Interactions and Assemblies
Session: Protein Structures Through the Lens of Machine Learning

ABS# 013 | Structure of SARS-CoV-2 Nsp1/5′-UTR Complex and Implications for Potential Therapeutics, Vaccine & Virulence

Naveen Vankadari[1]
[1]*Monash University (VIC, Australia)*

The SARS-CoV-2 is the cause of the ongoing Coronavirus disease 19 (COVID-19) pandemic around the world causing pneumonia and lower respiratory tract infections. Understanding the SARS-CoV-2 pathogenicity and mechanism of action, it is essential to depict the full repertoire of expressed viral proteins. Recent biological studies have highlighted the leader protein Nsp1 of SARS-CoV-2 importance in shutting down the host protein production. Besides it still enigmatic how Nsp1 regulate translation. Here we report the novel structure of Nsp1 from SARS-CoV-2 in complex with SL1 region of 5′UTR of SARS-CoV-2 and its factual interaction is corroborated with enzyme kinetics and experimental binding affinity studies. The studies also address how the leader protein Nsp1 of SARS-CoV-2 recognises its self RNA towards the translational regulation by further recruitment of 40S ribosome. With the aid of molecular dynamics and simulations, we also modelled the real-time stability and functional dynamics of the Nsp1/SL1 complex. The studies also report the potential inhibitors and their mode of action to block the viral protein/RNA complex formation. This built the fundamental in understanding the mechanism of the first viral protein synthesised in the human cell to regulate the translation of self and host. Understanding the structure and mechanism of SARS-CoV-2 Nsp1& its interplay with the viral RNA and ribosome will open the arena of exploring the development of live attenuated vaccines and effective therapeutic targets for this disease.References: (#Corresponding Author) Naveen V# et al,. "Structure of SARS-CoV-2 Nsp1/5′UTR Complex and Implications for Potential Targets, Vaccine and Virulence". ACS. J. Phys. Chem Lett (2020), 11(22): p9659-9668. (Recommended- F1000)

Track: Chaperones
Session: Allostery & Dynamics in Protein Function

ABS# 016 | DnaJC7 binds natively folded structural elements in tau to inhibit amyloid formation

Lukasz Joachimiak[1], Zhiqiang Hou[1], Pawel Wydorski[1], Valerie Perez[1], Ayde Oliva-Mendoza[1], Bryan Ryder[1], Hilda Mirbaha[1], Omar Kashmer[1]
[1]*University of Texas Southwestern Medical Center (TX, United States)*

Molecular chaperones, including Hsp70/Hsp40 families, play central roles in binding substrates to prevent their aggregation. How Hsp40s select different conformations of substrates remains poorly understood. Here, we report a novel interaction between the Hsp40 DnaJC7 and tau that efficiently suppresses tau aggregation in vitro and in cells. DnaJC7 binds preferentially to natively folded wild-type tau, but disease-associated mutants in tau reduce chaperone binding affinity. We identify that DnaJC7 uses a single TPR domain to recognize a b-turn element in tau that contains the 275VQIINK280 amyloid motif. Wild-type tau b-turn fragments, but not mutant fragments, can block full-length tau binding to DnaJC7. These data suggest DnaJC7 preferentially binds and stabilizes natively folded conformations of tau to prevent tau conversion into amyloids. This identifies a novel mechanism of tau aggregation regulation that can be exploited as both a diagnostic and a therapeutic intervention.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 017 | Unravelling Mechanism of Immune Evasion of SARS-CoV-2

Jennifer M. Bui[1], Joerg Gsponer[1]
[1]*University of British Columbia (British Columbia, canada)*

SARS-CoV-2 entry into host cells is mediated by the homotrimeric transmembrane spike protein, S via its interactions with the human protein angiotensin-converting enzyme 2 (ACE2). Specifically, the receptor binding domain (RBD) in the apex of S enables interactions with the ACE2. Recent CryO-EM studies show that Spike protein is predominantly in the closed state where the RBD stays in the down position. The hidden RDB contributes to the immune evasion of SARS-CoV-2 as one of the conformational masking strategies. Indeed, compared to SARS-CoV, many SARS-CoV-2 patients develop low levels of neutralizing antibodies and

suffer prolonged illness. These clinical features indicate that SARS-CoV-2 has evolved to evade the human immune surveillance more effectively than SARS-CoV does, and SARS-CoV-2 remains highly infectious. Using combined computational and biophysical approaches, we have been able to map a more complete structural landscape of protein S and reveal hinging motions as well as specific interactions that underlie transitions between closed and open states. Our study also provides insights into how some emergent variants (e.g. UK B.1.17 mutant) are advantages to SARS-CoV-2's interactions with ACE2.

Track: Folding

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 018 | Non-Refoldability is Pervasive Across the E. coli Proteome

Stephen Fried[1]
[1]*Johns Hopkins University (MD, United States)*

Decades of research on protein folding have primarily focused on a subset of small proteins that can reversibly refold from a denatured state. However, these studies have generally not been representative of the complexity of natural proteomes, which consist of many proteins with complex architectures and domain organizations. Here, we introduce an experimental approach to probe protein refolding kinetics for whole proteomes in cytosol-like environments using mass spectrometry-based proteomics. Our study covers the majority of the soluble E. coli proteome expressed during log-phase growth, and among this group, we find that one third of the E. coli proteome is not intrinsically refoldable on physiological timescales, a cohort that is enriched with certain fold-types, domain organizations, and other biophysical features. We also identify several properties and fold-types that correlate with slow refolding on the minute timescale. Hence, these results illuminate when exogenous factors and processes, such as chaperones or co-translational folding, might be required for efficient protein folding.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 019 | Designing thermostable DNA-binding proteins

Michelle McCully[1], Lauren Verheyden[1], Lily Schumacher[1], Andrew Bigler[1], Natali Gonzalez[1]
[1]*Biology Department, Santa Clara University (CA, USA)*

Protein designers are ever improving their ability to design proteins that fold stably and perform a function, yet meeting both of these goals at once has been a challenge. Here we investigate whether there is a trade-off between thermostability and function by designing chimeras of two three-helix bundle proteins. The Engrailed homeodomain is a DNA-binding protein that serves as a transcription factor in D. melanogaster development with a melting temperature of 52°C. Based on its backbone structure, a variant, UVF, was previously designed and found to be thermostable (Tm > 99°C) but was not functional. Molecular dynamics and coarse-grained simulations of chimeras of the two proteins suggest that UVF's fully hydrophobic core contributed entropically to its stability. Therefore, we designed chimeric proteins containing UVF's thermostabilizing core and EnHD's DNA-binding surface in attempts to rationally engineer a thermostable, functional protein. Here, we used molecular dynamics simulations of the proteins bound to DNA to predict the chimeras' DNA-binding function and the proteins in isolation to predict their thermostability. DNA-binding and thermostability were then tested experimentally using electrophoretic mobility shift assays and thermal melts monitored by circular dichroism.

Track: Enzymology

Session: Protein Evolution, Design and Selection

ABS# 020 | HutW from Vibrio cholerae is an Anaerobic Heme Degrading Enzyme with Unique Functional Properties

Marley Brimberry[1], Marina Toma[1], Kelly Hines[2], William Lanzilotta[1]
[1]*University of Georgia Department of Biochemistry and Molecular Biology,* [2]*University of Georgia Department of Chemistry (GA, United States)*

Increasing antibiotic resistance and a growing recognition of the importance of the human microbiome, demand that new therapeutic targets be identified. Characterization of metabolic pathways, that are unique to pathogens, represent a promising approach. Iron is often the rate-limiting factor for growth and Vibrio cholerae, the causative agent of cholera, has been shown to contain numerous genes that function in the acquisition of iron from the environment. Included in this arsenal of genes are operons dedicated to obtaining iron from heme and heme containing proteins. Given the persistence of Cholera, an important outstanding question is whether or not V. cholerae is capable of anaerobic heme degradation as was recently reported for the enterohemorrhagic Escherichia coli O157:H7. In this work, we demonstrate that HutW from

V. Cholerae is a radical S-adenosylmethionine (SAM) methyl transferase involved in the anaerobic opening the porphyrin ring of heme. However, in contrast to the previously characterized enzyme ChuW, found in the enterohemorrhagic E. coli O157:H7, there are notable differences in the mechanism and products of the HutW reaction. Of particular interest are data that demonstrate HutW will catalyze ring opening as well as tetrapyrrole reduction and can utilize reduced nicotinamide adenine dinucleotide phosphate (NADPH) as an electron source. A complete understanding of the mechanism, catabolites, and the modulation of the enzyme in anaerobic heme degradation will provide useful insight for future targeted pathogen antibiotic development.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 021 | Bringing Statistical Mechanical Rigor to Protein Design in Large Sequence Spaces with Multisite λ Dynamics

Ryan Hayes[1]
[1]*University of Michigan (MI, United States)*

There is growing interest in studying large protein sequence spaces encompassing many mutations both for protein engineering and design efforts as well as for biophysical insight into evolution. Computational studies have traditionally been limited to approximate rotamer-based methods such as Rosetta, but motivated by the success of alchemical free energy methods in computer-aided drug design, several recent studies have highlighted the improved accuracy possible for point mutations with alchemical free energy methods. Among alchemical free energy methods, multisite λ dynamics (MSλD) is unique in its ability to scale from point mutations to large combinatorial sequence spaces arising from many mutations. Building on studies of T4 lysozyme that demonstrate the accuracy of MSλD, we use MSλD to design chimeras mixing 15 mutations from E coli ribonuclease H and a consensus sequence, a space of 32768 sequences. The ability of MSλD to design a very stable sequence in this space and high Pearson correlation of 0.86 with experimental measurements contrast sharply with Rosetta. Not only can MSλD predict the effect of up to 15 mutations with kcal/mol level accuracy, but because the mutations considered are present in viable organisms, the observed effect of the mutations can constrain models of evolutionary selection for stability by quantifying a "selection temperature." Given the success of MSλD in studying ribonuclease H, we also apply MSλD to antibody design, computing the loss of affinity upon antigen mutation and proposing rescue mutations in the antibody. Together these studies showcase MSλD as a highly accurate emerging method for protein design.

Track: Synthetic Biology
Session: Protein Evolution, Design and Selection

ABS# 022 | Design of Red Light-Switchable Biosensors

Liangcai Gu[1]
[1]*University of Washington (WA, USA)*

Protein dimerization systems controlled by red light with increased tissue penetration depth are a highly needed tool for clinical applications such as cell and gene therapies. However, mammalian applications of existing red light-induced dimerization systems are hampered by limitations of their two components: a photosensory protein (or photoreceptor) which often requires a mammalian exogenous chromophore and a naturally occurring photoreceptor binding protein typically having a complex structure and nonideal binding properties. Here, we introduce an efficient, generalizable method (COMBINES-LID) for creating highly specific, reversible light-induced heterodimerization systems independent of any existing binders to a photoreceptor. It involves a two-step binder screen (phage display and yeast two-hybrid) of a combinatorial nanobody library to obtain binders that selectively engage a light-activated form of a photoswitchable protein or domain not the dark form. Proof-of-principle was provided by engineering nanobody-based, red light-induced dimerization (nanoReD) systems comprising a truncated bacterial phytochrome sensory module using a mammalian endogenous chromophore, biliverdin, and light-form specific nanobodies. Selected nanoReD systems were biochemically characterized, exhibiting low dark activity and high induction specificity, and further demonstrated for the reversible control of protein translocation and activation of gene expression in mice. Overall, COMBINES-LID opens new opportunities for creating genetically encoded actuators for the optical manipulation of biological processes.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 023 | The SARS-CoV-2 Nucleocapsid protein Binds RNA Multivalently

Heather Masson-Forsythe[1], Joaquin Rodriguez Galvan[1], Zhen Yu[1], Seth Pinckney[1], Patrick Reardon[2],

Richard B. Cooley[1], Phillip Zhu[1], Amber D. Rolland[3], James Prell[3], Elisar Barbar[1]
[1]*Biochemistry and Biophysics Department at Oregon State University,* [2]*Oregon State University NMR facility,* [3]*Department of Chemistry and Biochemistry, University of Oregon (OR, United States)*

The nucleocapsid phosphoprotein N plays critical roles in multiple processes of the SARS-CoV2 infection cycle: it protects and packages viral RNA in nucleocapsid assembly, interacts with the inner domain of spike protein in virion assembly, binds to structural membrane protein M during virion packaging and maturation, and binds to proteases causing replication of infective virus particle. Even with its importance, very limited biophysical studies are available on the N protein because of its high level of disorder, high propensity for aggregation and high susceptibility for auto-proteolysis. Here we successfully prepare the N protein and a 1000 nucleotide fragment of viral RNA in large quantities and purity suitable for biophysical studies. Using a combination of biophysical and biochemical techniques including multi-angle light scattering, analytical ultracentrifugation, native mass spectrometry, CD, and NMR, we show that the N protein is partially disordered and consists of an independently folded RNA binding domain and a dimerization domain, flanked by disordered linkers. The protein assembles as a tight dimer with a dimerization constant of sub micro molar, but can also form transient interactions with other N proteins facilitating larger oligomers. NMR studies on the ~100kDa dimeric protein identify a specific domain that binds 1-1000 RNA and show that the N/RNA complex remains highly disordered. Analytical ultracentrifugation, isothermal titration calorimetry, multi-angle light scattering, and cross-linking experiments identify a heterogeneous mixture of complexes with a core corresponding to at least 70 dimers of N bound to 1-1000 RNA. In contrast, very weak binding is detected for the RNA binding domain using similar experiments. A model that explains the importance of the bivalent structure of N to its binding on multivalent sites of the viral RNA is presented. This work is featured in the 2021 "Dance Your PhD" contest, winning the COVID19 prize.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 024 | Structural Characterization of a Hydroxyproline Dehydratase from C. difficile

Lindsey Backman[1], Yolanda Huang[2], Mary Andorfer[1], Brian Gold[1], Ronald Raines[1], Emily Balskus[2], Catherine Drennan[3]
[1]*Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139,* [2]*Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138,* [3]*Department of Chemistry, and of Biology, Howard Hughes Medical Instit (MA, USA)*

The glycyl radical enzyme (GRE) family utilizes a glycyl radical cofactor, installed by AdoMet radical activating enzymes, to catalyze difficult chemical reactions in a variety of microbial metabolic pathways. Although glycyl radical enzymes are widely encoded and expressed by bacteria found in the gut microbiome, these enzymes remain largely uncharacterized. Recently, a new glycyl radical enzyme was discovered to catalyze the dehydration of trans-4-hydroxy-L-proline (4-Hyp) to 1-pyrroline-5-carboxylic acid. Bioinformatics studies by the Balskus lab show that this hydroxyproline dehydratase (HypD) is the second most prominent GRE in the human gut microbiome and is encoded by 360 bacterial genomes, including the human pathogen C. difficile. HypD presents a pathway for bacteria to reverse 4-Hyp post-translational modifications, the most common post-translational modification in animals which was previously thought to be irreversible. Furthermore, the bacteria that encode HypD are known to use 4-Hyp as an electron acceptor during amino acid fermentation, their primary method of generating adenosine triphosphate (ATP). However, the enzyme responsible for assimilating 4-Hyp into this pathway has remained unknown until now. HypD could be the missing puzzle piece to understanding how these bacteria use the abundant metabolite 4-Hyp in energy production, while also symbiotically providing humans with a method for recycling this common amino acid. In order to elucidate the mechanism for how HypD performs the dehydration of hydroxyproline, we aimed to characterize HypD from C. difficile, in the presence of its substrate. Here, we have solved a 2.05-Å resolution structure for HypD by molecular replacement. Subsequently, a structure for HypD with its substrate 4-Hyp bound in the active site was solved to 2.52-Å resolution. These structures and accompanying biochemical studies have led us to identify key catalytic residues and have provided insight into the mechanism for 4-Hyp dehydration.

Track: Intrinsically Disordered Proteins
Session: New Protein Post-Translational Modifications

ABS# 026 | Breaking the "tubulin code": the case of intra-cellular trafficking

Lavi Bigman[1], Koby Levy[1]
[1]*Weizmann Institute of Science (Israel, Israel)*

Microtubules (MTs) are essential components of the eukaryotic cytoskeleton that serve as "highways" for intracellular trafficking. In addition to the well-known active transport of cargo by motor proteins, many MT-binding proteins adopt diffusional motility as a transportation mechanism. However, because of the limited spatial resolution of current experiments, the detailed mechanism of protein diffusion has not been elucidated. Possible contributors to the diffusion mechanism are tubulin disordered tails, which exist in several isoforms, undergo many post-translational modifications and give rise to the "tubulin code". However, the precise role of tubulin tails and tail modifications in the diffusion process is unclear Here, using coarse-grained and atomistic molecular dynamics simulations, we quantify the biophysical properties of tubulin tails and explore the molecular mechanism of protein diffusion along MTs. We found that electrostatic interactions play a central role in protein diffusion; the disordered tubulin tails enhance affinity but slow down diffusion, and diffusion occurs in discrete steps. While diffusion along wild-type MT is performed in steps of dimeric tubulin, the removal of the tails results in a step of monomeric tubulin. We found that the energy barrier for diffusion is larger when diffusion on MTs is mediated primarily by the MT tails rather than the MT body. In addition, globular proteins (EB1 and PRC1) diffuse more slowly than an intrinsically disordered protein (Tau) on MTs. We also found that polyglutamylation, but not poly-glycylation, expands the dimensions of the tubulin tails. Finally, polyglutamylation and poly-glycylation of tubulin tails lead to slower protein diffusion along MTs, although polyglycylation leads to faster diffusion across MT protofilaments. Our results explain experimentally observed data and shed light on the roles played by disordered tubulin tails and tail modifications in the molecular mechanism of protein diffusion along MTs. Reference: Bigman and Levy, PNAS , April 2020 117 (16)

Track: Proteomics
Session: Allostery & Dynamics in Protein Function

ABS# 027 | Investigation and Characterization of the Jumping Translocation Breakpoint (JTB) Protein using Mass Spectrometry based Proteomics

Madhuri Jayathirtha[1], Danielle Whitham[1], Devika Channaveerappa[1], Costel Darie[1]
[1]*Clarkson University (NY, United States)*

Human JTB (hJTB) is a gene located on the human chromosome 1 at q21 which is involved in the unbalanced translocation in various types of cancer. JTB protein is ubiquitously present in normal cells and is found to be overexpressed in various types of cancer including prostate and breast cancer. Hence this protein could be a biomarker for tumor malignancies and a potential target for their treatment. However, the biological function and the pathway through which this protein causes increased cell growth and proliferation is not entirely clear. Investigation and comparison of the proteomes of cells with upregulated and downregulated JTB can be a good approach to understand the function of the protein and also its contribution to tumorigenesis. In this study, MCF7 breast cancer cell lines were transfected with the sense orientation of the JTB cDNA in HA, His and FLAG tagged CMV expression vector as well as with shRNA plasmids. Proteins extracted from transient and stable transfected cells were separated using SDS-PAGE. The expression of JTB was confirmed by western blotting technique. In gel digested peptides were analyzed by a Nano Acquity UPLC coupled with QTOF Xevo G2 Mass Spectrometer. Data processing was done using Mascot 2.4 server and Scaffold 4.1 software. We found several proteins that were dysregulated. Furthermore, we will do Immunoprecipitation to look at JTB protein interacting partners. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the JTB protein as a potential drug target for malignancies with overexpression of the protein.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 028 | Natural polymorphisms affect structure and function of gC1q domain of human otolin-1

Rafał Hołubowicz[1], Martyna Uściła[1], Sylwia Groborz[1], Andrzej Ożyhar[1], Piotr Dobryszycki[1]
[1]*Department of Biochemistry, Molecular Biology and Biotechnology, Faculty of Chemistry, Wrocław University of Science and Technology (Wrocław, Poland)*

Otolin-1 is a C1q-like protein from the organic matrix of otoconia ("ear dust"), which are calcite biominerals localized in the vestibule of the inner ear. Otoconia, together with the semicircular canals, are responsible for sensing of balance. Degeneration of otoconia during life causes benign paroxysmal positional vertigo (BPPV), which is a balance disorder affecting the elderly, especially women. Although BPPV is not a direct life threat, it is a serious condition as it may lead to falls, bone fractures, and thus cause permanent disability. In the

Ensembl database, we identified several missense single nucleotide variants in the gC1q domain of human otolin-1, a fragment which is responsible for trimerization and binding of calcium ions by otolin-1. Here, we show how selected variants affect structure and function of gC1q otolin-1. We used circular dichroism spectroscopy, sedimentation velocity analytical ultracentrifugation, thermal shift assay, and terbium(III) binding assay and found that the mutations affected secondary structure, oligomerization propensity, thermal stability, and ability to bind calcium ions by the gC1q otolin-1. For example, R342W variant increased aggregation propensity of gC1q otolin-1 and decreased its affinity towards calcium ions. Strikingly, R402P variant prevented formation of gC1q trimers and completely disabled the ability of gC1q otolin-1 to bind calcium ions. Our study reveals that genetic variants identified in the population may severely affect the function of otolin-1 in vivo. Further studies are required to determine if the presence of the variants has medical consequences for sensing of balance.This work was supported by National Science Centre (Poland) grant 2017/27/N/NZ1/01319.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 029 | Investigating the interaction of enzymes with functionalized surfaces : Lessons from multiscale modeling approaches

Sophie Sacquin-Mora[1], Nicolas Bourassin[1], Marc Baaden[1]
[1]*Laboratoire de Biochimie Théorique, CNRS UPR9080 (Paris, France)*

Redox enzymes represent promising tools for H2-based technologies such as biofuel cells. However, many aspects of theses enzymes remain to be understood. In particular, designing efficient biofuel cells requires us to grasp details of the interaction between the enzymes and the electrode surfaces on the molecular level. 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. Applications on [NiFe]-hydrogenases (which catalyze hydrogen oxidation) and copper-billirubin oxidase (which catalyzes oxygen reduction) 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.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 033 | Potential Therapeutic Applications of Cyclodextrin Derivatives for the Remediation of Emerging Per-and Poly-fluoroalkyl substances (PFAS) from Human Serum Albumin (HSA) Protein

N.L Dilani Perera[1], Jaroslava Miksovska[2], Kevin E. O'Shea[2]
[1]*Florida international* , [2]*Florida international university (FL, United States)*

The presence of per-and poly-fluoroalkyl substances (PFAS), commonly referred to as forever chemicals, in the environment is a global problem. It is estimated that approximately 98% of the US population have a detectable level of PFAS in their blood, creating serious human health concerns. While remediation of PFAS from aqueous media has received tremendous attention, studies on their removal from biological systems are limited. The objective of this study was to investigate the binding of a series of emerging PFAS to the human serum albumin (HSA), an essential blood constituent. Further experiments were carried out with Gen X, a problematic emerging PFAS. We report herein the extraction of Gen X from HSA using β-cyclodextrin (β-CD) and heptakis(6-deoxy-6-amino)-β-cyclodextrin heptahydrochloride (NH2-β-CD). The addition of β-CD and NH2-β-CD to Gen X led to the dissociation of the HSA:Gen X complex. Upon addition of excess β-CD and NH2-β-CD the characteristic fluorescence structural features of undisrupted were observed. The interaction of Gen X with HSA was also probed by performing molecular docking (MD) studies, and monitored by fluorescence, and circular dichroism (CD) spectroscopies. Structural changes to HSA upon the addition of Gen X were evident by changes to the fluorescence and CD spectra. Competitive binding studies employing known site-specific HSA binding fluorophores, 8-anilinonaphthalene-1-sulfonic acid (1,8-ANS) and Warfarin, were performed to probe specific binding sites and interaction of Gen X and HSA. Fluorescence results indicated that Gen X replaced 1,8-ANS and Warfarin bound to the HSA. With the addition of β-CD and NH2-β-CD to GenX:HSA:1,8-ANS and GenX:HSA:Warfarin mixtures, the fluorescence emission returned to HSA:1,8-ANS and HSA:Warfarin respectively. These results illustrated the promising potential for β-CD

and NH2- β-CD in the therapeutic remediation of emerging PFAS from biological systems.

Track: Structure (X-Ray/NMR/EM)
Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 034 | Structural basis of the activation of the CC chemokine receptor 5 by a chemokine agonist

Polina Isaikina[1], Ching-Ju Tsai[2], Nikolaus Dietz[1], Filip Pamula[2,3], Ramon Guixà-González[2], Camila Branco[4], Gebhard F.X. Schertler[2,3], Oliver Hartley[4], Henning Stahlberg[5], Timm Maier[1], Xavier Deupi[2], Stephan
[1]Focal Area Structural Biology and Biophysics, Biozentrum, University of Basel, CH-4056 Basel, Switzerland, [2]Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland, [3]Department of Biology, ETH Zurich, CH-8093 Zurich, Switzerland, [4]Departme (Basel City, Switzerland)

The human CC chemokine receptor 5 (CCR5) is a G protein-coupled receptor (GPCR) that plays a major role in inflammation and is involved in the pathology of cancer, HIV, and COVID-19. Despite its significance as a drug target, the molecular activation mechanism of CCR5, i.e. how chemokine agonists transduce the activation signal through the receptor, is yet unknown. Here, we report the cryo-EM structure of wild-type CCR5 in an active conformation bound to the chemokine superagonist [6P4]CCL5 and the heterotrimeric Gi protein. The structure provides the rationale for the sequence-activity relation of agonist and antagonist chemokines. The N-terminus of agonist chemokines pushes onto specific structural motifs at the bottom of the orthosteric pocket that activates the canonical GPCR microswitch network. This activation mechanism differs significantly from other CC chemokine receptors that bind chemokines with shorter N-termini in a shallow binding mode involving unique sequence signatures and a specialized activation mechanism.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 037 | Simulations of Thrombin and the Role of Sodium Binding

Dizhou Wu[1], Freddie Salsbury[1]
[1]Wake Forest University (North Carolina, United States)

Thrombin is a Na+-activated serine protease existing in two forms targeted to procoagulant and anticoagulant activities, respectively. Previous molecular dynamics (MD) studies suggest that there are two Na+-binding sites in thrombin and that binding to different sites or to both sites may have conformational, dynamical, and ultimately functional significance.In this study, we performed 12 independent microsecond all-atom MD simulations for the wild-type (WT) thrombin, and we study the effects of the different Na+ binding modes on thrombin. Using structural and dynamical measures including root-mean-square fluctuations, correlation matrices as well as non-parametric clustering analysis, and PCA-construction of free energy surfaces, we identify conformational and dynamical changes that occur in different binding states and hypothesize a multistep kinetic scheme for the Na+ binding to thrombin.

Track: Chemical Biology
Session: Allostery & Dynamics in Protein Function

ABS# 038 | Disruption of a key ligand-H-bond network drives glucocorticoid receptor gene suppression without off-target gene activation

Xu Liu[1], Eric Hoffman[2], Eric Ortlund[1]
[1]Emory University, [2]Binghamton University, SUNY (GA, USA)

Duchenne muscular dystrophy is a genetic disorder that shows chronic and progressive damage to skeletal and cardiac muscle leading to premature death. Antiinflammatory corticosteroids targeting the glucocorticoid receptor (GR) are the current standard of care but drive adverse side effects such as deleterious bone loss. Through subtle modification to a steroidal backbone, a recently developed drug, vamorolone, appears to preserve beneficial efficacy but with significantly reduced side effects. We use combined structural, biophysical, and biochemical approaches to show that loss of a receptor-ligand hydrogen bond drives these remarkable therapeutic effects. Moreover, vamorolone uniformly weakens coactivator associations but not corepressor associations, implicating partial agonism as the main driver of its dissociative properties. Additionally, we identify a critical and evolutionarily conserved intramolecular network connecting the ligand to the coregulator binding surface. Interruption of this allosteric network by vamorolone selectively reduces GR-driven transactivation while leaving transrepression intact. Our results establish a mechanistic understanding of how vamorolone reduces side effects, guiding the future design of partial agonists as selective GR modulators with an improved therapeutic index.

Track: Folding
Session: New Protein Post-Translational Modifications

ABS# 039 | Assessing the Structures and Interactions of gammaD-Crystallin Deamidation Variants

Alex Guseman[1], Matthew Whitley[1], Jeremy Gonzalez[1], Nityam Rathi[1], Mikayla Ambarian[1], Angela Gronenborn[1]
[1]*University of Pittsburgh School of Medicine (Pennsylvania, USA)*

Cataracts, or the opacification of the eye lens, are the leading cause of visual impairment worldwide. Cataracts are formed by aggregation of the crystallin proteins, resulting in opacification of the eye lens, and in turn reduces the access of light to the retina. As there is essentially no protein turnover in the eye lens, crystallins have to last for the entire lifetime of the host organism. As a result, crystallins have evolved to possess great stability and solubility. Chronic exposure to chemical attacks, such as UV light or hazardous compounds, results in irreversible covalent modifications, which are hypothesized to decrease protein stability and solubility thus inducing aggregation. In this study, we focused on yD-crystallin, the most abundant y-crystallin in the lens nucleus, and how deamidation, the conversion of Asn residues to Asp, influences the structure and function of the protein. We used solution NMR to assess structural changes in Asn to Asp deamidation variants and solved the X-ray structures of two yD-crystallin deamidation variants. To complement our structural studies, biophysical characterization of yD-crystallin variants was done by DSC and DLS. We used DSC to evaluate thermal stability where all variants showed minimal changes in Tm. We used DLS to understand self-interactions probed by dynamic light scattering and diffusion interaction parameters (DIP), a measure similar to the 2nd viral coefficients, were determined for all mutants to be within the uncertainty of wild-type. Our results suggest that deamidation alone is likely not a driver of cataract formation.

Track: Membrane Proteins
Session: Measuring Forces of Biological Systems

ABS# 040 | Membrane binding of the S100B retinal protein

Paul Jaouen[1,2], Florence Guérin[1,2], Trystan Lessard[1,2], Élodie Boisselier[1,2]
[1]*Université Laval,* [2]*Centre de recherche du CHU de Québec (Québec, Canada)*

CONTEXTMuller glial cells, located in the retina, are responsible for the physiological function of the eye nervous system by maintaining tissue homeostasis and cellular function. Guanylate cyclase (GC1) is a transmembrane enzymatic protein that transduces cell synaptic signal. This transduction activity is actually regulated by various proteins, including S100B in Muller glial cells. Thus, alterations in their function could deregulate GC1 synaptic transduction activity leading to ocular pathologies such as diabetic retinopathy or glaucoma, where loss of vision is observed.GC1, as well as S100B, are membrane proteins and the regulatory activity of S100B on GC1 is directly influenced by the membrane composition. Yet, S100B has not been extensively studied in this area and no molecular information on its membrane behavior is known. Therefore, the study of the membrane binding of S100B is key to understand the regulation of GC1 as well as its role in the retinal synaptic activity.GOAL & METHODSThe objective of this research project is to characterize the interactions between the protein S100B and lipids. To do so, S100B transformation, overexpression, and purification has to be performed in order to study its interaction with the lipidic membranes using biophysical models and techniques. In this study, the Langmuir monolayer model was coupled to surface tensiometry for the characterization of the interactions between several specific phospholipids and S100B in different experimental conditions.RESULTS & CONCLUSIONIn this project, S100B was transformed, overexpressed and purified with a purity rate of more than 95%. Membrane interaction of S100B has shown that this protein preferentially interacts with unsaturated phosphoethanolamine lipids, which are abundantly found in the Muller glial cell inner membrane. Furthermore, those lipids are crucial for the cellular activity providing the membrane flexibility necessary for synaptic transduction in Muller glial cells.

Track: Proteostasis and quality control
Session: Targeted Protein Degradation

ABS# 042 | Functional cooperativity between the trigger factor chaperone and the ClpXP proteolytic complex

Walid Houry[1]
[1]*University of Toronto (Ontario, Canada)*

A functional association was uncovered between the ribosome-associated trigger factor (TF) chaperone and the ClpXP degradation complex. TF is an ATP-independent elongated chaperone that binds at the

ribosome exit tunnel. The ClpXP degradation complex consists of the hexameric ClpX unfoldase ATPase that binds to one or both ends of the ClpP tetradecameric cylindrical serine protease. ClpX binds to target proteins, unfolds them, and then threads them into the ClpP cylinder for degradation. Bioinformatic analyses demonstrated the conservation of the close proximity of tig, gene coding for TF, and genes coding for ClpXP suggesting a functional interaction. Effect of TF on ClpXP-dependent degradation varied based on the nature of substrate. While degradation of some substrates was slowed down or was unaffected by TF, surprisingly, TF increased the degradation rate of a third class of substrates. These include λ phage replication protein λO, master regulator of stationary phase RpoS, and SsrA-tagged proteins. Globally, TF was estimated to enhance the ClpXP-dependent degradation of about 2% of newly synthesized proteins. Using biophysical and NMR-based approaches, TF was found to interact through multiple sites with ClpX in a highly dynamic fashion to promote protein degradation. This chaperone-protease cooperation constitutes a unique and likely ancestral aspect of cellular protein homeostasis in which TF acts as an adaptor for ClpXP. Our data suggest the presence of co-translational degradation in bacteria.

Track: Membrane Proteins

Session: Allostery & Dynamics in Protein Function

ABS# 043 | Development and Application of an NMR-based High-Throughput Screening (HTS) Methodology to Discover Small-Molecule Binders of the Amyloid Precursor Protein (APP) Transmembrane C-Terminal Domain, C99.

Manuel Castro[1], Ilyas Beg[2], Michael Wolfe[2], Kristine Parson[3], Brandon Ruotolo[3], Charles Sanders[1]
[1]Vanderbilt University, [2]University of Kansas, [3]University of Michigan (Tennessee, United States)

There remains a compelling motivation to develop substrate-selective inhibitors of gamma-secretase that reduce cleavage of the amyloid precursor protein C-terminal domain (C99) by this protease while allowing normal processing of other potential substrates such as the Notch receptor to occur. Here we examined whether inhibitors of C99 cleavage can be found that act by directly binding to the C99 substrate rather than to gamma-secretase, selectively protecting C99 from cleavage while allowing normal cleavage of other protein substrates to proceed. Verteporfin was discovered to be the founding molecule of this class. Using NMR, Verteporfin was seen to form a direct 1:1 complex (KD of 15 μM) with C99 as verified in multiple model membrane conditions. In contrast, Verteporfin does not bind to another gamma-secretase substrate, the Notch-1 receptor transmembrane/juxtamembrane domain. Chemical cross-linking and mass spectrometry indicate that verteporfin binds to monomeric C99 but does not induce dimerization or covalently modify the protein. Biochemical assays showed that verteporfin inhibits gamma-secretase cleavage of C99 with a KI of 17 μM without inhibiting cleavage of the Notch receptor. These results provide proof-of-principle that substrate-selective inhibitors of gamma-secretase can be found that act by binding the substrate of interest.

Track: Dynamics and Allostery

Session: Allostery & Dynamics in Protein Function

ABS# 044 | Perturbing dimer interaction and allosteric communication alters the immunosuppressive activity of human galectin-7

Ngoc Thu Hang Pham[1], Myriam Létourneau[1], Marlène Fortier[1], Fabrizio Pucci[2], Benjamin Folch[1], Marianne Rooman[2], David Chatenet[1], Yves St-Pierre[1], Patrick Lagüe[3,4], Charles Calmettees[1,3], Nicolas Doucet[1,3]
[1]Centre Armand-Frappier Santé Biotechnologie, Institut national de la recherche scientifique (INRS), Université du Québec, 531 Boul. des Prairies, Laval, QC, H7V 1B7, Canada., [2]Computational Biology and Bioinformatics, Université Libre de Bruxelles, (QC, Canada)

Triple-negative breast cancer (TNBC) accounts for 15–20 % of all breast cancers. TNBC cells were found to abnormally overexpress galectin-7 (GAL-7), leading to metastasis. GAL-7 is a prototypical galectin, characterized by a b-galactoside binding site (GBS) and a homodimeric molecular architecture. This protein binds to glycoreceptors on the surface of activated T cells via its GBS and triggers T cell apoptosis, favoring tumor cell immune evasion. Consequently, GAL-7 represents a promising target for TNBC treatment. To this day, the development of GAL-7 modulators has almost exclusively focused on small-molecule GBS inhibitors aimed at perturbing glycoreceptor interactions. However, due to high GBS similarity among several galectin homologs in the cell, this remains a high-risk strategy because of unwanted off-target effects on other beneficial antitumoral galectins. Furthermore, GBS inhibitors are ineffective at targeting the glycan-independent functions of GAL-7. New approaches are thus required to develop effective and highly specific GAL-7 inhibitors. Since destabilization of the GAL-7 molecular architecture could

potentially alter its affinity towards glycoproteins and affect biological function, our main research objective aims to dissect the molecular and functional importance of GAL-7 homodimer organization and allosteric communication across the dimer interface and between opposite protomers. In this study, we present the impact of homodimer interface mutations on induction of Jurkat T cell apoptosis and describe the first allosteric network model defining function within GAL-7 protomers.

Track: Chaperones

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 045 | Nucleocytoplasmic Shuttling of the Glucocorticoid Receptor is Modulated by TPR-Domain Proteins

MARIO GALIGNIANA[1,2], GISELA MAZAIRA[2], PABLO ECHEVERRÍA[1]
[1]IBYME/CONICET, [2]University of Buenos Aires-FCEN (CABA, ARGENTINA)

HSP90 dimers shape an acceptor site for co-chaperones carrying tetratricopeptide-repeats (TPR), where they bind in vivo in a competitive and mutually exclusive manner. We aimed to study the impact of TPR protein overexpression on the biological properties of the GR•HSP90 complex. GR nucleocytoplasmic shuttling was analysed by confocal microscopy, steroid-dependent transcriptional regulation by gene-reporter studies, GR attachment to nucleoskeleton by biochemical copurification, and the heterocomplex composition by co-immunoprecipitation assays.It is demonstrated that the overexpression of the TPR domain of the Ser/Thr-phosphatase PP5 impairs GR nuclear translocation rate by preventing:a)-the assembly of FKBP52•dynein/dynactin complexes, b)-binding to the adaptor/transporter importin-b1, and c)-binding to the nuclear pore-associated glycoprotein Nup62. These disruptions slow-down the GR passage through the nuclear pore complex (NPC) and its nuclear anchorage to the nuclear matrix. Both FKBP52 and PP5 co-purify with the nucleoskeleton in a TPR-competed fashion and show colocalization with GR and NuMa in intact cells. TPR-peptide overexpression prompts the GR release from nuclear anchoring sites and its export rate. While leptomycin-B shows no effect on GR nuclear export, it abolishes the effect of TPR peptide overexpression, suggesting the existence of a TPR domain-dependent export mechanism. While FKBP52 favours GR transcriptional activity and its nuclear retrotransport, FKBP51 and 14-3-3sigma (whose 3D-structure mimics that of the TPR domain of PP5) impairs this effect. In turn, the TPR-protein SGT1α shows no effect on GR trafficking but inhibits its transcriptional activity like FKBP51. PP5 promotes an equal nuclear-cytoplasmic redistribution of GR that is independent of its phosphorylation status, while it shows biphasic transcriptional action.It is proposed that the pleiotropic actions of GR in different cell types may depend on the relative abundance of TPR-domain interacting co-chaperones, which affect the GR trafficking mechanism, its passage through NPC, the anchorage to the nuclear matrix, and steroid-dependent transcriptional regulation.

Track: Chemical Biology

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 046 | Versatile Interacting Peptide (VIP) Tags for Imaging Cellular Proteins

Kimberly Beatty[1]
[1]Oregon Health & Science University (OR, United States)

We are in a new era for cell cartography, wherein proteins and multi-protein complexes are being mapped onto cells using fluorescent protein fusions or immunolabeling. A central obstacle that has limited progress in this area is the shortage of methods for labeling cellular proteins for multi-scale microscopy. There are few genetic tags for electron microscopy (EM) and almost none for correlative fluorescence and electron microscopy. Versatile Interacting Peptide (VIP) tags were developed to remove this obstacle. VIP tags consist of a heterodimeric coiled-coil between a genetically-encoded peptide tag and a reporter-conjugated "probe peptide". Coiled-coils are a simple structural motif amenable to optimization by protein engineering. The dimerization specificity and affinity of VIP tags are dictated by the peptide sequence. To date, we have described three VIP tags: VIP Y/Z, VIPER, and miniVIPER. All three VIP tags enabled the selective labeling of a variety of cellular proteins and organelles. Importantly, this technology can be used to deliver a variety of biophysical reporters, including bright fluorophores (e.g., Cy5, BODIPY, xanthenes) and EM-optimized particles (e.g., gold or Qdots). VIP tags have been used in distinct applications, including pulse-chase labeling, observing endocytosis, and quantification of receptors in EM micrographs. For the 2021 Symposium of the Protein Society, we will describe two recent applications of VIP tags. First, we will demonstrate how VIP tags can be used to translocate proteins inside living cells. Second, we will describe how self-sorting (orthogonal)

VIP tags enable multiple proteins to be imaged at once. The latter application is enabling high-resolution studies on receptor-mediated sensing and signaling.

Track: Metabolic Engineering / energy applications

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 047 | Imaging brain metabolism in a mouse model of Huntington's disease

Farheen Farzana[1,2], Malcolm McConville[2], Anthony Hannan[1], Danny Hatters[2], Berin Boughton[3]
[1]Florey institute of Neuroscience & Mental health, University of Melbourne, [2]Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Victoria 3010, Australia, [3]Australian National Phenome Centre, Murdoch Universit (Victoria, Australia)

Introduction: Huntington's disease (HD) is a neurodegenerative disease, whose key pathological signature is the formation of intracellular inclusions. However, the exact role of inclusions in driving HD pathology remains to be clearly understood. Our lab has previously shown that the formation of huntingtin inclusions correlates with neuroblastoma cells becoming functionally quiescent and undergoing a slow death by necrosis. We hypothesize that inclusion formation establishes cellular quiescence in vivo. Our goal is to assess the extent to which neurons in vivo are metabolically quiescent and how this relates to the presence of inclusions in a transgenic mouse model (R6/1) of HD.Methods: We have studied the metabolic turnover of neuronal membrane lipids by feeding wild-type (WT) and HD mice with deuterated water at asymptomatic, pre-symptomatic &fully symptomatic ages of the disease. The left hemisphere of the brain was used for determining the spatial distribution and the abundance of neuronal lipids using MALDI-TOF imaging mass spectrometry (MALDI-IMS), while the right hemisphere was reserved for cross-validation using Liquid-Chromatography mass spectrometry (LC-MS). Results: Our data points towards alterations in neuronal lipids that play a critical role in neurotransmission, synaptic plasticity, myelination, and Endoplasmic reticulum (ER)- stress, thus providing lipid correlates for hippocampal-dependent cognitive deficits observed in HD pathology. Moreover, we found a remodeling of lipid synthesis in hippocampal areas that are densely populated by inclusions, detected using EM48-immunohistochemistry. We have also developed a novel bioinformatics tool to study in vivo kinetics using stable isotope labelling (Deuterium) coupled with a spatial metabolic approach.Conclusion: Collectively, this data reveals age-specific changes in brain lipids, providing mechanistic insights into the progressive changes observed in HD. Accelerated lipid synthesis observed in asymptomatic HD mice, hints at its possible role as an adaptive stress response to cope with ER-stress, thus providing an early biomarker for identification of HD.

Track: Amyloid and Aggregation

Session: Protein Structures Through the Lens of Machine Learning

ABS# 048 | Towards the understanding of the effects of DOPAC on α-synuclein biophysical and biochemical properties

Benedetta Fongaro[1], Luana Palazzi[1], Manuela Leri[2], Laura Acquasaliente[1], Monica Bucciantini[2], Patrizia Polverino De Laureto[1]
[1]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy, [2]Department of Biomedical, Experimental and Clinical Sciences, University of Firenze, Italy (VI, Italy)

A typical hallmark of Parkinson's disease (PD) is the presence in the dopaminergic neurons of intraneuronal inclusions containing aggregates of α-synuclein. The causative and the biochemical events underlying the aberrant aggregation of α-synuclein are not completely understood. The interplay between α-synuclein and dopamine derivatives, such as 3,4-dihydroxyphenylacetaldehyde (DOPAL), seems to be associated with oxidative stress-dependent neurodegeneration in the early onset of PD. Strong evidence correlates this process with the levels of dopamine metabolites in vivo. In vitro, DOPAL and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylathanol (DOPET), exhibit the property to inhibit the growth of mature amyloid fibrils of α-synuclein. The molecular basis of this inhibition is still unclear. Here, we have studied the interaction between α-synuclein and DOPAC and the effects of DOPAC on the aggregation properties of α-synuclein and its ability to interact with membranes. DOPAC dose-dependently inhibits α-synuclein aggregation. It stabilizes the monomeric form of α-synuclein and induces the formation of protein dimers and trimers. DOPAC-induced oligomers are not toxic, do not undergo a conformational transition in the presence of membranes, and penetrate the cell, where they triggered autophagic processes. Our findings show that the

early radicals resulting from DOPAC autoxidation produce covalent modifications of the protein, including methionine oxidation and Michael addition reactions. However, these covalent modifications are not by themselves a primary cause of either fibrillation or membrane binding inhibition. This study provides insight into the molecular mechanisms underlying DOPAC protection against α-synuclein aggregation and aggregate cytotoxicity. Our findings might contribute to increase and strengthen the knowledge on the beneficial effects of polyphenols against neurodegeneration encouraging the design of novel therapeutic strategies aimed at slowing down or reversing the clinical progression of PD and other chronic neurodegenerative disorders associated with aging.

Track: Membrane Proteins

Session: Allostery & Dynamics in Protein Function

ABS# 050 | Structural basis of metal import by NRAMP family transporters

Shamayeeta Ray[1], Rachelle Gaudet[2]
[1]*Postdoc,* [2]*Professor (MA, United States)*

Transition metal ions like Mn2+, Fe2+, Ni2+, Co2+, Cu2+, and Zn2+ are essential nutrients that play a crucial role in various metabolic processes in all living cells. Excess or deficiency of these metal ions leads to harmful diseases including cancer, anemia and immune deficiencies. Cellular levels of essential metal micronutrients are thus tightly controlled and regulated. NRAMPs (natural resistance-associated macrophage proteins) are a class of transition metal transporters present in all domains of life that regulate the levels of these essential divalent metal ions within cells and prevent human disorders related to metal insufficiency (like anemia) or overload. In humans, there exists two NRAMPs; NRAMP1 is expressed in macrophages and assists in immune response by preventing microbial access to Mn2+ and Fe2+ within pathogen-infected phagosomes, whereas NRAMP2 is widely expressed at low levels in the endosomes of all nucleated cells, and primarily involved in uptake of non-heme Fe2+ from the diet in the intestinal tract.NRAMP-mediated metal ion transport typically involves a co-transported proton. A few structures of bacterial NRAMPs are reported to date which reveal the overall fold (similar to LeuT permease) and provide a preliminary understanding of metal binding and proton co-transport. To obtain a better picture of metal ion selectivity, we obtained additional high-resolution crystal structures in multiple conformational states in apo and metal-bound forms of a bacterial NRAMP from Deinococcus radiodurans (DraNRAMP). To complement the structural data, we performed binding studies using ITC and proteoliposome-based transport assays to understand the thermodynamic parameters associated with metal binding and transport. Overall, the high-resolution structures of DraNRAMP provide a first look at the detailed coordination geometry for the bound metals in the transporter's metal-binding site with insights into metal selectivity determinants.

Track: Chemical Biology

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 051 | Discovery of ODC inhibitors simultaneously regulating ODC-OAZ1 interaction

Sen Liu[1], Xiaoying Chai[1], Jingqiong Zhan[1], Jing Pan[1], Mengxi He[1], Bo Li[1], Jing Wang[1], Hongyan Ma[1]
[1]*Hubei University of Technology (Hubei, China)*

Proteins play important roles in cellular signaling networks, and enormous of them are both enzymes and protein-protein-interaction (PPI) components. Frequently, the enzymatic activity and the PPI regulation of such "intersection proteins (ISPs)" are entangled. Therefore, it is of great importance to dissect these signals when targeting ISPs for network interrogation and drug discovery. Although the discovery of enzyme inhibitors has become routine, the discovery of ligands modulating PPIs is still difficult, and profoundly challenging is the rational forward discovery of PPI stabilizers. Taking Ornithine decarboxylase (ODC) as an example, we established a pipeline for the forward discovery of multi-purpose ligands that inhibit ODC's enzymatic activity, hinder ODC-OAZ1 interaction, and perturb ODC's monomer-dimer exchange process. To discover multipurpose ODC inhibitors, we established a computational pipeline by combining positive screening and negative screening. We used this pipeline for the forward screening of multipurpose ligands that might inhibit ODC's activity, block ODC-OAZ1 interaction, and enhance ODC non-functional dimerization. With a combination of different experimental assays, we identified three multipurpose ODC inhibitors. At last, we also verified such multi-purpose ligands are promising drug candidates through reconfiguring the polyamine network. Our work provided novel tools and insights for targeting ISPs in cellular signaling networks.

Track: Therapeutics and Antibodies
Session: Allostery & Dynamics in Protein Function

ABS# 052 | Discovery of novel AdoMetDC inhibitors with SCARdock

Sen Liu[1], Yan Zhang[1], Qiang Zheng[1], Zhaoxiang Liu[1]
[1]*Hubei University of Technology (Hubei, China)*

Spermidine and spermine are important polyamines for most cells and their biosynthesis are strictly regulated by the polyamine metabolic network. In cancerous cells and tumor environments, the concentrations of polyamines are much higher than in normal cells. During the synthesis of spermidine and spermine, an amino-propyl group is provided by decarboxylated S-adenosylmethionine, and the latter is generated from S-adenosylmethionine by AdoMetDC (AdoMet decarboxylase). Therefore, as a rate-limiting enzyme in the biosynthesis of spermidine and spermine, AdoMetDC has been an attractive drug target in cancer studies. In the last decades, many AdoMetDC inhibitors have been discovered, and several AdoMetDC inhibitors are under clinical trials, but unfortunately, none of them have been approved yet. To overcome the high costs in time and money for discovering de novo inhibitors, we set out to repurpose clinic drugs as AdoMetDC inhibitors. We used SCAR (steric-clashes alleviating receptors), a computer-aided drug discovery strategy developed by us recently for in silico screening of novel AdoMetDC inhibitors in this work. By combining computational screening and experimental validation, we successfully identified several new AdoMetDC inhibitors, including three covalent inhibitors, one non-covalent inhibitors, and two approved drugs.

Track: Therapeutics and Antibodies
Session: Measuring Forces of Biological Systems

ABS# 053 | Apoptosis inducing nanogels: Investigating caspase cargo and apoptotic combinations

Francesca Anson[1], S. Thayumanavan[1], Jeanne Hardy[1]
[1]*UMASS Amherst (MA, USA)*

The balance of pro-apoptotic and pro-survival proteins define a cell's fate. These processes are controlled through an inter-dependent, finely-tuned protein network that enables survival or leads to apoptotic cell death. The caspase family of proteases are central to this apoptotic network, with initiator and executioner caspases, and their interaction partners, regulating and executing apoptosis. In this work, we interrogate and modulate this network by exogenously introducing specific initiator or executioner caspase proteins. Each caspase is exogenously introduced using redox-responsive polymeric nanogels. Although caspase-3 might be expected to be the most effective due to the centrality of its role in apoptosis and its heightened catalytic efficiency relative to other family members, we observed that caspase-7 and caspase-9 are the most effective at inducing apoptotic cell death. By critically analyzing the introduced activity of the delivered caspase, the pattern of substrate cleavage as well as the ability to activate endogenous caspases, we conclude that the efficacy of each caspase correlated with the levels of pro-survival factors that both directly and indirectly impact the introduced caspase. These investigations have not only expanded therapeutic possibilities but also have revealed mechanistic insights into the pathways readily involved in disease, warranting future combinatorial therapies. These findings lay the groundwork for developing exogenous introduction of caspases as a therapeutic option that can be tuned to the apoptotic balance in a proliferating cell.

Track: Folding
Session: Allostery & Dynamics in Protein Function

ABS# 054 | Novel Dynamic Bivalent Interaction Mechanism: RAP and LDLR Family Receptors

Philip Olivares[1], Ekaterina Marakasova[1,2,3], Haarin Chun[1], Nancy Hernandez[1], James Kurasawa[1,4], Elena Karnaukhova[1], Gabriela Hassink[1,5], Svetlana Shestopal[1], Dudley Strickland[6], Andrey Sarafanov[1]
[1]*Center for Biologics Evaluation and Research, U. S. Food and Drug Administration,* [2]*George Mason University, School of Systems Biology,* [3]*Center for Devices and Radiological Health, U. S. Food and Drug Administration,* [4]*AstraZeneca, R&D, Antibod (Maryland, United States)*

Receptor-associated protein (RAP) is a molecular chaperone of low-density lipoprotein receptor-related protein 1 (LRP1), a member of the low-density lipoprotein receptor (LDLR) family of endocytic receptors. These receptors are involved in diverse processes such as hemostasis, metabolism, development, etc. in various tissues. In our study, we aimed to investigate RAP's role as chaperone for receptors of this family and investigate mechanisms of these interactions. RAP co-expression in an insect cell-based system, size-exclusion chromatography, PAGE, and Western Blotting were used to analyze the protein yields and molecular form distribution. Circular

dichroism spectroscopy was used to analyze protein folding, mutagenic analysis was used to determine importance of the interactive residues of the proteins, and surface plasmon resonance was used to analyze the binding affinities. Molecular modeling and docking were used to simulate protein interactions. We found that protein yields, folding quality, and binding characteristics were improved upon co-expression of RAP with clusters of complement repeats (CR) representing the ligand-binding portions of LDLR, vLDLR, and LRP1; simultaneously, while the yields of respective multimeric forms were decreased. These data suggest that RAP functions as a molecular chaperone for LDLR, vLDLR, and LRP1. Using LRP1 as a model receptor, we found that LRP1 and its recombinant CR-clusters II-IV and adjacent CR-doublets had similar affinities to RAP and its D1/D2 and D3 fragments. Mutational analysis confirmed both key roles of lysine pairs K60/K191 (D1/D2) and K256/K270 (D3), and conserved tryptophans on selected CR-doublets of LRP1 in respective interactions. In silico docking of a model CR-triplet and RAP confirmed the criticality of these residues, which switched upon alternative engagement of the overlapping CR-doublets in binding RAP. Taken together, these data indicate a dynamic bivalent mode of interaction between RAP and LDLR family receptors, a novel mechanism of biomolecules' interaction.

Track: Amyloid and Aggregation

Session: Allostery & Dynamics in Protein Function

ABS# 055 | Aggregation Inhibition: Mutant Gelsolin Peptides

Josephine Esposto[1], Colin Wu[2], Sanela Martic[1]

[1]Trent University, [2]Oakland University (Ontario, Canada)

Gelsolin protein is a calcium-activated, actin-modulating protein that plays a role in several biological processes present in cytosolic and secretory forms, most commonly linked to amyloidosis. Gelsolin amyloidosis is a disease characterized by several point mutations, leading to cleavage and fibrillization of the protein. This fibrillization has been linked to cardiac amyloidosis, where the D187 mutation to N or Y leads to aggregation of the peptide fragments through a specific peptide sequence: CFILDL. Recently, G167 mutation has also been identified as a relevant gelsolin mutation, leading to amyloid deposits in the kidneys, a mechanism much less understood. Thus, systematic in vitro studies were conducted in our lab to characterize the basic aggregation mechanisms of gelsolin peptides and the mutants, as well as the analyzation of the effects of the inhibitors on aggregation. The in vitro mechanistic evaluation of the aggregation propensities of specific gelsolin peptides (161RLFQVKG167, 184NNGDCFILDL) and their respective mutants were carried out by using Thioflavin T (ThT) fluorescence, turbidity assays, and transmission electron microscopy (TEM). The non-mutant peptides containing the CFILDL sequence aggregated into fibrillar network, while certain mutants modulated aggregation. In the presence of Methylene Blue (MB) and epigallocatechin gallate (EGCG) inhibitors, gelsolin peptide aggregation was largely reduced. Data indicate that the inhibitors have a dual functionality in the aggregation of the peptides, acting as both aggregation inhibitors and disaggregation promoters. The in vitro inhibition study of gelsolin peptide aggregation is a valuable model for future peptide studies and proved the feasibility of preventing and reversing gelsolin amyloidosis as a preliminary platform for screening therapies towards a viable treatment for amyloidosis.

Track: Amyloid and Aggregation

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 056 | Purification of Membrane Proteins Bereft of Membrane Mimics

James Hutchison[1], James Hayes[2], Ricardo Capone[1], Charles Sanders[1,2]

[1]Center for Structural Biology, Vanderbilt, [2]Biochemistry, Vanderbilt University (Tn, United States)

Membrane proteins are defined by hydrophobic regions that are integrated into or associated with lipid bilayers. Lipid substitutes such as detergents, polymers, and specialized amphipathic proteins have been widely used to study membrane proteins in vitro. We have developed a mutagenesis-free method to purify single pass membrane proteins with no membrane alternative present with the purified protein, here referred to as membrane-mimic free protein (MeMF protein). MeMF protein satisfies its hydrophobic needs by forming defined low-order oligomers that manifest as spherical particles that are easily reversed with the addition of detergent to a traditional "protein and micelle system". MeMF protein particles do not readily form classical protein aggregates and are stable in solution for weeks to months. It is normally difficult to deliver recombinant membrane proteins to mammalian cells due to the toxicity of membrane substitutes. MeMF protein particle size, stability and toxicity to mammalian cells are dependent on the membrane protein used to form the particles. Mammalian cells readily internalize the MeMF protein particles from solution, and we are currently exploring if these particles can be used to deliver functional protein. Regardless, the MeMF

protein particles represent a promising alternative to polymers and inorganics currently used to study endocytosis since the particles are comprised of protein and can reasonably be broken down by cellular pathways.

Track: Enzymology
Session: Allostery & Dynamics in Protein Function

ABS# 057 | Spare Parts for Oxygen-Damaged Enzymes

Mary Andorfer[1,2], Lindsey Backman[1], Sarah Bowman[3], Rebekah Bjork[1,2], Emily Ulrich[1,2], Phoebe Li[1], Santiago Yori[4], Catherine Drennan[1,2]
[1]Massachusetts Institute of Technology, [2]Howard Hughes Medical Institute, [3]Hauptman-Woodward Medical Research Institute, [4]University of California Berkeley (MA, USA)

The growing glycyl radical enzyme (GRE) superfamily plays important roles within anaerobic environments, such as the human gut microbiome. Within these O2-free environments, GREs use a glycyl radical cofactor to catalyze challenging reactions. Once installed, the glycyl radical is stable for days in vitro under strictly anoxic conditions; however, even small amounts of molecular oxygen can oxidatively cleave the polypeptide at the site of the glycyl radical and render it inactive. An abundant GRE located within many facultative anaerobes (i.e. microbes that can survive in aerobic or anaerobic conditions) is known as pyruvate formate-lyase (PFL) and is key in anaerobic metabolism. Because PFLs exist in environments that can occasionally be exposed to microaerobic conditions, a unique rescue mechanism has evolved to overcome this obstacle. A small, spare part protein termed YfiD is able to form a complex with O2-damaged PFL and, in doing so, replace the glycyl radical domain. Until recently, very little was known about the molecular details of this repair mechanism. We have used a variety of techniques, including nuclear magnetic resonance (NMR) spectroscopy, circular dichroism (CD), electron paramagnetic resonance (EPR) spectroscopy, UV-Vis spectroscopy, and isothermal calorimetry (ITC) to investigate this mechanism. Our data have led us to propose a new model for O2-damaged PFL rescue of activity by YfiD. In this model, we propose that the N-terminal half of YfiD recognizes and binds to a small region of O2-damaged PFL before the C-terminal half of YfiD can replace the glycyl radical domain of PFL. As O2-damaged PFL is highly susceptible to further truncations, this recognition mechanism could prevent YfiD from binding to severely damaged PFLs and thus maintain tight control over PFL:YfiD radical chemistry.

Track: Protein Interactions and Assemblies
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 058 | In Vitro Evaluation of Variants of Unknown Significance in the Tumor Suppressor Protein, PALB2

Davis Martin[1], Jane Lee[1], Mikaela Stewart[1]
[1]Texas Christian University (Texas, USA)

The proper functioning of the protein PALB2 is vital to preventing breast cancer formation. Upon the detection of DNA damage, PALB2 and BRCA1 interact to recruit BRCA2 to the site of DNA damage. This complex then promotes DNA double-strand break repair in order to prevent the accumulation of DNA damage that leads to breast cancer. BRCA1 and PALB2 bind via PALB2's coiled-coil domain; however, how variants of unknown significance (VUS) in this region of PALB2 affect the affinity of this binding interaction are largely unknown. Further, while some of these VUS have been studied in vivo and in cells, quantitative in vitro methods to measure their effect on binding affinity have yet to be described. Here we present an in vitro testing method for assessing the effects of VUS within the coiled-coil domain of PALB2 using isothermal titration calorimetry (ITC) titrations of the intrinsically disordered region of BRCA1. We tested the efficacy of this method by measuring the binding event of seven separate variants of the PALB2 coiled-coil domain as well as the binding event of PALB2 to BRCA1 via ITC. Previously published data for PALB2 mutant homologous recombination deficiency correlates with the defects we observe in BRCA1 binding affinities. We also observe varying thermodynamic behavior for some of the binding events between PALB2 VUS and BRCA1. We combine this data with insights from nuclear magnetic resonance to predict structural changes that occur in the BRCA1 intrinsically disorder region upon binding PALB2.

Track: Membrane Proteins
Session: Protein Evolution, Design and Selection

ABS# 059 | Design of a Chimeric KCNQ1 Channel for E. coli Expression and Studies of LQTS Variants

Kathryn Brewer[1,2], Georg Kuenze[3], Hope Woods[2,4], Hui Huang[1], Jens Meiler[2,3,5], Charles Sanders[1,2]
[1]Department of Biochemistry, Vanderbilt University, Nashville, TN 37240, USA, [2]Center for Structural Biology, Vanderbilt University, Nashville, TN 37240, USA, [3]Institute

for Drug Discovery, Leipzig University, Leipzig, SAC 04103, Germany, [4]Che (Tennessee, United States)

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 (SAD). Screens for LQTS-associated KCNQ1 mutations would be advantageous for preventing SAD. However, numerous patients carry variants of unknown significance (VUS) for which pathogenicity has not been determined. This precludes risk assessment and preventative care for at-risk patients. Previous work has been conducted on the voltage sensor domain (VSD) of KCNQ1 with nuclear magnetic resonance spectroscopy to elucidate molecular mechanisms of LQTS pathogenesis and aid VUS classification, revealing VSD destabilization as a common mechanism. The aim of this work is to conduct similar studies with LQTS variants located in the pore domain (PD). Toward this end, chimeras containing the KCNQ1 channel domain (VSD+PD) and a coiled-coil to promote tetramerization have been designed using the Rosetta modeling software suite. Models were generated using cryo-electron microscopy structures of hKCNQ1 along with three coiled-coils: the KCNQ1 HC coiled-coil, GCN4-LI, and RHCC. The latter two were previously used to guide tetramerization in Shaker and/or KcsA channels. Chimera models lacking a linker between KCNQ1 and coiled-coil domains contained distorted S6 helix bundles in the PD, hinting at strain in S6 caused by oligomerization of the coiled-coils. Introducing polyglycine linkers between the PD and coiled-coils relieved structural distortion. Perturbations in the PD could be relieved in RHCC chimeras with shorter linkers than GCN4 and HC, likely because RHCC and the S6 helix bundle share coil handedness. Work is currently underway to express promising constructs in E. coli and develop a purification scheme. Once established, PD variants will be studied to explore structural perturbations in LQTS.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 061 | The Effects of Load on the Conformations of A6 TCR in Complex with Peptide-Bound MHC

Ana C. Chang-Gonzalez[1], Wonmuk Hwang[1,2,3,4], Robert J. Mallis[5,6,7], Matthew J. Lang[8,9], Ellis L. Reinherz[7,10,11]

[1]Dept. of Biomedical Engineering, Texas A&M University, [2]Dept. of Materials Science & Engineering, Texas A&M University , [3]Dept. of Physics and Astronomy, Texas A&M University, [4]School of Computational Sciences, Korea Institute for Advanced St (TX, United States)

Mounting experimental evidence suggests that the αβT-cell receptor (αβTCR) acts asa mechanosensor when bound to an antigenic peptide-loaded major histocompatibilitycomplex (pMHC). This interaction exhibits a "catch bond" behavior, where the lifetimeof the TCRαβ-pMHC bond increases upon application of physiological piconewton-levelforce. In our recent work using molecular dynamics (MD) simulations of the JM22 TCRcomplexed with HLA-A2 with a peptide from an influenza matrix protein, we found thatthe subdomain motion within TCRαβ influences the pMHC binding behavior, which iscontrollable by the applied load. This model elucidates how an applied load allostericallyinfluences the binding between TCRαβ and pMHC in forming a catch bond. To examinehow this mechanism applies to other TCRαβ-pMHC systems, we perform all-atom MDsimulations of the A6 TCR with Tax peptide mutants that vary in equilibrium bindingaffinities. We apply realistic loads (10–15 pN) in our simulations and analyze the in-terfacial contacts as well as intra-TCRαβ domain contacts. Relations between contactdynamics and the subdomain motion are also analyzed. Our results indicate contact in-stability at the TCR-pMHC interface with the low load weak agonist similar to the JM22TCR study. This work expands on our understanding of the role of mechanical forcein αβTCR peptide discrimination, illuminating the prior conundrum of nearly identicalA6 TCR structural interactions among the Tax-related pMHC ligands in crystallographiccomplexes despite their promulgation of vastly different T cell activation signals.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 062 | Dynamic features of the unactivated c-Cbl

Tianyi Yang[1], Shuanghong Huo[1]
[1]Clark University (MA, United States)

c-Cbl is a single-subunit RING E3 ubiquitin ligase. It attenuates receptor tyrosine kinase signal transduction. X-ray structures show that in the absence of E2 c-Cbl adopts a closed conformation before it binds with its substrate while after the substrate binding, the RING

is partially open, which is the first step of phosphorylation-dependent activation of c-Cbl. Previous NMR studies of Cbl-b, another protein in the Cbl family, have shown that the RING domain is in a dynamic equilibrium between several forms. As a first step to elucidate the details of the phosphorylation-dependent activation mechanism, molecular dynamics simulations have been used to investigate the dynamic features of the closed form of c-Cbl and the partially open state bound with the substrate at neutral pH and different salt concentrations. We have found that salt concentrations play an important role in the conformations of the RING domain. The high salt concentration (4.2 M) under which the X-ray structure was resolved favors the closed-form of free c-Cbl. At a lower salt concentration, the closed-form is in equilibrium with the partially-open form. Substrate binding stabilizes the partially-open form. Simulations of mutants help to pinpoint the roles of key residues in the conformational transitions. Our simulations provide new insights into the c-Cbl conformational transitions.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 063 | Structure Based Design of a Photoswitchable Affibody Scaffold

Ryan Woloschuk[1], Maximilian Reed[1], Anna Jaikaran[1], Voula Kanelis[1], Maruti Uppalapati[2], Andrew Woolley[1]
[1]University of Toronto, [2]University of Saskatchewan (Ontario, Canada)

Affibodies are small 3 helix binding proteins that can be evolved using high-throughput techniques, such as phage or yeast display to bind a variety of medically and industrially relevant target proteins. In applications like cell signalling or drug delivery, the ability to control when and where an affibody makes binding interactions may be useful. Such control of affibody activity could be achieved if binding occurred in a light dependent manner, where affibody binding is abolished or decreased in the dark and restored upon exposure to light. To this end, we developed a line of affibody photoswitchable protein fusions with the goal of creating a light-controlled affibody. We chose to engineer the Z domain, a well characterized affibody which binds the Fc portion of human IgG and fuse it to photoactive yellow protein (PYP) a blue light absorbing photoswitchable protein. To test our hypothesized designs, several Z domain PYP fusions were created and characterized in vitro and in vivo in yeast. The chimeric proteins were found to undergo light mediated changes in binding affinity in vitro and allowed for light-controlled gene expression in yeast when used in a yeast two hybrid format. These initial results appear encouraging and following future refinement could eventually result in the creation of useful affibody based light controlled tools.

Track: Computational Modeling/Simulation

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 064 | DES3PI: a Fragment-based Approach to Design Peptides Targeting Protein-Protein Interactions

Maxence Delaunay[1], Tâp Ha Duong[1]
[1]Université Paris-Saclay (Ile-De-France, France)

Protein-protein interactions (PPI) play crucial roles in many cellular processes and their deregulation often leads to cellular dysfunctions. One promising way to modulate PPI is to use peptide derivatives that bind their protein target with high affinity and high specificity.Peptide modulators are often designed using secondary structure mimics. However fragment-based design is an alternative emergent approach in the PPI field. Most of the reported computational fragment-based libraries targeting PPI are composed of small molecules or already approved drugs, but according to our knowledge, no amino acid based library has been reported yet. In this context, we developed a new fragment-based approach called DES3PI (Design of Peptides targeting Protein-Protein Interactions) with a library composed of single amino-acids. Our goal is find the optimal sequence of cyclic peptides that will bind a given protein surface with high affinity.Each amino acid of the library is docked into the target surface using Autodock Vina. The resulting binding modes are geometrically clustered, and in each cluster, the most populated amino acids are determined and form the hot spots that will compose the optimal cyclic peptide.This approach has been applied on three proteins: Mcl1, Ras, and Abeta. For each target, the five best peptides determined by DES3PI have been tested in silico. First, the peptides were blindly redocked on their target. Between two and four of the five peptides have binding modes close to the DES3PI predictions among the top 2 % of the all the redocked conformations. Secondly, the stability of the "good" redocked complexes has been verified using 200 ns MD simulations.DES3PI shows encouraging results with almost all the peptides that sucessfully pass the redocking step.

Track: Intrinsically Disordered Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 065 | Diversity of tau protein: microtubule binding, phosphorylation and aggregation

Sanela Martic[1]
[1]*Trent University (Ontario, Canada)*

Tau is a neuronal protein and one of the biomarkers of neurodegenerative diseases, such as Alzheimer's disease. Tau protein maintains microtubule structure, undergoes post-translational modifications, aggregation, and is a viable drug target. In addition, this protein is a vital biomarker in biological fluids towards early detection of neurodegenerative diseases. Given the diversity of tau structure and function, we explored various aspects of tau biochemistry: 1) microtubule (MT)-tau interactions, 2) tau phosphorylation, and 3) tau aggregation. The biophysical studies were used to probe how phosphotau proteins modulate MT formation. Using various protein kinases, MARK-4, Fyn and GSK-3β, phosphotau proteins were generated and characterized for their ability to aggregate. We discovered that MT formation is dependent on the phosphotau forms. In addition, using antitau antibodies and small molecules we illustrated that phosphorylation may be inhibited and aggregation prevented. By using novel electrochemical methods, the following events were monitored: tau-tau, tau-ferritin, tau-transferrin, and tau-heparin interactions, and phosphorylations. Electrochemical methods are useful tools for analysis of various aspects of tau biochemistry, including phosphorylation, aggregation, as well as tau biomarker detection.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 066 | Proline-rich motifs exploit sequence context to target actin remodeling Ena/VASP proteins

Theresa Hwang[1], Sara Parker[2], Samantha Hill[2], Meucci Ilunga[1], Robert Grant[1], Venkatesh Sivaraman[1], Ghassan Mouneimne[2], Amy Keating[1]
[1]*MIT,* [2]*University of Arizona (MA, United States of America)*

Protein interactions involving short, linear motifs (SLiMs) underlie diverse cellular processes, yet it remains unclear how SLiMs discriminate between paralogous but biologically distinct SLiM-binding proteins. The Ena/VASP proteins ENAH, VASP, and EVL bind to a 5-residue SLiM prevalent in the proteome and perform distinct cellular functions despite sharing high sequence similarity. To interrogate how the sequence context of SLiMs impacts Ena/VASP interaction specificity, we performed a proteomic screen against the ENAH EVH1 domain. We discovered unexpected ways in which local and distal motif-flanking sequence modulates binding. Particularly notable is a peptide from PCARE that achieves paralog specificity by stabilizing an ENAH-specific conformation. A PCARE-derived peptide selectively recruits ENAH and blocks its activity in cells, establishing it as a valuable reagent to disentangle the roles of Ena/VASP paralogs and a potential agent for modulating ENAH function in breast cancer. Using information about flanking sequences, we designed the tightest known ENAH EVH1 binder with a dissociation constant of 50 nM and 400-600-fold selectivity over EVL and VASP. Our work demonstrates that sequence context plays a prominent role in dictating SLiM interactions and can inform the design of molecules that target SLiM-based interactions.

Track: Enzymology
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 067 | Identification of Cancer-Associated Mutations that Disrupt Protein Arginine Methyltransferase 1 (PRMT1) Activity and Oligomerization

Owen Price[1], Abhishek Thakur[2], Ariana Ortolano[1], Arianna Towne[1], Orlando Acevedo[2], Joan Hevel[1]
[1]*Utah State University,* [2]*University of Miami (Utah, United States)*

Arginine methylation is a set of widespread post-translational modifications used to help control a variety of signaling processes, including processes that regulate transcription, DNA repair, and apoptosis. These modifications are mediated by the Protein Arginine Methyltransferase (PRMT) family of enzymes. Aberrant arginine methylation occurs in many cancer cells but the mechanisms by which the PRMTs contribute to cancer pathogenesis are still poorly characterized. PRMT1 is the predominant PRMT in mammalian cells which targets histones, transcription factors, splicing factors, and other proteins. [JH1] This enzyme has been shown to form at least a dimer through interactions occurring between a Dimerization Arm of one

monomer, and a Rossman Fold on the other monomer. Although the mechanisms that underlie the requirement for dimerization remain unclear, it is well documented that mutations to the dimer interface often disrupt oligomerization and activity. We searched the Catalogue of Somatic Mutations in Cancer (COSMIC) database to identify potentially inactivating mutations occurring in the PRMT1 dimerization arm. We identified three mutations corresponding to W197L, Y202N, and M206V substitutions (numbering for rat PRMT1 V1). Using a combination of site-directed mutagenesis, analytical ultracentrifugation, native PAGE, and activity assays, we found that these substitutions disrupt oligomer formation and substantially impair activity. Molecular dynamics trajectories suggest that these substitutions induce structural perturbations across the entire protein and likely disrupt activity by altering conformational dynamics. These studies provide a rationale for the contribution of these mutations to cancer pathogenesis in which PRMT1 inactivation results in perturbed cell signaling processes. Further studies will be needed to elucidate which of the pathways mediated by PRMT1 are most significantly affected. [JH1]The way this sentence is structured implies that PRMT1 methylates multiple histones but I think H4 is the major one, correct? Perhaps take out the word "variety"

Track: Enzymology

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 068 | Investigating the Impact of N-Terminal Alterations of PRMT1 on Oligomeric Structure

Arianna Towne[1], Owen M Price[1], Joan M Hevel[1]
[1]*Utah State University (Utah, United States)*

Protein arginine methylation is catalyzed by a family of protein arginine methyltransferases known as the PRMTs. These enzymes play a critical role in eukaryotic cell health by modulating transcription, DNA repair, cell cycle progression, and the oxidative stress response. Key to understanding the basis for how PRMT enzymes manipulate cellular processes is understanding how PRMT activity is regulated in the cell. One mechanism that has been proposed to regulate PRMT activity is protein oligomerization. This study aims to clarify the relationship between N-terminal alterations of the PRMT1 isoforms and their oligomerization using physiologically relevant concentrations of protein. Several studies suggest that at least a dimer is required for PRMT1 enzymatic activity, and that higher order PRMT1 oligomers may display increased activity; however, many of the studies have been performed at concentrations outside of what is physiologically relevant (e.g., 10-100 mM rather than the physiological ~100 nM). Characterization of the oligomeric state of various PRMT1 N-terminal splice variants and fusion constructs were performed at physiological concentrations of protein using Native PAGE. These studies will serve as a baseline for understanding how changes to the N-terminus of the PRMT1 protein may affect oligomerization and activity.

Track: Enzymology

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 069 | Characterization of Physiological Concentrations of Protein Arginine Methyltransferase 1 (PRMT1), Oligomerization, and Associated Methyltransferase Activity

Drake Comer[1], Ariana Ortolano[1], Owen Price[1], Joan Hevel[1]
[1]*Utah State University (Utah, United States)*

Protein arginine methyltransferases (PRMTs) catalyze the addition of a methyl group onto the terminal guanidino nitrogens of arginine residues in their protein targets. These enzymes play a crucial role in biology, however much of their basic biochemistry remains uninvestigated. The expression of the predominant isoform, PRMT1, varies dramatically in different cellular states. However, the endogenous concentration of PRMT1 present in mammalian cells has never been quantified. Because oligomerization as a dimer is critical for baseline PRMT1 activity and higher order oligomers have been suggested to enhance activity, knowledge of the physiological PRMT1 concentration and an understanding of how oligomerization affects activity at this concentration is important. Through quantitative western blotting we determined that concentration of PRMT1 in several human cell types is in the nanomolar range. We used a combination of sedimentation velocity and Native PAGE along with strategically designed oligomeric state protein standards to show that the major oligomeric species for PRMT1 at concentrations ranging from 20 nM to 3 uM is a tetramer. Intriguingly, at physiological concentrations, the in vitro methyltransferase activity of wild-

type (tetrameric) PRMT1 was identical to a dimeric PRMT1 variant using saturating R3 peptide or hnRNPK protein as substrates. These findings provide evidence of the predominant oligomer adopted by PRMT1 at physiological concentrations, and suggest that formation of the tetramer is not necessary for at least some PRMT1 targets.

Track: Enzymology
Session: Allostery & Dynamics in Protein Function

ABS# 070 | Is Protein Arginine Methyltransferase 1 (PRMT1) Activity Regulated by Oligomerization?

Joan Hevel[1]
[1]*Utah State university (UT, United States)*

Protein arginine methyltransferases (PRMTs) are integral to mammalian cell function, impacting nearly every aspect of cellular biology and cell health. Determining how PRMT activity is regulated in cells is important not only for understanding normal cell function and communication but also could lead to new avenues to manipulate PRMT activity. Given this and recent reports of PRMT1 oligomerization, we have sought to characterize the oligomeric species that exists at physiological concentrations and understand how oligomerization may control methyltransferase activity. Using quantitative Westerns, we show that PRMT1 is present in low nM quantities in a handful of cultured mammalian cells. Using analytical ultracentrifugation and Native PAGE we observe a tetrameric species at a range of concentrations up to low uM. Methyltransferase activity of the tetramer on a single peptide and protein substrate is identical to activity of a tetramer-interface mutant that exists as a dimer. Mutations of the dimer interface as represented by three single amino acid substitutions deposited in the Catalogue of Somatic Mutations in Cancer (COSMIC) database show impaired dimerization and a corresponding impaired methyltransferase activity. Computational modeling suggests that even a single mutation in the dimerization interface is sufficient to alter the geometry of dimer formation, which may explain why these cancer-related mutants do not form tetramers. Our studies define the dimer of PRMT1 as the minimal unit for maximal activity for the peptide and protein substrates studied. However, given the breadth of PRMT1 targets, we are currently investigating if dimeric and tetrameric PRMT1 preferentially methylate specific substrates. Our studies also suggest that the higher-order structures observed in previous studies only exist in vitro, and are likely not involved in regulating PRMT1 activity in vivo.

Track: Evolution
Session: Protein Evolution, Design and Selection

ABS# 071 | Alterations in the stability and hetero-oligomerization of the p53 family in vertebrates

Shuya Sakaguchi[1], Natsumi Nakagawa[1], Haytham Wahba[2], Rui Kamada[1,3], James G. Omichinski[2], Kazuyasu Sakaguchi[1,3]
[1]*Laboratory of Biological Chemistry, Division of Chemical Sciences and Engineering, Graduate School of Chemical Sciences and Engineering, Hokkaido University, Sapporo, Japan,* [2]*Department of Biochemistry and Molecular Medicine, University of Montreal (Hokkaido, Japan)*

The p53 family includes the tumor suppressor protein p53 and two related proteins, p63 and p73. Tetramer formation of p53/p63/p73 proteins is essential for their functions. The tetramerization domain (TD) of human p53 is composed of a β strand, a tight turn, and an α helix. Human p63 and p73 form hetero-oligomers and negatively regulate the function, while p53 does not form hetero-oligomers with p63 or p73. In this study, we analyzed changes in the structure and thermostability of the TD of p53 and p63 from different vertebrate species, ranging from the jawless fish Lamprey through to birds and mammals. Our results indicate that the p53 TDs of lamprey, shark, gar, catfish and zebrafish contain a 2nd α-helical segment, but the TD of coelacanth has lost the second segment. Thermostability assays demonstrate that despite losing the 2nd α-helical segment during evolution, the p53 protein of higher organisms possess more stabile tetrameric structures. Interestingly, the ability to form p53/p63 hetero-oligomers also changes during the evolutionary process as lamprey and gar form the hetero-oligomers, but zebrafish, coelacanth, frog and human do not. Our results strongly suggest that changes in the structure and thermostability of the p53 TD have occurred in response to important environmental changes such as exposure to UV irradiation and higher oxygen concentration. In addition, the loss in ability of p53 and p63 to form hetero-oligomers between each other may have played an impotant evolutionary role in p53 and p63 aquiring new functions.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 072 | Computational design of bioactive protein switches with multi-logics for cell-based therapeutics

SAILAN SHUI[1], Pablo Gainza[1], Leo Scheller[1], Che Yang[1], Bruno Correia[1]
[1]*École Polytechnique Fédérale de Lausanne (LAUSANNE, Switzerland)*

Small-molecule responsive protein switches are crucial components to control synthetic cellular activities. However, the repertoire of small-molecule protein switches is insufficient for many applications, including those in the translational spaces, where properties such as safety, immunogenicity, drug half-life, and drug side-effects are critical. Here, we present a computational protein design strategy to repurpose drug-inhibited protein-protein interactions as OFF- and ON-switches. Our previous work on computationally designed small-molecule responsive switches led to the development of chemically disruptable heterodimers (CDHs), consisting of a pair of designed interacting domains, which dissociates in the presence of a specific small molecules. The 1st generation of CDHs was used to safeguard the CAR-T cell functions in vivo1. To design ON-switches, we converted the CDHs into a multi-domain architecture which we refer to as activation by inhibitor release sensors (AIR) that incorporate a rationally designed drug-insensitive receptor protein. The AIRs assemble upon the release of designed binders from the pre-fused CDHs. CDHs and AIRs showed excellent performance as drug responsive switches to control diverse combinations of synthetic circuits, including transgene expression and endogenous receptor signaling. The switches respond to several pre-clinical and clinically approved drugs, and are exciting candidates for controlling the activity of synthetic cells with human clinical relevance.

Track: Membrane Proteins
Session: New Protein Post-Translational Modifications

ABS# 073 | Rapid Investigation of Terminal Oxidase Functionality in Escherichia coli

Hojjat Ghasemi Goojani[1], Henk Hakvoort[1], Holger Lill[1], Dirk Bald[1]
[1]*Department of Molecular Cell Biology, Amsterdam Institute of Molecular & Life Sciences Faculty of Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands. (North Holland, The Netherlands)*

In the bacterial respiratory chain electrons are transferred from reducing equivalents along respiratory complexes onto terminal acceptors. In case of oxygen as electron acceptor the enzymes catalyzing the final step are called terminal oxidases. Next to this importance in fundamental biology, terminal oxidases in various bacteria have been implied in bacterial biofilm formation, pathogenicity and the defense against antibacterials. Here, we introduce an experimental system for rapid investigation of terminal oxidase functionality in Escherichia coli. E. coli employs three terminal oxidases, one heme-copper type enzyme (cbo3) and two cytochrome bd-type quinol oxidases, called bd-I and bd-II. We utilize E. coli strain MB43, which due to multiple knock-outs in the respiratory chain displays strongly reduced growth. Expression of cytochrome bd-I in MB43 significantly increased growth yield, revealing that terminal oxidase functionality can be evaluated by measuring bacterial growth, without the need for time-consuming purification of the expressed protein. First, we used this experimental system to assess the functionality of heterologously expressed cytochrome bd from Mycobacterium tuberculosis and of E. coli cytochrome bd-II. The former was not functional, while the latter significantly enhanced bacterial growth. We were able to purify both cytochrome bd-I and bd-II, but not the enzyme from M. tuberculosis, validating our approach. This experimental system allowed for rapid characterization of cytochrome bd-I mutants, involving various sequences, domains and subunits of this enzyme. Our results revealed that the cytochrome bd-I Q-loop, a domain important for substrate binding, displays surprisingly low plasticity. We also showed that neither bd-I nor bd-II are functional in the absence of cydDC, an ABC transporter has been implied in biogenesis of cytochrome bd. Taken together, an E. coli strain lacking terminal oxidases may be a useful tool for a variety of purposes, including characterization of mutants, heterologously expressed respiratory proteins or assembly factors.

Track: Bioinformatics
Session: Protein Evolution, Design and Selection

ABS# 074 | Database of Causitive Genes/Proteins for Common "Rare" Monogenic Mendelian Disorders

Tucker Apgar[1], Charles Sanders[2]
[1]*Vanderbilt University College of Arts & Science,*
[2]*Department of Biochemistry and Center for Structural*

Biology, Vanderbilt University School of Medicine Basic Sciences (Massachusetts, United States)

The objective of this study is to create a database of common "rare" Mendelian disorders and their associated genes and encoded proteins to aid future drug discovery efforts. "Simple" Mendelian diseases are rare genetic disorders with phenotypes linked to pathogenic variants in single genes. Although these disorders are considered promising drug targets due to their simple etiology, their rare nature has historically discouraged drug discovery efforts in the pharmaceutical industry. However, a number or these diseases are not that rare. We initially compiled a list of common "rare" disorders documented in ORPHANET, which we define as those disorders that have a prevalence or incidence of higher than 5.0 (1 in 20,000 people) and that are known to be caused by single mutations in any one or more genes. After narrowing down the list of diseases, we catalogued the associated proteins targets, gene targets, and relevant nonsense/missense mutations from the Human Gene Mutation Database (HGMD). Clinical genetics reviews were also used to confirm the identities of genes implicated in the specific disorder. To confirm the Mendelian basis for each disorder, disease etiology was corroborated based on data compiled from genome and rare disease databases (ORPHANET, Online Mendelian Inheritance in Man (OMIM), Genetic and Rare Diseases Information Center (GARD), PubMed, and GeneReview and relevant literature reviews. 68 common "rare" Mendelian disorders have been documented with associated gene/protein targets and the number of single nucleotide variants (SNVs) documented in the most commonly-causitive gene. The current database provides list of simple Mendelian disorders that have a high enough incidence in the population to motivate drug discovery efforts even by those who, for various possible reasons. wish to conduct drug discovery to target "rare" disorders that afflict a relatively high number of people.

Track: Single Molecule Studies

Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 076 | The 3'-5' Exonuclease Dis3L2 Undergoes Conformational Dynamics During Structured RNA Degradation

Katarina Meze[1,2], Armend Axhemi[1], Dennis Thomas[1], Leemor Joshua-Tor[1,3]

[1]W. M. Keck Structural Biology Laboratory, Cold Spring Harbor Laboratory, [1]Bungtown Road, Cold Spring Harbor, New York 11724, USA, [2]Cold Spring Harbor Laboratory School of Biological Sciences, [1]Bungtown Road, Cold Spring Harbor, New York 11724 (NY, USA)

Effective RNA degradation is crucial to achieving timely control of gene expression and maintaining cellular homeostasis. The 3'-5' exonuclease Dis3L2 plays a central role in the 3'-end degradation of many coding and non-coding cytoplasmic RNAs. Loss of Dis3L2 causes the congenital fetal overgrowth disorder Perlman Syndrome, and its dysregulation has been implicated in the pathogenesis of various cancers including hepatocellular carcinoma. Dis3L2 is a homolog of the RNA exosome associated nucleases Dis3 and Dis3L, and is structurally highly similar to them as it contains two cold shock domains (CSD), an RNA binding domain (RNB) and an S1 domain. Unlike Dis3 and Dis3L, which function predominantly as part of the RNA exosome, Dis3L2 acts independently. Direct comparison of these homologs has shown that Dis3L2 degrades structured RNA substrates much more efficiently. We sought to determine the mechanism of structured RNA degradation by Dis3L2, given its unique functional characteristics among the Dis3 family nucleases. To this end, we combined single particle electron cryo-microscopy (cryoEM) studies and pre-steady state kinetic analysis at single nucleotide resolution to build a step-by-step model of structured RNA degradation. Together, these methods have enabled us to reconstruct a detailed model of structured RNA degradation by Dis3L2. We have defined the kinetic parameters governing each step of degradation, determined several structures representing snapshots along the degradation pathway, discovered a novel conformational change and the key subdomain involved in strand separation. This has allowed us to understand how efficient degradation of structured RNA is achieved, and the factors responsible for the observed differences between Dis3L2 and its homolog Dis3.

Track: Computational Modeling/Simulation

Session: Allostery & Dynamics in Protein Function

ABS# 077 | Development of an Accelerated Molecular Dynamics of Proteins based on the Bond-boost Method

Soon Woo Park[1], Moon-ki Choi[2], Byung Ho Lee[1], Woo Kyun Kim[3], Moon Ki Kim[1]

[1]Sungkyunkwan University, [2]University of Minnesota, 3University of Cincinnati (Gyeonggi, South Korea)

Over the last few decades, molecular dynamics (MD) has become a powerful tool to calculate various

thermodynamic properties of biomolecules. Despite increasing the simulation techniques and the computing power, however, it is still hard to run MD simulations of large deformations of proteins for a long time because of their high computational cost. To overcome this limitation, various accelerated MD techniques have been developed and among them, the bond-boost method (BBM) has been widely used in material science by adding a bias potential to accelerate the escape from energy basin. But, it is still limited to be adopted in protein simulation due to the complexity of protein structures.In this study, we present a modified BBM that is optimized to sample the conformational space of proteins. There is a bias potential defined by the dihedral angles of backbone which is dominant in the conformational change of proteins. Moreover, in order to validate the proposed BBM, we carried out accelerated MD simulation for adenylate kinase and compared its catalytic cycle with the conventional MD simulation results. Remarkably, the proposed accelerated MD simulation reproduced a transition pathway from closed to open form in a timely fashion although it didn't happen for 100 ns in the conventional MD simulation. Consequently, the modified BBM is expected to play a key role in protein dynamics simulation.

Track: Therapeutics and Antibodies
Session: Protein Evolution, Design and Selection

ABS# 079 | Tuning Antibody-Antigen Binding through Glycan Masking

Cristina Martina[1], Clara Schoeder[1], Iuliia Gilchuk[2], James Crowe[2], Jens Meiler[1]
[1]Department of Chemistry and Center for Structural Biology, Vanderbilt University, Stevenson Center, Nashville, USA, [2]Vanderbilt Vaccine Center, Vanderbilt Medical Center, Nashville, Tennessee, USA (TN, USA)

Viruses are able to escape recognition by the immune system by masking their glycoproteins with a glycan shield that blocks the binding of antibodies to amino acid residues. This technique, referred as glycan masking, was recently adopted in the vaccinology field to accomplish a similar result: hiding epitopes of low interest from the immune system.In this work the ability and efficiency of the glycan masking technique is tested to selectively tune the binding of different antibodies to the same antigen. The head domain of the hemagglutinin protein (HA) of influenza A H7 was chosen as antigen. This molecule presents four distinct major antigenic sites, targeted by four monoclonal antibodies whose structures in complex with HA are publicly available on RCSB-Protein Data Bank: FluA-20 (pdb ID: 6OCB), H7.5 (6MLM), H7.167 (5V2A) and H7.200 (6UIG).With the use of Rosetta, a software for protein modeling and design, each of the four binding interfaces was analyzed, 10 key residues on the H7-HA head domain were selected and glycan chains were modeled in each position individually. In order to mimic glycan flexibility, each carbohydrate chain was modeled 100 times. Antibodies then were docked to the antigens in the presence of the glycan chain. In all 10 cases, the resulting binding energy was worse compared to the wild-type HA, as expected. Six out of the 10 designs of H7-HA head domain were selected for experimental validation and were successfully expressed in human embryonic kidney (HEK) cells. Binding to the antibodies was tested through an enzyme linked imunosorbent assay (ELISA) and four out of the six proteins successfully blocked the binding of the corresponding antibody. This work demonstrates that glycan masking can specifically regulate antigen-antibody binding, and the success of this approach opens new perspectives in the field of vaccine design.

Track: Proteomics
Session: Protein Evolution, Design and Selection

ABS# 080 | Proteomic Analysis of Human Breast Milk to Reveal Potential Protein Biomarkers for Breast Cancer

Danielle Whitham[1], Roshanak Aslebagh[1], Devika Channaveerappa[1], Brian Pentecost[2], Kathleen F. Arcaro[2], Costel C. Darie[1]
[1]Clarkson Univeristy, [2]University of Massachusetts Amherst (New York, United States)

Breast cancer (BC), one of the most common cancers, is a leading cause of death for women in the United States. An estimated 1 in 8 women in the United States will develop BC in their lifetime. Early diagnosis and treatment of BC is crucial, and protein biomarkers for this disease could make this possible. Mass spectrometry (MS)-based proteomic methods are ideal for the investigation of protein biomarkers. This study employs MS-based proteomics to study the protein differences in human breast milk from women with BC and matched controls. If significant protein dysregulations are revealed, they could be considered potential future protein biomarkers of BC for diagnosis

and treatment. In this study, six human breast milk samples were analyzed by performing a 2D-SDS-PAGE and further analyzed via nanoLC-MS/MS. The human breast milk samples consisted of three comparison pairs of BC vs. control. There were one Coomassie blue-stained gel (6 total gels) and two replicate silver-stained gels (12 total gels) for each sample of breast milk. The silver-stained gels were scanned using a laser densitometer and the images were analyzed using Progenesis Same Spots and Progenesis PG240 software. The spot percentages were measured for each dysregulated spot., An in-gel trypsin digestion was performed for each spot with statistically significant dysregulation. This was followed by nanoLC-MS/MS, data processing, database search and statistical analysis. The dysregulated proteins found in this study can be investigated as BC biomarkers for future clinical methods for early diagnosis and treatment of BC.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 081 | Can we design highly active de novo enzymes by focusing exclusively on the active site?

Niayesh Zarifi[1], Joey Olmos[2], James S. Fraser[2], Roberto A. Chica[1]

[1]Department of Chemistry and Biomolecular Sciences, University of Ottawa, 10 Marie Curie, Ottawa, ON, K1N 6N5, Canada, [2]Department of Bioengineering and Therapeutic Science, University of California, San Francisco, San Francisco, CA, 94158, USA (Ontario, Canada)

De novo enzyme design has broad applications in biotechnology, medicine, and industry. Despite significant advancements in this field, the activity levels of designed enzymes are orders of magnitude lower than natural enzymes. To increase activity, designed enzymes have been subjected to directed evolution, resulting in activity-enhancing mutations both within and far from the active site. Here, we investigate the impact of distal mutations on the catalytic efficiency of artificial enzymes to evaluate whether it is possible to design highly active de novo enzymes by focusing solely on the active site. To do so, we created variants of de novo designed Kemp eliminases that contain active-site mutations found during evolution but not distal ones. Kinetic analyses of these enzymes, which we call "core variants", revealed that they display 10 to 1,000-fold higher catalytic efficiencies than the original designs. We also show that it is possible to recapitulate the active site structure of several evolved enzymes using ensemble-based computational design methods. Taken together, our results demonstrate that it should be possible to design de novo enzymes with higher activities than previously shown.

Track: Membrane Proteins

Session: Allostery & Dynamics in Protein Function

ABS# 082 | Molecular determinants of lipid-modulation of the cyclic nucleotide-gated channel SthK

Philipp Schmidpeter[1], Di Wu[2], Jan Rheinberger[1], Haiping Tang[2], Carol Robinson[2], Crina Nimigean[1]

[1]Weill Cornell Medicine, [2]University of Oxford (NY, USA)

Hyperpolarization activated and cyclic nucleotide-modulated (HCN) channels are key players in electrical signaling related to pacemaking in the heart and brain. Analysis of these proteins under defined conditions is obstructed by the amounts of purified protein available from heterologous expression systems. To that end, a bacterial homolog from Spirochaeta thermophila, SthK, is used as model system to study the regulation of these proteins in vitro, using purified protein under controlled conditions. SthK shows high sequence and structural homology to its eukaryotic counterparts, can be easily obtained from bacterial expression hosts and is amenable to many biophysical and structural assays. Here, we systematically study how different lipids modulate SthK channel activity. A stopped-flow flux assay revealed that negatively charged lipids increase SthK channel activity. Single-channel recordings showed that these lipids increase the open probability of the channel. Mass-spectrometry based lipidomics identified that anionic lipids are copurifed with the protein from the bacterial membrane and native mass spectrometry suggested that one lipid in particular is important for the structural stability of tetrameric SthK. cryoEM was employed to structurally resolve SthK-lipid interactions. Based on the location of the lipids a mutational approach combined with functional experiments led to the hypothesis that a salt-bridge at the bundle crossing is responsible for lipid sensitivity. This interaction is modulated by anionic lipids explaining the differential effect of lipids on SthK channel activity. The conservation of this salt-bridge between SthK and HCN but not the closely related CNG channels, might explain differences in lipid modulation of these proteins.

Track: Intrinsically Disordered Proteins
Session: Measuring Forces of Biological Systems

ABS# 083 | Protein-RNA phase-separated condensates are viscoelastic fluids with tunable material properties

Ibraheem Alshareedah[1], Priya R Banerjee[1]
[1]*University at Buffalo (NY, United States)*

Liquid phase condensation of intrinsically disordered polypeptides underlies numerous physiological functions in the cell such as stress response and transcriptional control. The material state of a protein condensate dictates the rate of molecular exchange with its environment and the rate of molecular diffusion within the condensates. Importantly, the material properties control the response of condensates to external mechanical stress and deformation. However, the molecular features of a protein chain that determine the material state dynamics of its condensates across different time scales remain far from understood. Here, we implement passive microrheology with optical tweezers to characterize the dynamical behavior of designer protein-RNA condensates in vitro. Utilizing synthetic disordered polypeptides with stickers-and-spacers architecture, we show that protein-RNA condensates exhibit time-dependent viscoelastic properties similar to a Maxwell Fluid. At short time scales, these condensates display elastic behavior while at long time scales, viscous properties dominate. The temporal behavior and the viscosity of these condensates are strongly governed by the identity of the sticker as well as the spacer residues, pointing to unique roles of composition and patterning of amino-acids in polypeptide chains in determining the fluid properties of their condensates. Collectively, our findings shed light on the molecular features that give rise to viscoelasticity in protein-RNA condensates and pave the way towards mapping sequence-viscoelasticity relations for multicomponent biomolecular condensates.

Track: Computational Modeling/Simulation
Session: Protein Structures Through the Lens of Machine Learning

ABS# 084 | Functional Dynamics Recognition within beta-lactamase

Christopher Avery[1], Donald Jacobs[2]
[1]*University of North Carolina Charlotte Department of Bioinformatics,* [2]*University of North Carolina Charlotte Department of Physics and Optical Science (North Carolina, United States)*

Beta-lactamase mediated resistance to beta lactam antibiotics is among the foremost threats to public health. Despite the ever-increasing number of antibiotics being developed, beta-lactamase's high susceptibility to mutations allows it to rapidly adapt to new drugs. In this work we explore the role internal dynamics play in substrate specificity in class A extended spectrum beta-lactamase. We use our new algorithm, SPLOC, to extract the functional motions that differentiate wild type from extended spectrum mutants. SPLOC is a novel supervised projection pursuit machine learning method based on a Recurrent Neural Network (RNN). SPLOC performs a projection pursuit to find generalized degrees of freedom which capture the most discriminant or indiscriminate motions between two molecules. The two molecules can represent a "functional" and "non-functional" variant of an enzyme. SPLOC can be used to perform dimensionality reduction and classification. We have simulated eight 500 nanosecond all-atom Molecular Dynamics (MD) Simulations of TEM-1, TEM-2, and TEM-52 beta-lactamase mutants. Using these simulations, we have constructed data packets which summarize the conformational diversity of each mutant across all the simulations. SPLOC was used to find motions which can differentiate the mutants. Using SPLOC's novel feature space we can see that the differentiating motions exist in all three mutants, but with varying expression. We found that when all but the discriminating motions were filtered from the MD trajectories, the remaining fluctuations were most prevalent near catalytic residues which suggests these motions impact the molecules functional capacity. Finally using SPLOCs internal classification ranking system, based upon a discovery likelihood, the three mutants were ranked in terms of likelihood that they share similar properties to the functional class, or TEM-1 for this case. We find TEM-52 to be less like TEM-1 than TEM-2 through the lens of functional motions, which mirrors the reality of their binding specificities.

Track: Amyloid and Aggregation
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 085 | Real-time Characterization of Cell Membrane Disruption by Alpha-Synuclein Aggregates in Parkinson's Disease

Jacob Parres-Gold[1], Andy Chieng[1], Stephanie Wong Su[1], Yixian Wang[1]
[1]*California State University, Los Angeles (CA, United States)*

Aggregation of the natively unfolded protein alpha-synuclein has been widely correlated to the neuronal death associated with Parkinson's disease. Mutations, protein overaccumulation, and other forms of cellular dyshomeostasis can promote the aggregation of alpha-synuclein into oligomers and fibrils. Recent work has suggested that alpha-synuclein oligomers can permeabilize the neuronal membrane to small ions, promoting calcium influx and cell death. However, the mechanism of this permeabilization has been difficult to elucidate, especially in live cell systems. This work uses scanning ion conductance microscopy (SICM) to image, in real time and without the use of chemical probes, the topographies of live SH-SY5Y neuroblastoma cells after exposure to alpha-synuclein oligomers. Substantial changes to the membrane morphology were observed, with micrometer-scale curvature changes observed at lower alpha-synuclein concentrations (1.00 μM) and additional large, transient pores observed at higher alpha-synuclein concentrations (6.0 μM). These findings suggest that alpha-synuclein oligomers may permeabilize the neuronal membrane by restructuring its architecture, such as by increasing membrane curvature and stabilizing large pores. Future work aims to further characterize the roles of various protein/membrane interactions in membrane disruption.

Track: Chaperones

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 088 | Interaction Between Ribosome-Bound Nascent Proteins and a Specific Region of the Ribosome in the Vicinity of the Exit Tunnel

Valeria Guzman-Luna[1], Andrew M. Fuchs[1], Ana Allen[1], Rayna M. Addabbo[2], Alexios Staikos[1], Silvia Cavagnero[1]
[1]University of Wisconsin, [2]University of Minnesota (WI, United States)

An accurate balance between protein synthesis, folding and degradation is needed to prevent accumulation of toxic aggregates in the cell. This balance may depend in part on how the ribosome interacts with nascent chains during the early stages of a protein's life. The influence of the ribosome on ribosome-bound nascent chains (RNCs) devoid of signal or arrest sequences is particularly poorly understood. Here, we provide explicit evidence for the RNC-ribosome interactions involving RNCs derived from the intrinsically disordered protein PIR or from the globular signaling protein apoHmpH. Both nascent proteins lack N-terminal signal sequences. We employed chemical crosslinking, Western-blotting, mutagenesis and a range of Mg2+ concentrations to explore the interaction network encompassing RNCs, chaperones and ribosomal proteins in bacteria. We found that PIR and apoHmpH RNCs interact with ribosomal proteins L23 and L29 in a chain-length dependent fashion. The interacting ribosomal proteins are clustered on one region of the ribosome, close to the exit tunnel vestibule. Mutational and Mg2+-dependence studies on PIR showed that the interactions between both negatively charged ribosomal proteins and nascent chains are Mg+2-mediated. The amino acid composition analysis of the interacting ribosomal proteins suggests the involvement of nonpolar interactions. Also, in the absence of the trigger-factor (TF) chaperone, nascent apoHmpH interacts only with ribosomal proteins, suggesting a role of the ribosome in cotranslational protein folding. In case of apoHmpH RNCs, the extent of interaction made with the ribosomal proteins affects the chain propensity to aggregate, suggesting a physiological role of the detected interactions. These findings are significant because they unravel a novel role of the ribosome, which is shown to actively engage with the nascent protein chain and might indicate a mechanism to prevent cotranslational aggregation of foldable nascent chains.

Track: Intrinsically Disordered Proteins

Session: New Protein Post-Translational Modifications

ABS# 089 | Decoding substrate selection by the Proteasome

AMIT KUMAR SINGH GAUTAM[1]
[1]The university of texas at Austin (TX, United States)

The ubiquitin-proteasome system controls cellular protein concentrations by selecting specific proteins for degradation. Proteins are targeted to the proteasome through a two-part degradation signal, consisting of a proteasome-binding tag, typically a polyubiquitin chain, and a disordered region in the substrate at which the proteasome initiates degradation. The proteasome has preferences for the amino acid sequences of the initiation region. To characterize these preferences in vivo, we developed a fluorescence-based degradation assay. We found that initiation sequences tune protein abundance over two orders of magnitude, which is the same dynamic range as obtained by controlling the ubiquitination of a classic N-end rule degron. These sequence preferences are evolutionarily conserved from yeast to mammalian cells. We have tested the entire human proteome for its ability to act as initiation regions. This peptide library was fused to the C-terminus of fluorescence substrate (YFP) and targeted to the proteasome by an N-terminal ubiquitin-like domain

(UBL). The library was expressed in yeast and sorted on 14 bins based on YFPintensity using flow cytometry. A lower YFP signal will indicate efficient initiation and vice versa. The peptide sequences in different fluorescence bins were identified using next-generation sequencing and a stability value was assigned for each sequence. The sequence and stability data were used to characterize the sequence preference for the initiation region by the proteasomal. The physicochemical property, the length, secondary structure, the position of certain residues in the sequence, and the composition complexity of the initiation sequence contribute to defining the initiation code. We build a model to predict the ability of a given sequence to initiate degradation. The initiation code that in parallel with the ubiquitin code could determine substrate selection for proteasomal degradation.

Track: Folding

Session: Allostery & Dynamics in Protein Function

ABS# 090 | Folding of Peripheral Myelin Protein 22 and Its Charcot-Marie-Tooth Disease Mutant Forms

Geoffrey Li[1], Charles Sanders[1]
[1]*Vanderbilt University (Tennessee, United States)*

Peripheral myelin protein 22 (PMP22) is a tetraspan integral membrane protein that is highly abundant in compact myelin of the peripheral nervous system. Missense mutations of PMP22 give rise to a hereditary neuropathy known as Charcot-Marie-Tooth Disease (CMTD), for which no treatment is yet available. PMP22 mutations result in dysmyelination of peripheral nerve axons due to the failure of the mutant forms to properly fold and, hence, failing to traffic efficiently within the cell and promoting CMTD. Previous studies from our lab (Schlebach et al. JACS 2015) have established a linear correlation between the thermodynamic folding stability of the different mutants and both the cell surface trafficking efficiencies and nerve conduction velocities of patients. Here, we investigated the folding of wild type PMP22 and nine of its CMTD mutant forms in detergent micelles using NMR spectroscopy. The mutations are distributed throughout the four transmembrane helices and span a broad range of neuropathy phenotypes. Using both amide and methyl TROSY experiments, we found that mutants causing severe neuropathy are all misfolded, and all adopt a similar unfolded structure, irrespective of which transmembrane helix the mutation is situated on. On the other hand, mutants that cause mild or intermediate neuropathy are either partially misfolded or are seen to have a fold that resembles wild type. These results provide insight into the issue of whether all disease mutant forms of PMP22 misfold via the same mechanism and into the same misfolded state.

Track: Computational Modeling/Simulation

Session: Protein Structures Through the Lens of Machine Learning

ABS# 092 | Simultaneous tracking of multiple dynamic microtubules decorated by the microtubule-associated protein EB1

Wonmuk Hwang[1], Goker Arpag[2], Veronica Farmer[2], Elizabeth Lawrence[2], Marija Zanic[2]
[1]*Texas A&M University,* [2]*Vanderbilt University School of Medicine (TX, United States)*

Tracking time-lapse images (e.g., taken by total internal reflection fluorescence microscopy) of dynamic microtubules (MTs) is a challenging task which has relied heavily on using kymographs of manually selected MTs. The task is more difficult in experiments involving MT-associated proteins (MAPs), where the percentage of fluorescently labeled tubulins is kept low to avoid influencing MT dynamics and interaction with MAPs, which yields noisier images. We introduce a computational method that overcomes these issues and reliably tracks most of the MTs in the field of view. Our approach involves progressive refinement based on local pixel information, and represent MTs using beads-on-chain models. Additional error correction is made by leveraging spatio-temporal continuity of MTs. We demonstrate its utility by analyzing images of MTs decorated with the MAP EB1 that tracks the MT plus end. Our method is fast, handles low signal-to-noise images, and allows simultaneous tracking of multiple MTs that cross and un-cross. We also discuss quantitative measurements by using this method.

Track: Membrane Proteins

Session: Measuring Forces of Biological Systems

ABS# 093 | Coordination of -1 Programmed Ribosomal Frameshifting by Transcript and Nascent Chain Features Revealed by Deep Mutational Scanning

Patrick Carmody[1], Matthew Zimmer[1], Charles Kuntz[1], Haley Harrington[1], Kate Duckworth[1], Wesley Penn[1], Suchetana Mukhopadhyay[1], Thomas Miller[1], Jonathan Schlebach[1]
[1]*Author (Indiana, United States)*

Coordination of -1 Programmed Ribosomal Frameshifting by Transcript and Nascent Chain Features Revealed by Deep Mutational Scanning Patrick J. Carmody,1† Matthew H. Zimmer,2† Charles P. Kuntz,1 Haley R. Harrington,1 Kate E. Duckworth,1 Wesley D. Penn,1 Suchetana Mukhopadhyay,3* Thomas F. Miller III,2* andJonathan P. Schlebach1* 1Department of Chemistry, Indiana University, Bloomington, IN USA 474052Division of Chemistry and Chemical Engineering, California Institute of Technology,Pasadena, CA USA 911253Department of Biology, Indiana University, Bloomington, IN USA 47405 †Authors contributed equally *Corresponding authors: jschleba@indiana.edu, tfm@caltech.edu, sumukhop@indiana.edu -1 Programmed ribosomal frameshifting (-1PRF) is a translational recoding mechanism that enables the synthesis of multiple polypeptides from a single transcript. In the alphavirus structural polyprotein, -1PRF is coordinated by a "slippery" sequence in the transcript, an RNA stem-loop, and a conformational transition in the nascent polypeptide chain. To characterize each of these effectors, we measured the effects of 4,530 mutations on -1PRF by deep mutational scanning. While most mutations within the slip-site and stem-loop reduce the efficiency of -1PRF, mutagenic effects upstream of the slip-site are far more variable. Coarse grained and atomistic molecular dynamics simulations of polyprotein biogenesis suggest many of these mutations alter pulling forces on the nascent chain by perturbing its interactions with the ribosome, the translocon, and the lipid bilayer. Finally, we provide evidence suggesting the coupling between cotranslational folding and -1PRF depends on the translation kinetics upstream of the slip-site. These findings provide unprecedented insights into how -1PRF is coordinated by the interplay between structural elements within the transcript and nascent polypeptide chain.

Track: Enzymology

Session: Measuring Forces of Biological Systems

ABS# 095 | Investigating the rate of dopamine oxidation by newly synthesized copper-peptide complex

Ngoc Kim Nguyen[1], Samir Das[1], Arundhati Nag[1]
[1]*clark university (Massachusetts, United States)*

Dopamine is a type of neurotransmitter that carries many important roles in the brain and body such as movement, pleasure, attention, mood, and motivation. Dopamine can be oxidized by the metal-catalyzed oxidation reactions that lead to the formation of reactive oxygen species such as aminochrome. Excess of reactive oxygen species increases the level of oxidative stress, which is considered a major contributor to a variety of neurodegenerative diseases like Parkinson's.In the oxidation of dopamine, tyrosinase participates in the oxidation pathway of tyrosine residue and forms reactive oxygen species such as aminochrome. Tyrosinase is a copper metal-catalyzed enzyme that oxidizes phenols into quinones. In the neurodegeneration of dopaminergic systems in Parkinson's disease, the tyrosinase enzyme oxidizes dopamine into dopamine o-quinone. Dopamine o-quinone cyclizes at physiological pH to leucoaminochrome that auto oxidizes to aminochrome. However, the subsequent steps of dopamine oxidation in a controlled way are still not characterized.We aimed to find a copper-small molecule complex that can act effectively as tyrosinase mimic and oxidize dopamine to different quinone forms. This complex is synthesized and kept on resin beads, as the solid support allows proximity of multiple copper centers and therefore allows its function as an enzyme mimic. We synthesized and examined the oxidation of phenolic substrate by different copper(II) complexes on resin. The oxidation of phenol and Tyr-containing peptides by the copper complex on beads was examined using spectroscopic techniques in phosphate buffer pH 9. Our results indicate that phenol and the peptide GYYY are oxidized to quinone forms when reacted with the copper complex. Our synthesized solid-supported copper(II)complex can therefore act as a tyrosinase mimic successfully.

Track: Peptides

Session: Protein Evolution, Design and Selection

ABS# 097 | Programmed Assembly of Coiled-Coil Peptides

Linhai Jiang[1], Xiaobing Zuo[2], Kent Kirshenbaum[1]
[1]*New York University,* [2]*Argonne National Laboratory (NY, United States)*

Despite recent progress, it remains challenging to program biomacromolecules to fold and assemble to discrete nanostructures. Heterodimeric coiled coils are highly attractive folding motifs and building blocks for synthetic biology, supramolecular chemistry, and materials science. We have discovered that discrete assemblies with novel topologies can be predictably obtained by using multiple pairs of heterodimeric coiled-coil peptides that are covalently linked via strategic end-to-side conjugations between the non-registered pairs. Using this strategy, we have created several unusual discrete supramolecular assemblies that are composed of a defined number of coiled-coil

components. SEC-MALS (size exclusion chromatography in-line with multi-angle laser scattering) analysis confirmed the homogeneity and anticipated size (Mw: 21 kDa, 32 kDa, or 41 kDa) of the designed assemblies. Circular dichroism studies show that each of the coiled-coil components retains its native folding abilities and binding specificity within the supramolecular context. On-going efforts using SAXS (small-angle X-ray scattering), MD (molecular dynamics) simulations, and TEM (transmission electron microscopy) will further elucidate the conformational properties of these unusual coiled-coil assemblies. Our discoveries expand the design paradigms and topological space of peptide-based supramolecular assemblies.

Track: Enzymology
Session: Protein Evolution, Design and Selection

ABS# 099 | Determination of The Rate Limiting Step During Zearalenone Hydrolysis by ZenA

Sebastian Fruhauf[1], Andreas Hoebartner-Gußl[1], Jiri Damborsky[2], Wulf-Dieter Moll[1], Zbynek Prokop[2]
[1]BIOMIN Research Center, Austria, [2]Loschmidt Laboratories, Masaryk University, Czech Republic (Tulln, Austria)

Zearalenone (ZEN) is a mycotoxin produced by different Fusarium species, including Fusarium graminearum and Fusarium culmorum which infect major crop plants worldwide. Biological decontamination is a favorable method to reduce ZEN concentration in contaminated feed. Previously we identified, cloned and produced a ZEN-hydrolyzing alpha/beta hydrolase from Rhodococcus erythropolis. This enzyme was named ZenARe. ZenARe hydrolyses zearalenone to hydrolysed zearalenone (HZEN) which then decarboxylates further to decarboxylated hydrolyzed zearalenone (DHZEN). Both, HZEN and DHZEN are non-estrogenic. Due to low heat stability and susceptibility to low pH ZenARe was considered unfit for application as feed additive active in the animal gastrointestinal tract. The homologous enzyme from Streptomyces coelicoflavus (ZenAScfl) showed higher thermostability, but with higher Km and lower kcat. It was used as starting point for enzyme engineering, resulting in a variant with lower Km and increased catalytic efficiency (ZenAScfl041).All three enzymes were subjected to pre-steady state enzyme kinetic analysis to elucidate their reaction mechanism and identify rate limiting steps as a basis for further targeted engineering.It was shown that substrate binding and affinity of all variants is significantly driven by conformational change (enzyme closure). ZenARe showed highest substrate affinity. This is partially related to increased binding affinity but also to the fast kinetics of the consecutive enzyme closure which brings the complex to the reactive mode. A six-step kinetics mechanism was proposed for all three variants. The rate-limiting steps differ between ZenAScfl variants and ZenARe. Both, ZenAScfl and ZenAScfl041, are limited by the first chemical step forming the intermediate form. ZenARe is different, above 298 K it is limited by the second chemical step (hydrolysis of the intermediate). Below 298 K the rate-limiting step is shifted to enzyme opening. Results showed that different ZenA variants may need different approaches for improvement of kinetics by enzyme engineering.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 101 | Coupling between local hydration and protein motion in determining the free energy of ligand binding

Jie Shi[1], Jae-Hyun Cho[1], Wonmuk Hwang[1]
[1]Texas A&M University (TX, United States)

The contribution of surface hydration to protein-protein recognition has been difficult to characterize since both proteins and water molecules move. To mitigate this issue, previous computational approaches relied mostly on simulations where the protein atoms are positionally restrained. However, the extent to which protein motion influences surface hydration remains not well-understood. We introduce a computational method to calculate the hydration energy of proteins in all-atom molecular dynamics simulation. We tested our method with two protein-ligand complexes, one formed between the Src homology 3 (SH3) domain and a ligand from the cell signaling protein cAbl, and the other formed between the SH3 domain and a ligand from the nonstructural protein 1 (NS1) of the 1918 Spanish influenza A virus. Surface water density map was calculated by counting the number of water molecules within a protein-locked cubic grid created with the stably folded core of the SH3 domain as the orientational reference. We find the hydration energy around the complex with the viral NS1 ligand is lower, which contributes to its higher binding affinity compared to the cellular cAbl ligand. The difference in hydration energy arises from the differences in the side-chain mobility of certain residues of SH3 domain that in turn reduce the density of the surrounding water molecules. This result highlights the importance of the coupling between local protein dynamics and the surface hydration structure in determining protein-ligand recognition.

Track: Design/engineering

Session: Designer Proteins Through Genetic Code Expansion

ABS# 102 | Protein-stabilised nanoclusters as novel MRI contrast agents

Gabriela Guedes[1], Antonio Aires[1], Aitziber L. Cortajarena[1,2]

[1]Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance, Donostia-San Sebastian, Spain, [2]Ikerbasque, Basque Foundation for Science, 48009 Bilbao, Spain (Donostia, Espanha)

In the last decades, due to its outstanding and diverse properties, nanomaterials have been widely studied for numerous applications, namely as magnetic resonance imaging (MRI) contrast agents. However, despite the great advances in this field, the development of straightforward methodologies and stable and safe contrast agents is still incipient. Protein-stabilised nanomaterials emerged recently and have been raising high interest in the biomedical field due to its biocompatibility, stability, and ease of preparation, thereby promising to overcome the main drawbacks of the traditional nanomaterials. The use of engineered proteins as scaffolds, such as consensus tetratricopeptide repeat (CTPR) proteins allows the tailoring of the nanomaterials' properties by tuning the CTPR sequence. For instance, the CTPR proteins used herein were engineered to display metal-binding sites by replacing some non-structural amino-acids by cysteines or histidines. Additionally, the CTPR scaffolds can also be engineered to have distinct binding modules or therapeutic moieties, imparting them with multiple functionalities.In this work, we exploit the development of protein-stabilised nanoclusters (NCs) through green methodologies and tailor their properties for the development of MRI contrast agents.

Track: Enzymology

Session: Allostery & Dynamics in Protein Function

ABS# 103 | An Evolutionary Non-conserved Motif in Helicobacter pylori Arginase Mediates Positioning of the Loop Containing the Catalytic Residue for Catalysis

Ditsa Sarkar[1], Ankita Dutta[1], Satya Narayan[2], Mohit Mazumder[3], Sri Rama Koti Ainavarapu[2], Samudrala Gourinath[3], Apurba Kumar Sau[1]

[1]National Institute of Immunology, [2]Tata Institute of Fundamental Research, [3]Jawaharlal Nehru University (Delhi, India)

The binuclear metalloenzyme Helicobacter pylori arginase is important for pathogenesis of the bacterium in the human stomach. Despite the conservation of its catalytic residues, the single Trp enzyme has an insertion sequence (--153ESEEKAWQKLCSL165--) that is vital for catalytic function. This stretch is reported to be present only in the arginase of Helicobacter gastric pathogens. The insertion motif contains the critical residues (Glu155, Trp159, and Cys163), which are conserved in the homolog of other Helicobacter gastric pathogens. Regardless of structural studies, the underlying basis for the role of this motif in catalytic function is not completely understood. Here, we used a combination of biochemical, biophysical, and molecular dynamics simulations studies to determine that Glu155 of this stretch interacts with both Lys57 and Ser152. The interaction involving Lys57, Glu155 and Ser152 is essential for positioning of the motif through Trp159, which is located near Glu155 (His122-Trp159-Tyr125 contact is essential to tertiary structural integrity). The individual or double mutation of Lys57 and Ser152 to Ala considerably reduces catalytic activity with Lys57 to Ala being more significant, indicating these residues are crucial for enzyme function. Our data suggest that the Lys57-Glu155-Ser152 interaction influences the positioning of the loop containing the catalytic His133 so that this His can participate in catalysis, thereby providing a mechanistic understanding into the role of this motif in catalytic function. Lys57 is also found only in the arginases of Helicobacter gastric pathogens. Thus, the present study provides a molecular basis into the role of this motif in function and offers an opportunity for the design of specific inhibitors.

Track: Folding

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 104 | Potential Self-Peptide Inhibitors of the SARS-CoV-2 Main Protease

Arkadeep Banerjee[1], Shachi Gosavi[1]

[1]National Centre for Biological Sciences (Karnataka, India)

The main protease (Mpro) of SARS-CoV-2 plays an essential role in viral replication, cleaving viral polyproteins into functional units. It consists of an N-terminal

catalytic domain (MproN), a linker, and an α-helical C-terminal domain (MproC). Mpro is active as a homodimer formed partly through a MproC dimer interface. Previous studies have shown that peptides derived from a given protein sequence (self-peptides) can affect the folding of that protein and in turn, affect its function. Since MproC stabilizes Mpro in the homologous SARS-CoV-1, we wanted to understand if MproC self-peptides can affect the folding of Mpro in SARS-CoV-2. We studied the folding of MproC in the presence of several MproC self-peptides using molecular dynamics simulations of coarse-grained structure-based models. When peptides containing the loop between α4 and α5 (loop4) were present along with the unfolded MproC in simulations, MproC had a tendency to fold upon the peptide instead of its own corresponding region, in a manner which prevented the protein's own C-terminus tail from participating in the dimer interface. In addition to loop4 self-peptides, those containing the α1 helix could reach another peptide-folded state. If such a peptide folded MproC structure is present in the context of full length Mpro, the presence of the free native α1 elongates the inter-domain linker which could disturb the allosteric communication between the two domains and in turn Mpro function. Mutational studies on SARS-CoV-1 Mpro indicate that interactions involving the linker are important for activity and their absence in the peptide-folded state may inhibit dimerization and catalytic activity of the protease. If these peptides are observed to specifically inhibit Mpro in vitro, they may be potential therapeutic agents against SARS-CoV-2. Further, as MproC is well conserved among and unique to the coronaviruses, self-peptides derived from it may be useful as broad-spectrum coronavirus inhibitors.

Track: Membrane Proteins
Session: Measuring Forces of Biological Systems

ABS# 105 | Membrane binding of the retinal S100B protein

Paul Jaouen[1,2], Florence Guérin[1,2], Trystan Lessard[1,2], Élodie Boisselier[1,2]
[1]*Université Laval, faculté de médecine, département d'ophtalmologie et d'oto-rhino-laryngologie – chirurgie cervico-faciale,* [2]*CHU de Québec, axe médecine régénératrice (Québec, Canada)*

CONTEXTMuller glial cells, located in the retina, are responsible for the physiological function of the eye nervous system by maintaining tissue homeostasis and cellular function. Guanylate cyclase (GC1) is a transmembrane enzymatic protein that transduces cell synaptic signal. This transduction activity is actually regulated by various proteins, including S100B in Muller glial cells. Thus, alterations in their function could deregulate GC1 synaptic transduction activity leading to ocular pathologies such as diabetic retinopathy or glaucoma, where loss of vision is observed.GC1, as well as S100B, are membrane proteins and the regulatory activity of S100B on GC1 is directly influenced by the membrane composition. Yet, S100B has not been extensively studied in this area and no molecular information on S100B membrane interaction is known. Therefore, the study of membrane binding of S100B is key to understand the regulation of GC1 as well as its role in the retinal synaptic activity.GOAL & METHODSThus, the goal of this research project is to characterize lipid membrane interactions of the retinal protein S100B. To do so, S100B transformation, overexpression, and purification will be performed in order to study its interaction with the lipidic membranes using biophysical models and techniques. In this study, the Langmuir monolayer model was coupled to surface tensiometry in order to characterize the interactions between phospholipids and S100B in different experimental conditions.RESULTS & CONCLUSIONIn this project, S100B was transformed, overexpressed and purified with a purity rate of 95%. Membrane interaction of S100B have shown that this protein preferentially interacts with phospholipids that are a major component of the Muller glial cell inner membrane (unsaturated phosphoethanolamine lipids). Furthermore, those lipids provide the membrane flexibility that may be necessary for synaptic transduction in Muller glial cells.

Track: Intrinsically Disordered Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 106 | Effect of Phosphorylation of Nucleolar Protein Nucleophosmin on Nucleolar Formation

Itsumi Tani[1], Shogo Ito[1], Yui Oikawa[2], Rui Kamada[1,2], Kazuyasu Sakaguchi[1,2]
[1]*Graduate School of Chemical Sciences and Engineering, Hokkaido University, Sapporo, Japan,* [2]*Laboratory of Biological Chemistry, Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo, Japan (Hokkaido, Japan)*

Nucleolus, a membrane-less structure in nucleus, is the place of ribosome biogenesis and RNA processing. It is also known that the nucleolus acts as stress sensor and induces cell cycle arrest. The nucleolar formation is

driven by "Liquid-liquid phase separation (LLPS)". Phase Separation in the nucleolus is induced by various nucleolar molecules including Nucleophosmin (NPM) and Fibrillarin, ribosomal proteins, and ribosomal RNAs. Recently, we reported that overexpression of Ser/Thr phosphatase PPM1D induces aberrant nucleolar formation through phosphorylation on Ser4 and Thr199 of NPM. The results suggested that these phosphorylation of NPM are important for regulation of nucleolar formation. However, the regulatory mechanism of nucleolar formation still remains obscure. Here, we report the role of NPM phosphorylation on controlling nucleolar formation. By immunocytochemistry, we found that small bright spots containing NPM, termed "small bodies", were formed in the PPM1D overexpressing cells. We found that the formation of small bodies was dependent on PPM1D expression level. Also, the phospho-mimetic and Ala mutation for Ser4 and/or Thr199 of NPM resulted in different formation of small bodies. SEC-MALS analysis showed that phosphorylation on Ser4 and/or Thr199 greatly contributed to the oligomer formation of NPM. Furthermore, LLPS activity was reduced by the phosphorylation on Ser4 and/or Thr199. The results suggested that the phosphorylation of Ser4 and Thr199 reduces the phase separation activity, prevents the formation of mature nucleoli, and promotes the formation of small bodies. Our study suggests that nucleolar maturation is regulated by the phase separation activity of NPM through Ser4 and Thr199 phosphorylation.

Track: Folding

Session: Protein Evolution, Design and Selection

ABS# 107 | The stability landscape of de novo TIM barrels explored by a modular design approach

Sergio Romero-Romero[1], Miguel Costas[2], Daniel-Adriano Silva Manzano[3], Sina Kordes[1], Erendira Rojas-Ortega[2], Cinthya Tapia[2], Yasel Guerra[2], Sooruban Shanmugaratnam[1], Adela Rodriguez-Romero[2], David Baker[3], Birte Höcker[1],
[1]University of Bayreuth, [2]Universidad Nacional Autónoma de México, [3]University of Washington (Bayern, Germany)

The ability to design stable proteins with custom-made functions is a major goal in biochemistry with practical relevance for our environment and society. Understanding and manipulating protein stability provide crucial information on the molecular determinants that modulate structure and stability, and expand the applications of de novo proteins. Since the (β/α)8-barrel or TIM-barrel fold is one of the most common functional scaffolds, in this work we designed a collection of stable de novo TIM barrels (DeNovoTIMs) using a computational fixed-backbone and modular approach based on improved hydrophobic packing of sTIM11, the first validated de novo TIM barrel. DeNovoTIMs were subjected to thorough biochemical and folding analyses using computational, biophysical, structural, and thermodynamic methods. DeNovoTIMs navigate a region of the stability landscape previously uncharted by natural TIM barrels, with variations spanning 60 degrees in melting temperature and 22 kcal per mol in conformational stability throughout the designs. Significant non-additive or epistatic effects were observed when stabilizing mutations from different regions of the barrel were combined. The molecular basis of epistasis in DeNovoTIMs appears to be related to the extension of the hydrophobic cores. This study is an important step towards the fine-tuned modulation of protein stability by design.

Track: Enzymology

Session: New Protein Post-Translational Modifications

ABS# 108 | Interaction of Arginine Residues with phosphate group in the Active Site of Ser/Thr Phosphatase PPM1A

Shogo Ito[1], Itsumi Tani[1], Yukiko Shirahata[1], Yasuyuki Shimohigashi[2], Rui Kamada[1], Kazuyasu Sakaguchi[1]
[1]Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo, Japan, [2]Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka, Japan (Hokkaido, Japan)

Metal-dependent Ser/Thr phosphatase PPM family is involved in a wide variety of cellular functions. PPM phosphatases recognize their substrates as a monomeric enzyme, unlike other Ser/Thr phosphatase family. A PPM family member, PPM1A, is highly conserved from prokaryotes to eukaryotes. PPM1A have many roles including angiogenesis, immune responses, cell differentiation and tumor suppression. PPM1A is known to dephosphorylate various proteins, including ERK, SMAD and IKKB. However, a particular motif cannot be found in the sequences around dephosphorylation site in these proteins. It is not clear how PPM1A can recognize various types of substrate sequences. Here, we report analysis of two Arg residues, Arg33 and Arg186, located in the catalytic center of PPM1A in substrate recognition. In the dephosphorylation of an artificial substrate p-nitrophenyl phosphate (pNPP), PPM1A mutants, R33Q and R33Q/R186Q, drastically decreased kcat/Km value compared to

the wild-type PPM1A while R186Q mutant retained some activity. This result showed that Arg33 is important for the recognition of pNPP as reported previously. Interestingly, in the case of Ac-Gly-pThr-Gly-NH2 peptide used as a substrate, R186Q mutant showed lower kcat/Km value than R33Q mutant. Furthermore, the mutations of R33 and R186 showed different effects for longer phosphopeptide substrates. Docking model structure of PPM1A complexed with Ac-Gly-pThr-Gly-NH2 peptide showed that the phosphate group of the substrate was located closer to Arg186 than Arg33. These results suggested that Arg186 interact with the substrate phosphate group as well as Arg33 and that the contribution of these two Arg residues are different depending on substrate sequence. This study provides a new insight into the mechanism by which PPM1A recognizes substrates.

Track: Protein Interactions and Assemblies

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 110 | 3D Hydropathic Interaction Maps as a Novel Motif for Protein Structure Description: A Case Study of Phenylalanine, Tyrosine, and Tryptophan Residues

Mohammed AL Mughram[1], Claudio Catalano[1], Martin K. Safo[1], Glen E. Kellogg[1]
[1]*Department of Medicinal Chemistry and Institute for Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, Richmond, Virginia, USA. (Virginia, United States)*

Hydropathic environments of protein amino acid residues are information-rich, revealing structural details on multiple levels. Phenylalanine, tyrosine, and tryptophan residues are classified as aromatic amino acids with distinct sidechain characters that enable them to engage in various non-covalent hydrophobic and polar interactions. Extensive sets of the studied residues were binned by backbone ϕ and ψ and sidechain chi1 angles. 3D residue-based maps probing each residue's interaction environment were calculated using the Hydropathic INTeraction (HINT) force field. Calculated maps were clustered using the k-means algorithm based on map-map pair similarities. As a result, the ~31,000 phenylalanines, ~29,000 tyrosines and ~12,000 tryptophans in the data set were clustered, respectively, into 607, 609, and 457 unique sidechain-dependent environments. Furthermore, interactions above and below the space of sidechain rings were sampled to evaluate the magnitude/occurrence of π-cation, π-π stacking, or other classes of interactions. A common map motif of unfavorable hydrophobic-hydrophobic interactions along the CA-CB axis was shared by the three aromatic residues. The phenylalanines showed a common motif of favorable hydrophobic-hydrophobic interactions surrounding the aromatic ring, which is more ubiquitous in β-pleat region than in the left-hand and right-hand α-helix regions. A common motif in the tyrosines showed favorable hydrophobic-hydrophobic interactions of tyrosine rings and favorable polar-polar interactions of phenolic hydroxyls with their environments. The latter motif was common in tryptophans, where favorable hydrophobic-hydrophobic interactions of the indole ring and favorable polar-polar interactions of the indole NH with their surroundings were consistently observed. Around half of the residues studied showed evidence of π-π interactions (PHE: 53%, TYR: 34%, TRP: 56%); however, this accounted for only 4% of the total energy contribution. Evidence for π-cation interactions was seen in 14% of PHE, 8% of TYR and 27% of TRP residues, but this contributed only ~1% of the total energy between the residue and environment.

Track: Membrane Proteins

Session: Protein Evolution, Design and Selection

ABS# 111 | Challenges with the Preparation of a Soluble and Active Form of Lecithin Retinol Acyltransferase

Sarah Roy[1], Line Cantin[1], Jordan Grondin[1], Vincent Boulanger[1], Marie-Eve Gauthier[1], Stéphane M. Gagné[1], Christian Salesse[1]
[1]*Université Laval (QC, Canada)*

Lecithin retinol acyltransferase (LRAT) is a membrane protein that transforms all-trans retinol into all-trans retinyl ester. LRAT mutations lead to vision loss. High activity is obtained with truncated LRAT (tLRAT; LRAT 30-196) purified in the presence of sodium dodecyl sulfate (SDS) whereas its natural mutations lead to a loss of its activity. However, our data show that tLRAT is not properly structured at the high concentrations necessary for the NMR structural studies. Structural information of LRAT must be obtained to better understand the mechanism of its enzymatic activity. The objective of this study was thus to purify a form of tLRAT with high yield, high activity, and that is stable enough to determine its tertiary structure using NMR. The approaches used to achieve this aim included 1) testing various detergents and 2) modifying the primary sequence of LRAT and tLRAT to obtain a protein that can be purified without detergent. tLRAT was overexpressed in E. coli and then purified

using affinity chromatography. The only detergent that is capable of properly solubilizing tLRAT is SDS. Different detergents were used during the steps of washing and eluting. Small detergents that are either anionic or zwitterionic gave the highest protein yields. tLRAT has the highest activity when purified with Lyso-myristoyl-phosphoglycerol (LMPG), having an activity comparable to that obtained with SDS. Different constructions of LRAT and tLRAT were also produced. Many constructions were designed by either further truncating tLRAT or by adding hydrophilic residues on the N- or C-terminus. The solubility of these constructs was predicted using bioinformatic tools and those with the highest predicted solubility were then engineered. Our results suggest that adding hydrophilic residues produces more soluble forms of LRAT.

Track: Bioinformatics
Session: Targeted Protein Degradation

ABS# 112 | Development of Flexible Receptor Docking and its Application in Identifying Putative Inhibitors Against TMPRSS2, as a Potential Anti-CoV-2 Therapeutic.

Yujin Wu[1], Charles Brooks[1]
[1]*University of Michigan, Ann Arbor (Michigan, United States)*

The binding of small molecule ligands to receptor targets is important to numerous biological processes. When multiple ligands with different sizes are docked to a target receptor it is reasonable to assume the residues in the binding pocket may adopt alternative conformations. It has also been suggested that the entropic contribution to binding can be important. In the work presented here, we discuss a new physics-based scoring function that includes both enthalpic and entropic contributions to binding that consider the conformational variability of the flexible side chains within the ensemble of docked poses. To accommodate the additional searching requirements for flexible receptor docking studies, we also describe a novel hybrid searching algorithm that combines both molecular dynamics (MD) based simulated annealing and a continuous genetic algorithm. These advances further the development of the Flexible CDOCKER docking methods in the CHARMM software package. We benchmark our developments using 6 varied receptor targets: thrombin, dihydrofolate reductase (DHFR), T4 L99A, T4 L99A/M102Q, PDE10A and the riboswitch. These cover a wide range of enzyme classes with different binding pocket environments and the novel aspects of RNA receptor targets. We demonstrate improved accuracy in flexible cross-docking experiments compared with rigid cross-docking. The largest improvement in top ranking accuracy is 22% for ligands binding to DHFR. Moreover, by using advances in GPU accelerated sampling, we reduce the wall-time required for such calculations so that flexible receptor ligand docking can be applied in high-throughput experiments.As a practical example of this methodology, we worked with a team of experimental colleagues to identify potential therapeutics for the host transmembrane serine protease TMPRSS2, a promising antiviral target that plays a direct role in SARS-CoV-2 infections. From our in silico docking trials using the flexible docking method noted above, novel non-covalent inhibitors were identified and verified with biochemical assays.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 113 | Protein local conformations analyses in ordered and intrinsically disordered proteins in the light of a structural alphabet

Alexandre G de Brevern[1]
[1]*INSERM, Univ Paris (Ile-de-France, France)*

Protein structures are highly dynamic macromolecules. Molecular dynamics (MDs) simulations were performed on a large set of 169 representative protein domains. Protein flexibility was assessed with B-factors, RMSf and solvent accessibility, but also innovative approaches such as entropy. Concerning the helical structures, only 76.4% of the residues associated to α-helices retain the conformation; this tendency drops to 40.5% for 310-helices and surprisingly too for π–helices showed behaviors (Narwani et al, Arch Biol Sci, 2018). The rigidity of β-sheet was confirmed, as well as its capacity to transform into turns. Finally, while the dynamics between turns (with hydrogen bond) and bends (without hydrogen bond) have some strong similarities; they also showed differences as turns convert easily to helical structures while bends prefer extended conformations.Analyses were similarly performed using a structural alphabet, namely the Protein Blocks (PBs, de Brevern et al, Proteins, 2000). For half of the PBs, to be buried or exposed does not change at all their dynamics. Majority of PBs remains as their original conformation, or at least with a high frequency. Few PBs have a higher tendency to be more flexible. The intriguing fact is that the change from a PB to another one does not correspond to a simple geometrical evolution. It is more frequent to go to an unexpected PB than an

expected one (Narwani et al, J Biomol Struct Dyn, 2019). To go further, a dataset of disorder protein ensembles was analyzed with the PB. Using a PB derived entropy index, we quantify, for the first time, continuum from rigidity to flexibility and finally disorder. We also highlight non-disordered regions in the ensemble of disordered proteins (Melarkode Vattekatte et al, J Struct Biol, 2020 & Data in Brief, 2020). These results have been compared to different types of prediction (de Brevern, Biomolecules, 2020 & Biochimie, 2020).

Track: Membrane Proteins

Session: Protein Evolution, Design and Selection

ABS# 114 | Computationally designed GPCR quaternary structures bias signaling pathway activation

Robert Jefferson[1], Xiang Feng[2], Patrick Barth[1], Justine Paradis[3], Michel Bouvier[3], Andy Chevigne[4], Martyna Szpakowska[4]
[1]EPFL, [2]Van Andel Institute, [3]Université de Montréal, [4]Luxembourg Institute of Health (Vaud, Switzerland)

Communication across membranes controls critical cellular processes and is achieved by receptors translating extracellular signals into selective cytoplasmic responses. While receptor tertiary structures can now be readily characterized, receptor associations into quaternary structures are very challenging to study and their implications in signal transduction remain poorly understood. Here, we report a computational approach for predicting membrane receptor self-associations, and designing receptor oligomers with various quaternary structures and signaling properties. Using this approach, we designed chemokine receptor CXCR4 dimers with reprogrammed stabilities, conformations, and abilities to activate distinct intracellular signaling proteins. In agreement with our predictions, the designed CXCR4s dimerized through distinct conformations and displayed different quaternary structural changes upon activation. Consistent with the active state models, all engineered CXCR4 oligomers activated the G protein Gi, but only a few specific dimer structures also recruited b-arrestins. Overall, we demonstrate that quaternary structures represent an important unforeseen mechanism of receptor biased signaling and reveal the existence of a conformational switch at the dimer interface of several G protein-coupled receptors including CXCR4, mu-Opioid and type-2 Vasopressin receptors that selectively control the activation of G proteins vs β-arrestin-mediated pathways. The approach should prove useful for predicting and designing receptor associations to uncover and reprogram selective cellular signaling functions.

Track: Evolution

Session: Protein Evolution, Design and Selection

ABS# 115 | Marginal specificity in protein-protein interactions constrains the evolution of a paralogous family

Dia Ghose[1], Amy Keating[1], Michael Laub[1]
[1]Department of Biology, MIT (MA, United States)

One of the most prevalent mechanisms used by nature to allow organismal evolution is the duplication and divergence of genes to adopt novel functions. This evolutionary mechanism of continually repurposing proteins with similar structures and sequences for multiple functions presents certain problems: how is the specificity of interactions maintained? Does the need to maintain distinct specificities constrain evolution? To investigate this question, we studied a large paralogous family of bacterial signaling proteins, two-component systems. These systems consist of a sensor histidine kinase (HK) and partner response regulator (RR) that form strictly specific pairs. Although crosstalk between two-component systems is detrimental to fitness, the degree to which this presents a barrier to evolution remains unclear. The answer to this lies in the nature of local sequence spaces surrounding two-component systems. Sequence spaces that are crowded, such that many mutations introduce non-cognate interactions, constrain evolution in ways that sparsely occupied spaces do not. We performed a screen of the local sequence space surrounding a model HK, testing a library of single mutants for interaction with its cognate RR, and two non-cognate RRs. This involved a high-throughput assay utilizing fluorescence-based sorting, coupled with deep sequencing. This screen revealed rampant crosstalk in the local sequence space surrounding the HK, with ~30% of single mutations causing a 5+-fold increase in crosstalk towards one or both non-cognate RRs, including mutations distal from the binding site that likely influence the conformational equilibrium of the kinase. This result demonstrates that these paralogous proteins only show marginal specificity, suggesting that two-component systems are not selected to separate their niches in sequence space post-duplication, resulting in a crowded local space that eventually constrains their evolution. This finding highlights how the nature of evolution, in only selecting for 'good enough', leads to restrictions on subsequent evolvability of paralogous families.

Track: Computational Modeling/Simulation
Session: Protein Structures Through the Lens of Machine Learning

ABS# 116 | How good are protein structure prediction methods at predicting folding pathways?

Carlos Outeiral Rubiera[1], Charlotte Deane[1]
[1]*University of Oxford (Oxfordshire, United Kingdom)*

Deep learning has achieved unprecedented success in predicting a protein's crystal structure, but whether this achievement relates to a better modeling of the folding process is an open question. In this work, we compare the dynamic pathways from six state-of-the-art protein structure prediction methods to experimental folding data. We find evidence of a weak correlation between simulated dynamics and formal kinetics; however, many of the structures of the predicted intermediates are incompatible with available hydrogen-deuterium exchange experiments. These results suggest that recent advances in protein structure prediction do not provide an enhanced understanding of the principles underpinning protein folding.

Track: Enzymology
Session: Protein Evolution, Design and Selection

ABS# 117 | Structural Insights into Secoiridoid Production by Secologanic Acid Synthases (CYP72As) in Camptotheca acuminata from Ancestral Sequence Reconstruction

Justin Miller[1], Mary Schuler[1]
[1]*University of Illinois Urbana-Champaign (Illinois, United States)*

Terpene indole alkaloids (TIAs) are a major class of plant-derived specialized metabolites widely used in medicine. A conserved early pathway derives their secoiridoid core from geraniol and condenses it with tryptamine producing the strictosidine core from which species-specific TIAs are produced. However, metabolomics and biochemical work have shown that (at least for camptothecin-producing Camptotheca acuminata) strictosidine is not the universal precursor of TIAs. The point of divergence is at loganic acid: the conserved pathway converts loganic acid to loganin then to secologanin; Camptotheca utilizes loganic acid and directly produces secologanic acid. This divergence is due to the inability of the cytochrome P450 (CYP) secologanin synthase (SLS; exemplified by Catharanthus roseus CYP72A1) to utilize loganic acid as a substrate. Conversely, at least two Camptotheca secologanic acid synthases (SLASs; CYP72A564, CYP72A565) produce secologanic acid from loganic acid and secologanin from loganin in vitro. Utilizing the phylogenetic technique of ancestral sequence reconstruction to compare various CYP72As in combination with molecular modeling, we identified key residues that distinguish SLS from SLAS activity. Mutations of the Camptotheca SLASs at any of four residues identified from ancestral sequence reconstruction immediately surrounding the active site perturbed the binding and/or turnover of loganin and loganic acid. Of foremost interest, one mutation (H131F) increased the Camptotheca SLASs' affinity for loganin, improved their production of secologanin, and abolished loganic acid turnover. These studies facilitate rational engineering of these CYPs to optimize the heterologous production of strictosidine and other TIAs. The identification of the residues essential for the broadened substrate scope of the SLASs additionally supplies a framework for tailoring these enzymes to utilize other substrates. Such reactions have significant potential as the starting point for the production of unprecedented natural product-like compounds.

Track: Protein Interactions and Assemblies
Session: Protein Evolution, Design and Selection

ABS# 118 | Biophysical and structural characterization of novel RAS-binding domains (RBDs) of PI3Kα and PI3Kγ

Nicholas G. Martinez[1,2], David F. Thieker[1,2], Leiah M. Carey[1,2], Juhi A. Rasquinha[1], Samantha K. Kistler[1], Brian A. Kuhlman[1,2], Sharon L. Campbell[1,2]
[1]*University of North Carolina at Chapel Hill,* [2]*UNC Lineberger Comprehensive Cancer Center (North Carolina, United States of America)*

Phosphatidylinositol-3-kinases (PI3Ks) are lipid kinases that phosphorylate phosphatidylinositol 4,5-bisphosphate to generate a key lipid second messenger, phosphatidylinositol 3,4,5-bisphosphate. PI3Kα and PI3Kγ require activation by RAS proteins to stimulate signaling pathways that control cellular growth, differentiation, motility and survival. Intriguingly, RAS binding to PI3K isoforms likely differ, as RAS mutations have been identified that discriminate between PI3Kα and PI3Kγ, consistent with low sequence homology (23%) between their RAS binding domains (RBDs). As disruption of the RAS/PI3Kα interaction reduces tumor growth in mice with RAS- and epidermal growth factor receptor driven

skin and lung cancers, compounds that interfere with this key interaction may prove useful as anti-cancer agents. However, a structure of PI3Kα bound to RAS is lacking, limiting drug discovery efforts. Expression of full-length PI3K isoforms in insect cells has resulted in low yield and variable activity, limiting biophysical and structural studies of RAS/PI3K interactions. This led us to generate the first RBDs from PI3Kα and PI3Kγ that can be expressed at high yield in bacteria and bind to RAS with similar affinity to full-length PI3K. We also solved a 2.31 Å X-ray crystal structure of the PI3Kα-RBD, which aligns well to full-length PI3Kα. Structural differences between the PI3Kα and PI3Kγ RBDs are consistent with differences in thermal stability and may underly differential RAS recognition and RAS-mediated PI3K activation. While these high expression, functional PI3K RBDs will aid in interrogating RAS interactions and could aid in identifying inhibitors of this key interaction, further investigation into how oncogenic KRAS mutants bind and activate full-length PI3Ka will allow us to connect meaningful data from studies with our PI3Kα-RBD.

Track: Membrane Proteins

Session: Protein Structures Through the Lens of Machine Learning

ABS# 119 | Generalizable Predictions for the Effects of Missense Mutations on Membrane Protein Expression from Deep Mutational Scanning Data

Charles Kuntz[1], Hope Woods[2], Andrew McKee[1], Nathan Zelt[1], Jeffrey Mendenhall[2], Jens Meiler[2], Jonathan Schlebach[1]
[1]Indiana University, [2]Vanderbilt University (Indiana, United States)

Missense mutations that compromise the plasma membrane expression (PME) of integral membrane proteins are the root cause of numerous genetic diseases. Differentiation of this class of mutations from those that specifically modify the activity of the folded protein has proven useful for the development and targeting of precision therapeutics. Nevertheless, it remains challenging to predict the effects of mutations on membrane protein stability and/ or expression. In this work, we utilize deep mutational scanning data to train a series of artificial neural networks (ANNs) to predict the effects of mutations on the PME of rhodopsin from structural and/ or evolutionary features. We find that ANNs trained exclusively with structural features generate the most accurate predictions, and that the inclusion of evolutionary features biases scoring towards functional effects. We show that our best performing ANN generates statistically significant predictions for the quantitative effects of mutations on the expression of the β2-adrenergic receptor. Moreover, this ANNs generally retains the ability to discriminate between functionally neutral and deleterious variants. Our final ANNs are uniquely designed to predict the effects of missense variants on the expression of membrane proteins at the plasma membrane, and their performance will likely improve with the incorporation of new training data. Our findings highlight important considerations for the design of mechanistic genetic predictors.

Track: Chemical Biology

Session: Allostery & Dynamics in Protein Function

ABS# 120 | Understanding the Catalytic Mechanism of Spl DnaX intein-mediated Protein Splicing guided by Solution NMR Spectroscopy and Molecular Dynamics Simulations

Soumendu Boral[1], Tushar Kushwaha[2], Krishna K. Inampudi[2], Soumya De[1]
[1]School of Bioscience, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal 721302, India, [2]Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110029, India (West Bengal, India)

Inteins or intervening proteins are the enzymes that catalyze their excision from a precursor protein and facilitate peptide bond formation between the external proteins (exteins). More than 600 intein genes have been reported, but only a few have been thoroughly characterized. Understanding the structural basis of their catalysis is important to engineer intein enzymes with novel applications.The Spl DnaX intein is derived from the cyanobacterium Spirulina platensis. It shares significant structural homology with other inteins despite having a low sequence identity (<33%). The NMR structure of this 136-residue protein has a backbone RMSD of 0.27 Å and heavy atom RMSD of 0.52 Å (PDB code: 7CFV) across all ordered residues.1 The Spl DnaX intein has a highly symmetric fold. It has a very stable core as determined by NMR-based hydrogen exchange experiments. Backbone 15N-dynamics experiments showed the presence of conserved motions. These dynamic motions were found in symmetric positions in the intein structure, which is most likely a

result of its symmetrical structure. To assess in vivo activity, the intein enzyme was fused with two small soluble proteins at its N- and C-termini. The precursor protein was not detected, indicating its rapid splicing immediately upon synthesis in the cell. In order to expand the conformational landscape of the intein, the solution structure was subjected to molecular dynamics simulation for a duration of 1.5μs using GROMACS. The dominant motions identified through principal component analysis corroborated with the NMR studies. Possible catalytic residues were further identified from the protein-energy network and their functional roles in the splicing mechanism were confirmed by site-directed mutagenesis. References:1. Boral S, Maiti S, Basak AJ, Lee W, De S. Structural, Dynamic, and Functional Characterization of a DnaX Mini-intein Derived from Spirulina platensis Provides Important Insights into Intein-Mediated Catalysis of Protein Splicing. Biochemistry. 2020;59:4711–4724. https://pubs.acs.org/doi/10.1021/acs.biochem.0c00828

Track: Transcription/translation/post-translational modifications

Session: New Protein Post-Translational Modifications

ABS# 121 | Investigating Functional Conservation of BRCA1/BARD1 E3 Ligase Activity

Russell Vahrenkamp[1], Sam Witus[1], Rachel Klevit[2], Mikaela Stewart[3]
[1]Graduate Student , [2]Faculty Member, [3]Principle Investigator (Texas, United States)

Breast cancer susceptibility gene 1 encoded BRCA1 and its heterodimeric partner BARD1 play an essential role in genomic stability by regulating DNA damage repair, cell-cycle checkpoints, and transcription regulation. Germline mutations in either of these genes expose individuals to a higher risk of developing breast and ovarian cancer. The Caenorhabditis elegans orthologs, brc-1 and brd-1, also regulate DNA damage repair and cell cycle checkpoints. However, the role these proteins play in regulating gene transcription is still unknown. We hypothesize that brc-1 and brd-1 function in a mechanistically homologous manner to their human counterparts in that they form a heterodimeric complex (CeBCBD) that acts as an E3 ligase. This CeBCBD complex ubiquitinates histone H2A resulting in epigenetic transcriptional regulation in C. elegans. We show that mutations in the CeBCBD complex inhibit ubiquitination of H2A in nucleosomes. These mutations are analogous to those in humans that inhibit the E3 enzymatic function and result in loss of transcription regulation. Our results suggest that regulation of transcription is a conserved function of brc-1 and brd-1, and further substantiate the use of C. elegans as a model organism for studying BRCA1 function.

Track: Proteins in Cells

Session: Targeted Protein Degradation

ABS# 122 | A System of Auxin-inducible Degradation of Human Ku70 Protein to Study Its Role in HIV-1 Replication

Anton Sorkin[1], Olga Shadrina[1], Maria Kapitonova[1], Marina Gottikh[1]
[1]Moscow State University (Moscow, Russia)

The human Ku70 protein is thought to be involved in several stages of the human immunodeficiency virus (HIV) replication. Thus, it is not easy to assess the Ku70 effect at individual steps. In this regard, a system of quick and reversible Ku70 degradation is needed. Previously we established the 293T-Ku70-AID cell line, in which the endogenous Ku70 is fused with the part of an auxin-inducible degron (AID). The second component of the auxin-inducible degradation system is an osTIR1 protein. Initially, the osTIR1 gene was introduced into cells under the CMV promoter. However, it led to Ku70 degradation even without auxin activation. To avoid background degradation, we created a cell line with osTIR1 under the control of a doxycycline-inducible promoter. In these cells, Ku70 degradation was observed only after doxycycline and auxin addition.The study of degradation and recovery kinetics showed that the osTIR1 protein was detectable on a blot starting 2 h after induction and reached its maximum after 24 h. A gradual decrease of Ku70 level started at the 6 h mark, and after 24 h, the protein was not detectable. Removal of inductors caused the concentration of osTIR1 to decrease by 70% in 16 h. Meanwhile, the Ku70 level began to recover 20 h after inductor removal and reached its native level by 40 h. We compared these results with the kinetics of Ku70 knockdown by siRNAs in 293T cells: a significant decrease was observed only 48 h after transfection and continued for the next 96 h. Thus, the developed system of auxin-inducible degradation of Ku70 allows to reduce and restore the protein level quickly and could be used to assess the effect of Ku70 on individual stages of HIV replication. The work was supported by RSF grant 17-14-01107

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 123 | Alteration of the Specificity of a Quorum Quenching Lactonase Using Rational Design

Joseph Bravo[1], Mikael Elias[1]
[1]*BioTechnology Institute, University of Minnesota, St. Paul 55108 (MN, United States)*

Quorum sensing refers to bacterial systems in which a signal molecule is produced by a bacteria and diffuses into the environment, so that behavior may be controlled in a cell density dependent manner. Quorum sensing systems utilizing Acyl Homoserine Lactones (AHLs) are prominent in many microbial organisms and communities of high impact to human interests, such as agriculture, biofilm production, and certain pathogens. Interestingly, AHL chemical structures are variable and determines the specificity of the signal. Several strategies that interfere with quorum sensing are naturally used by various organisms. One such strategy is to use enzymes which degrade the AHL signal, known as Quorum Quenching (QQ). We undertook biochemical and structural studies of various QQ enzyme representatives, that all exhibit broad range AHL specificity. In order to target QQ approaches, we decided to create QQ enzymes with greater AHL specificity. We used the broad spectrum, thermostable lactonase from Geobacillus caldoxylosilyticus, dubbed GcL, as a starting point of our rational design approach. Key residues interacting with the AHL substrate acyl chains were identified by structural studies, and were used as saturation library targets. Several key mutations were identified in a screening step, and confirmed with purified enzyme and kinetic characterization. Several of these mutations were combined and the best mutants obtained thus far have shifted substrate preference by up to 200-fold in favor of short chain AHLs. These mutants were crystallized and their structures revealed a reshaping of the active site binding cleft, as well as an alternate substrate binding conformation for a prominent active site loop.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 124 | Regulation of G-Protein Signaling: Physical Studies on the Interaction of RGS10 and Calmodulin

Cynthia Tope[1], Ramona Bieber Urbauer[1], Shelley Hooks[1], Jeffrey Urbauer[1]
[1]*The University of Georgia (GA, USA)*

Approximately 35% of all drugs currently in clinical use target a single family of proteins: G-Protein Coupled Receptors (GPCRs). Due to the therapeutic importance of this class of proteins, it should be no surprise that proteins involved in regulating signaling initiated by GPCRs are gathering attention as potential drug targets. One such family of proteins is known as the Regulators of G-protein Signaling (RGS). These proteins function as negative regulators for G-protein signaling by binding to the GTP-bound G-alpha subunit, accelerating the hydrolysis of GTP to GDP. In turn, RGS proteins are themselves regulated by direct interactions with other biomolecules. For instance, RGS10, which is integral for neural and cardiovascular function, is differentially regulated by a sophisticated and poorly understood competitive binding to the membrane lipid phosphatidylinositol (3,4,5)-triphosphate (PIP3) and the ubiquitous calcium-signaling protein, calmodulin (CaM). Our current focus is on the interaction of the RGS10 protein with CaM and how this interaction affects the RGS binding to G-alpha. We have thus far produced recombinant full-length RGS10 and the RGS domain only (RGS10 lacking N- and C-terminal regions) and purified them to homogeneity. By monitoring the intrinsic tryptophan fluorescence of RGS10, we have measured the affinities of the RGS10 proteins for CaM and their dependence on ionic strength. Here, the full-length RGS10 binds only marginally tighter to CaM than the RGS domain alone at low ionic strength and the affinities of both complexes decrease with increasing ionic strength. The results indicate 1:1 stoichiometry of the complexes. NMR spectra reveal the expected slow-exchange behavior when isotopically labeled RGS proteins are titrated with unlabeled CaM and demonstrate that RGS10 preferentially binds to the C-domain of CaM.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 125 | Comparative Analysis of Strain-Dependent Inter-Domain Binding Free Energies of the Influenza A Virus Non-Structural Protein 1

James Gonzales[1,2], Jie Shi[1,2], Jae-Hyun Cho[2,3], Wonmuk Hwang[1,2,4,5,6,7]
[1]*Department of Biomedical Engineering,* [2]*Texas A&M University,* [3]*Department of Biochemistry and Biophysics,* [4]*Department of Materials Science and Engineering,* [5]*Department of Physics and Astronomy,* [6]*School of Computational Sciences,* [7]*Kore (TX, United States)*

Non-structural protein 1 (NS1) of influenza A virus is a multifunctionalprotein that is responsible for several

functions including viral proteinsynthesis and suppression of host immune response. NS1 has two distinctdomains that are strain-specific, an RNA binding domain (RBD) and aneffector domain (ED), which are connected by a linker region (LR) that alsovaries between strains. NS1 has the ability to form higher-order structuresthrough forming RBD-RBD dimers and two types of ED-ED dimers. The firsttype of ED-ED interaction occurs between the long alpha-helices of thetwo monomers (Type A ED dimer), while the other interaction involvesbeta-strand 1 and the following loop region (Type B ED dimer). Thevarious dimers and higher-order structures have been suggested to becrucial for multiple functions of NS1 during the infection cycle, includingthe inhibition of signaling proteins and dsRNA sequestration. We performall-atom molecular dynamics simulations of the RBD and Type A ED dimersfrom H5N1 and H6N6 strains of influenza A virus and Type B ED dimer fromH6N6, to calculate the relative binding free energies of the interfaces. Wefind that the binding free energy for RBD dimers is comparable between thetwo strains. For Type A ED dimers, the binding free energy for H6N6 is stronger than that of H5N1, while H6N6 Type B ED dimers have comparable binding free energy to H5N1 Type A ED dimers. Differences in the inter-domain bindingfree energies of EDs can influence the propensity of NS1 to form higher-orderstructures, which has implications on the infection cycle kinetics andvirulence.

Track: Amyloid and Aggregation
Session: Measuring Forces of Biological Systems

ABS# 126 | The Aggregation Propensity of N184K and D187Y Mutant Plasma Gelsolin Peptides

Ayan Niazi[1], Josephine Esposto[2], Colin Wu[3], Sanela Martic-Milne[2]
[1]Department of Biology, Trent University, ON, Canada, K9L 0G2, [2]Department of Forensic Science, Environmental and Life Science Program, Trent University, ON, Canada, K9L 0G2, [3]Department of Chemistry, Oakland University, MI, USA, 483092 (Ontario, Canada)

Gelsolin amyloidosis is a disease caused by point mutations which cause the protein to partially misfold and aggregate resulting in the formation of amyloid fibrils. The D187 mutation causing Aspartate (D) to be replaced by Asparagine (N) or Tyrosine (Y) leads to aggregation of peptide fragments and has been found to deposit in the eyes, skin and blood vessel walls. Recently, the N184 mutation, in which Asparagine (N) is replaced by Lysine (K) has been discovered in renal amyloidosis. However, its aggregation remains to be much less understood. It was hypothesized that aggregation of gelsolin is due to hydrophobic sequences and that mutations around such regions will modulate aggregation. Additionally, it was posed that mutation within and proximal to hydrophobic sequences will modulate aggregation due to additional electrostatic, hydrogen bonding or hydrophobic interactions. Herein, in vitro mechanistic evaluation of the aggregation propensities of the following gelsolin peptides: 184NNGYCFILDL193 and its respective mutants was carried out by using spectroscopic methods such as Thioflavin T (ThT) and turbidity assays. Furthermore, mutant peptides were characterized as a function of various environmental factors: heat, agitation, aging time, chemical additives and acidic pH. Findings indicate that the peptides containing CFILDL sequence aggregated into fibrillar networks. However, at low concentrations the N184K and D187Y mutants did not aggregate regardless of the environmental factors used. Future studies will investigate the effects of seeding on gelsolin amyloid formation at low concentrations.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 127 | Production of STIM2 Coiled-Coil Region and Its Interaction with Calmodulin

Karen Ramirez Quintero[1], Ramona Bieber Urbauer[1], Cynthia Tope[1], Shelley Hooks[1], Jeffrey Urbauer[1]
[1]The University of Georgia (GA, USA)

Stromal Interaction Molecules (STIMs) are transmembrane proteins located in the endoplasmic reticulum (ER) that act as calcium sensors. STIM proteins play a key role in the store-operated calcium entry (SOCE) that facilitates the replenishment of calcium reserves in the cell upon depletion. The STIM family is composed of STIM1 and STIM2, which work together forming homo and heterodimers to fulfill their functions as calcium regulators. The STIM2 protein is essential for sustaining life and has been shown to be highly involved in the nervous and immune systems, while the mechanisms for it remain largely unknown. Our current focus is on the production of STIM2 and its domains for studies of its interaction with regulator molecules, including the calcium-binding protein, calmodulin (CaM). Here, we show the production of the coiled-coil regions 1 and 2 of STIM2 (residues A234-S392) located in the cytoplasmatic region of the protein. The gene encoding this domain was cloned into an expression vector that coded for an N-terminal histidine tag followed by a TEV-protease cleavage site. The protein was overexpressed in bacteria then

purified using immobilized metal affinity chromatography. Electrophoretic analysis showed the protein was greater than 90% pure and its mass was confirmed by mass spectrometry. Far UV circular dichroism analysis suggests that the construct is properly folded. By monitoring the intrinsic tryptophan fluorescence of STIM2, we have determined that CaM binds tightly to the coiled-coil regions 1 and 2 of STIM2 in a calcium dependent manner. We currently are studying the interaction of the STIM2 coiled-coil region with other proteins using various methods, such as isothermal titration calorimetry.

Track: Evolution

Session: Designer Proteins Through Genetic Code Expansion

ABS# 128 | Engineering and Directed Evolution of Cellular Protein Translation

Ahmed Badran[1]
[1]*The Broad Institute (MA, USA)*

Rapid methods to engineer evolve and cellular translation machinery would enable applications beyond canonical protein synthesis. Unsurprisingly, manipulations to cellular translation often yield pleiotropic results, confounding experimental interpretation as researcher-dictated activities cannot be easily uncoupled from cellular fitness. Here, we develop a series of systematic advances to engineer and evolve novel translational capabilities in living cells. We apply orthogonal translation to define general requirements for efficient heterologous rRNA processing. We discover that supplementation with a small subset of cognate r-proteins enhances heterologous ribosome activity for rRNAs derived from organisms with as little as 76.1% 16S rRNA sequence identity to E. coli. We leverage these advances to develop oRibo-PACE, a novel method for continuous evolution of orthogonal rRNAs. Using oRibo-PACE, we evolved orthogonal ribosomes from E. coli, P. aeruginosa and V. cholerae for >260 generations under increasing selection regimes. Evolved o-ribosomes exhibited improved protein translation activities, up to 400% of corresponding wild-type ribosomes, at similar or reduced cellular burdens. Finally, we develop an engineering approach that affords quadruplet-decoding tRNAs using representative scaffolds for all 20 canonical amino acids, and employ novel PACE methodologies to substantially improve their translation efficiency without compromising amino acid selectivity.Collectively, our results simplify translational engineering and directed evolution pipelines, and provide extensible strategies for affecting ribosome function in vivo.

Track: Transcription/translation/post-translational modifications

Session: Protein Evolution, Design and Selection

ABS# 129 | Illuminating an Optogenetics Blind Spot: Tools to Control Eukaryotic Translation via EIF4E

Danlin Zhen[1], Andrew Woolley[1]
[1]*University of Toronto (Ontario, Canada)*

Protein synthesis and translation are highly spatiotemporally regulated processes which also play critical roles in key biological processes such as memory formation, embryonic development and tumour formation. Unfortunately, there exist very few optogenetics tools for controlling translation. We sought to fill in this blind spot by designing LITRs (LOV2 insertion in Translational Regulators) based on EIF4E, which is a central player in eukaryotic translation initiation, to couple blue light irradiation to cap-dependent protein synthesis. Using a yeast model, we screened for and characterized LITR function. By optimizing the linker between LOV2 and EIF4E using a structure-guided approach we constructed variants which up-regulated translation under blue light (LITUP) and variants which down-regulated translation in blue light (LITDOWN). LITRs were found to modulate generation time, and protein synthesis via the incorporation of a bio-orthogonal probe, TePhe. While we have observed that LITRs affect protein synthesis, cell growth, and cell division in yeast more work to elucidate the exact mechanism of LITR is needed. We observed multiphasic growth curves, longer generational times compared to wild type EIF4E and light-dependent steady-state quantities of LITRs. This suggests that some LITRs may not recapitulate all EIF4E functions and mechanisms other than allostery may contribute to LITR functionality. Further work in human cell lines is needed to fully characterize LITR mechanism and reversibility.

Track: Peptides

Session: Designer Proteins Through Genetic Code Expansion

ABS# 130 | Side-chain cyclization to generate fibrillation-resistant glucagon analogues

Nicolas Varas[1], Rachel Grabowski[2], Shayan Poordian[2], Nelson Phillips[2], Mark Jarosinski[1], Faramarz Ismail-Beigi[2], Michael Weiss[1]
[1]*Indiana University,* [2]*Case Western Reserve University (Indiana, United States)*

Insulin treatment is lifesaving for type 1 diabetic patients, but unfortunately, comes with an ever-present threat of hypoglycemic episodes caused by over-insulinization. Most of these episodes are manageable by sugar ingestion, but their continuous occurrence increases the probability of ischemic events, arrhythmias, neurological damage, coma, and even death. Consequently, the risk of hypoglycemia remains the most significant obstacle that prevents insulin-dependent patients from attaining adequate metabolic control. Glucagon is insulin's natural counter-regulatory hormone and therefore is used in emergency settings to treat severe hypoglycemia. Glucagon binds in an α-helical structure to its receptor and stimulates hepatic glycogenolysis and gluconeogenesis in the liver. Previous clinical studies have shown a significant decrease in hypoglycemic events when using a dual-hormone, insulin/glucagon, artificial pancreas, and recent seminal studies have shown prevention of hypoglycemia when glucagon is co-administrated with insulin. But, despite its great potential as a drug, native glucagon is chemically and physically unstable with an intrinsic propensity to rapidly form β-amyloid fibrils, which nullify its activity and can make it cytotoxic. To address this problem, our group is developing fibrillation-resistant glucagon analogues through side-chain cyclization with the purpose of lock-in an α-helix turn and prevent β-sheet formation. Particularly we are exploring lactam side-chain bond at positions i,i+4 and disulfide bridges between D-Cys at position i and L-Cys at position i+3. These strategies allowed us to generate analogs resistant to fibrillation for at least two weeks at 100μM in PBS buffer, under agitation and 37°C. One particular lactam-restrained analog proved to be fully active in rats after two weeks of agitation at 45°C. These results confirm side-chain cyclization as a valid approach to develop fibrillation-resistant glucagon analogues and give hope in the search for treatments that allow better glycemic control in Diabetes Mellitus.

Track: Membrane Proteins

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 131 | Profiling Retinal and Temperature Sensitive Variants of Rhodopsin by Deep Mutational Scanning

Andrew McKee[1], Charles Kuntz[1], Joseph Ortega[2], Hope Woods[3], Francis Roushar[1], Jens Meiler[3], Beata Jastrzebska[2], Jonathan Schlebach[1]
[1]Indiana University, [2]Case Western Reserve University, [3]Vanderbilt University (Indiana, United States)

Membrane protein variants with diminished conformational stability often exhibit enhanced cellular expression at reduced growth temperatures. The expression of "temperature-sensitive" variants is also typically sensitive to corrector molecules that bind and stabilize the native conformation. In this work, we employ deep mutational scanning to compare the effects of reduced growth temperature and an investigational corrector (9-cis-retinal) on the plasma membrane expression of 700 rhodopsin variants in HEK293T cells. We find that the change in expression at reduced growth temperatures is correlated with the response to retinal among variants bearing mutations within a hydrophobic transmembrane domain (TM2). The most sensitive variants within this helix appear to disrupt a network of hydrogen bonds that stabilizes a native helical kink. By comparison, mutants that alter a polar transmembrane domain (TM7) exhibit weaker responses to temperature and retinal that are poorly correlated. Statistical analyses suggest this insensitivity primarily arises from an abundance of mutations that enhance its membrane integration, stabilize its native conformation, and/ or perturb the retinal binding pocket. Finally, we show that the characteristics of purified temperature- and retinal-sensitive variants suggest that the proteostatic effects of retinal may be manifested during translation and cotranslational folding. Together, our findings elucidate various factors that mediate the sensitivity of genetic variants to temperature and to small molecule correctors.

Track: Chemical Biology

Session: Allostery & Dynamics in Protein Function

ABS# 133 | Rational Screening for Inhibitors of the HIV Integrase-Ku70 (XRCC6) Interaction

Ekaterina Ilgova[1], Simon Galkin[1], Andrey Anisenko[1], Marina Gottikh[1]
[1]Lomonosov Moscow State University, Faculty of Bioengineering and Bioinformatics, Chemistry Department and A.N. Belozersky Institute of Physico-Chemical Biology (Moscow, Russia)

HIV epidemic largely affects economic growth and is likely to exacerbate income inequality and increase poverty. Current ways to fight the virus include prevention measures and usage of Highly Active Antiretroviral Therapy aimed to suppress viral replication. HIV has extremely high mutation rates, and resistant strains have been developed for almost every available drug. Consequently, there is a continuous demand to search for novel anti-HIV drugs that will be insensitive to the virus's high

mutation rate. Targeting virus-host protein-protein interactions is a promising strategy to develop such drugs due to the genetic conservativeness of host proteins. A variety of cellular proteins are needed for the successful integration of viral cDNA. Among them, Ku70/Ku80 heterodimer facilitates post-integrational DNA repair. The interaction of IN and Ku70 has been shown. Disruption of the IN-Ku70 complex by point mutations at IN suppresses HIV replication. Therefore, the IN-Ku70 complex is a promising target for the development of novel anti-HIV drugs. Here we present a screening for inhibitors of IN-Ku70 interaction. We have identified Ku70 amino acids crucial for HIV integrase binding. These residues are part of a cavity suitable for molecular docking. At first, about 50,000 structurally diverse compounds from the ChemDiv vendor were submitted to the docking analysis. For the highest-ranking compounds, the solvent-accessible surface area of IN binding site within Ku70 was calculated. 31 compounds shielding IN-interacting residues the most were selected for further biological evaluation. From 31 chosen molecules, two hit compounds moderately inhibited IN-Ku70 interaction (IC50 = 70-80 μM). These compounds provide a proof of concept that inhibition of IN-Ku70 interaction is possible. However, further structure optimization is needed to develop compounds with higher inhibitory activity. The work was supported by RFBR grant 18-29-08012.

Track: Protein Interactions and Assemblies

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 134 | XL-MS for novel HIV-1 integrase partners detection

Anna Rozina[1], Andrey Anisenko[1,2,3], Simon Galkin[1], Marina Gottikh[2,3]

[1]Lomonosov Moscow State University, faculty of bioengineering and bioinformatics, [2]Belozerskiy Research Institute for Physical and Chemical Biology, [3]Lomonosov Moscow State University, chemistry department (Moscow, Russian Federation)

RNA viruses, in pursuit of genome miniaturization, tend to employ cellular proteins to facilitate their replication. HIV-1, one of the most studied retrovirus, is not an exception. Search for HIV-1 cellular partners and their examination is a prevailing area of research. One of the probable applications is a development of anti-HIV drugs that hamper cell-virus protein-protein interactions. One of HIV-1 proteins, integrase, is a key virus enzyme since it catalyzes HIV integration. To carry out its functions, integrase, seemingly, employs a great number of cellular factors. Despite years of extensive research, there are processes where cellular cofactors employed have not been determined yet or where their interplay is not fully understood, including integrase degradation, its interactome during PIC nuclear import, its role in post-integrational repair machinery recruitment and many others. Expanding our understanding of the integrase interactome would potentially open up new opportunities for drug development. Our work aims at capturing transient partners of HIV-1 integrase that would enhance our understanding of the molecular pathways involved in the integrase functioning. To do that, we employed XL-MS technique in HA-integrase plasmid transfected cells. We have conducted four repeats of the experiment and as a result have detected 18 potential previously undescribed partners, as well as several well-known partners. Among the former, we observed several proteins involved in HIV-1 replication but not known as the integrase partners. Also, we have found a previously undescribed in HIV-1 integrase context E3-ubiquitin ligase, a protein involved in tethering a specific complex to an actively transcribed region and several other interesting proteins. Their interaction with integrase has been tested in vivo. The work was supported by RSF grant 19-74-10021

Track: Proteins in Cells

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 135 | Luciferase-based SARS-CoV-2 Replicon System for Studying Viral Replication and Discontinuous Transcription

Simon Galkin[1], Andrey Anisenko[1], Olga Shadrina[1], Marina Gottikh[1]

[1]Lomonosov Moscow State University, Faculty of Bioengineering and Bioinformatics, Chemistry Department and A.N. Belozersky Institute of Physico-Chemical Biology (Moscow, Russia)

Coronavirus disease 2019 (COVID-19) is induced by the SARS-CoV-2 novel betacoronavirus. Due to the COVID-19 pandemic, the WHO has declared a worldwide public health emergency. Almost 3 million covid-19 deaths were reported by the end of March 2021. Despite the recent advances in vaccine development, COVID-19 remains a serious threat due to controversial information about immunity duration and herd immunity attainability. SARS-CoV-2 is a (+) single-stranded RNA virus containing multiple open reading frames in its genome. During viral transcription, shorter subgenomic RNAs

(sgRNAs) are produced. These RNAs are used to translate viral 3′ distal genes. Our knowledge about the molecular basis of SARS-CoV-2 replication and discontinuous transcription is still limited. In particular, it is unknown whether or which cellular proteins are involved in these processes. Largely, it is due to the lack of simple and safe replicon systems, which allow the performing of high-throughput screenings. In this work, we have developed a reporter replicon system consisting of 5′ coronaviral untranslated region (UTR), Firefly luciferase (Fluc), Puromycin resistance gene, Transcriptional Regulation Sequence of SARS-CoV-2 essential for viral RNA-dependent RNA polymerase jump, Renila luciferase (Rluc), and 3′ coronaviral UTR. If all conditions for discontinuous transcription are met an increased Rluc/Fluc signal ratio is registered. We have identified a minimal list of viral proteins from orf1a/b sufficient for discontinuous transcription. The created system can be used for the high-throughput screening of cellular proteins and drugs affecting SARS-CoV-2 replication and sgRNAs synthesis. The work was supported by RFBR grant 20-04-60216.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 136 | The Mosquito Protein AEG12 as a Scaffold for Novel Antiviral Therapeutucs

Alexander Foo[1], Shih-Heng Chen[1], Negin Martin[1], Geoffrey Mueller[1]
[1]*NIEHS (North Carolina, USA)*

The mosquito protein AEG12 is upregulated in response to viral infection, though its specific contribution and mechanism of action remained unknown. As a member of the insect major allergy (MA) domain family, the structure of AEG12 is defined by a large central cavity which can bind a range of hydrophobic ligands, potentially giving rise to its biological activity. Using mass-spectrometry and solution-NMR, we identified a mixture of saturated and unsaturated fatty acids as the likely native ligands of AEG12. Fluorescence assays indicate that these fatty acid ligands could be exchanged with phospholipids from biological membranes, destabilizing them in the process. This lipid-exchange mechanism enables AEG12 to disrupt the lipid bilayer of both mammalian cells and enveloped viruses, resulting in broad-spectrum viral suppression with micromolar IC50's against flavivirus, dengue virus, lentivirus, and human coronaviruses, albeit at the cost of significant hemolytic and cytotoxic activity. In addition to fatty acids, the unique MA domain fold enables AEG12 to accommodate a range of non-natural ligands such as lysophosphatidylcholine (LPC) and ginkgolic acid (GA); both of which have been shown to specifically inhibit viral membrane fusion, reducing infectivity independent of membrane destabilization and its associated cytotoxic effects. The resulting complexes displayed robust antiviral activity. In contrast to free-LPC or free-GA, these AEG12 complexes retained their activity even in the presence of serum albumins and other lipid-sequestering moieties, highlighting its utility as a delivery vehicle for hydrophobic antiviral compounds. These studies provide valuable insight into the biophysical mechanisms underlying AEG12's biological functions, with implications for the design of novel therapeutics leveraging the unique structure and ligand delivery capabilities of AEG12 and other MA domain proteins.

Track: Motors & Machines
Session: Allostery & Dynamics in Protein Function

ABS# 138 | Insights into the mechanism of temperature compensation in the cyanobacterial circadian clock

Andy LiWang[1], Ning Zhang[1], Archana Chavan[1]
[1]*University of California (CA, United States)*

Circadian clocks provide an internal representation of local time as an evolutionary adaptation to daily swings in ambient temperature and light. These oscillating biochemical systems prepare organisms for sunrise and sunset by controlling gene networks in metabolism, physiology, and behavior. Although the rates of most biochemical reactions are sensitive to temperature, circadian clocks reliably maintain a circadian rhythm under a range of different physiological temperatures. However, the molecular mechanisms by which clocks compensate for such variations in temperature remain only partially resolved in any organism. Here, we will present biochemical, biophysical, and structural data supporting the idea that cyanobacterial circadian clock proteins KaiA, KaiB, and KaiC shift their active ⇌ inactive conformational equilibria to compensate for changes in ambient temperature. Guided by our hypothesis that temperature compensation in KaiA involves a competition between the N- and C-terminal domains for the linker that connects them, we can abolish and rescue temperature compensation in this protein by mutagenesis. For KaiB, we will show that the equilibrium free energy (ΔG) between its active and inactive folds changes such that the active fold becomes, respectively, more and less accessible at lower and higher temperatures. The enzymatic activities

of KaiC likewise shift to compensate for temperature changes. Thus, each of the core oscillator proteins plays an important role in the overall mechanism of temperature compensation in the cyanobacterial circadian clock.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 140 | De novo Protein Design of Vascular Endothelial Growth Factor Inhibitors

Kateryna Maksymenko[1], Julia Skokowa[2], Andrei Lupas[1], Patrick Mueller[3], Mohammad ElGamacy[1]
[1]Max Planck Institute for Developmental Biology, [2]University Hospital Tübingen, [3]Friedrich Miescher Laboratory of the Max Planck Society (BW, Germany)

Growth factors are signaling molecules coordinating the complex functionality of multicellular organisms during development and homeostasis. The activity of signaling networks needs to be tightly controlled; otherwise, aberrant expression of growth factors can cause diverse disorders such as cancer, autoimmune and cardiovascular diseases. Being highly specific and able to target various molecular surfaces, protein-based binders constitute a powerful means to manipulate signaling interactions. In contrast to the common antibody development technologies, which are empirically guided and often yield binders to irrelevant epitopes, de novo protein design methods allow targeting arbitrarily selected functional sites. Additionally, de novo design offers control over protein sequence and topology, facilitating improvements in folding kinetics, protein stability, and solubility. Here, we present our work on in silico design of the epitope-directed inhibitors against vascular endothelial growth factor (VEGF), a key modulator of tumor progression. Taking advantage of a new computational approach for massive-scale docking of a target epitope against a protein structure database, we selected several scaffolds with high shape complementarity to the receptor binding site of VEGF. After further interface design, aiming to minimize the estimated binding free energy, a small set of best candidates (16 proteins) was experimentally evaluated. Biophysical measurements revealed that the binding affinities of the designs to VEGF ranged from nano- to micromolar levels. X-ray structure determination of one of the candidates showed atomic-level agreement with the design model. Moreover, in vitro assays showed the ability of the best designs to inhibit proliferation of VEGF-dependent acute myeloid leukemia cells. Thus, our results not only provide potential anti-cancer therapeutic candidates, but also demonstrate the feasibility of the rational approach to design high-affinity protein binders against predefined conformational motifs of the target molecules. This generalizable approach can be deployed to generate novel leads for future research and therapeutic purposes.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 141 | Conformational Dynamics and Allostery in Yeast Chorismate Mutase

Dennis Winston[1], Scott Gorman[1], David Boehr[1]
[1]Pennsylvania State University (PA, USA)

Chorismate mutase catalyzes the conversion of chorismate to prephenate in the biosynthesis of phenylalanine and tyrosine via the shikimate pathway. Because humans lack this pathway, chorismate mutase is a potential target for fungicides and herbicides. The chorismate mutase from Saccharomyces cerevisiae (ScCM) is allosterically regulated by tryptophan and tyrosine. It was initially proposed that ScCM in the absence of effector fluctuates between activated and inhibited conformations according to the Monod-Wyman-Changeux (MWC) model, although later mutagenesis and kinetic data suggest that the regulation is more complex. The aim of this study was to understand the conformational fluctuations that occur in ScCM in the absence of effector and how those conformational fluctuations change upon effector binding. We used solution NMR spectroscopy to study conformational exchange on the μs-ms timescale via Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion. We found that apo ScCM is dynamic on the μs-ms timescale and much of this motion is quenched upon saturation with tyrosine, which decreases enzymatic activity. Upon saturation with tryptophan, which increases enzymatic activity, faster conformational exchange was observed. In order to determine whether ScCM is exchanging between activated and inhibited conformations in the absence of either effector, we compared chemical shift differences between exchanging states from relaxation dispersion to chemical shift differences between each effector-bound state for residues away from the effector binding site. We found that there is a correlation between the chemical shift differences, but the conformational fluctuations undergone by apo ScCM appear to be more complicated than a two-state model, suggesting that apo ScCM is not exclusively exchanging between activated and inhibited conformations. The changes in conformational dynamics on the μs-ms timescale by effector binding may be important for understanding the mechanism of allosteric regulation in ScCM.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 142 | Damietta: A Tensorised Protein Design Engine, and Its Application in Therapeutic Protein Engineering

Mohammad ElGamacy[1], Birte Hernandez Alvarez[1], Julia Skokowa[2], Andrei Lupas[1]
[1]*Max Planck Institute for Developmental Biology,* [2]*University Hospital Tübingen (BW, Germany)*

Protein-based therapeutics can carry out complex pharmacological roles that has broadened the range of treatable disorders. The main challenge in the face of designing or engineering proteins is the breadth of the protein sequence and structure spaces. Experimental approaches of directed-evolution easily run into limits of tractability beyond a few amino acid positions to combinatorially mutate. In contrast, computational protein design has recently made great leaps in handling much larger sequence spaces, resulting in novel or enhanced therapeutic protein candidates from first principles. These advances in rational design came through the two-pronged progress in: i) scoring functions could make good predictions of the mutational fitness, and ii) advanced sampling algorithms could "prune" vast sequence sub-spaces that would otherwise provably yield poor solutions. Despite its larger capacity for sequence sampling, computational protein design runs into the same problem of sequence space intractability, as it is limited by the finite computing resources available to a particular design problem. Hence comes the motivation for innovative scoring and sampling algorithms that are several orders of magnitude faster. Ideally, these algorithms should also be compatible with cutting-edge hardware accelerators (e.g. GPU and TPU), and capable of multi-level parallelisation.Herein, we developed a new mathematical and computational framework (Damietta) aiming to accelarate the rotamer/mutant scoring and sampling. We then retrospectively validated the accuracy of the energy function using mutants thermostability datasets. Lastly, we prospectively validated the Damietta design energy function by deploying it to redesign the recombinant human granulocyte-colony stimulating factor (rhG-CSF), with the goal of creating a thermostable immunotherapeutic protein. So far, we experimentally tested two designs, which showed more than 10-fold higher expression yield in E. coli, enhanced thermostability, and higher proteolytic resistance. Our present work is focused on evaluating in cell proliferative and differentiation activities of these designs.

Track: Membrane Proteins
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 143 | Delivery of Recombinant SARS-CoV-2 Envelope Protein into Human Cells

Ricardo Capone*[1], James M. Hutchison*[2], Dustin D. Luu[3], Arina Hadziselimovic[1], Wade D. Van Horn[3], Charles R Sanders[4]
[1]*Department of Biochemistry, & Center for Structural Biology, Vanderbilt University, Nashville, TN, USA,* [2]*Chemical and Physical Biology Graduate Program, & Center for Structural Biology, Vanderbilt University, Nashville, TN, USA,* [3]*School of Molec (TN, USA)*

The SARS-CoV-2 envelope protein (S2-E) is a conserved membrane protein in the β-coronavirus family, which is responsible for the COVID-19 pandemic. S2-E is efficiently retained in the Golgi and ERGIC membranes during infection of human cells, where this localization is essential to viral assembly and budding. Here, we describe the recombinant expression and purification of the SARS-CoV-2 envelope protein (S2-E) into amphipol-class amphipathic polymer solutions. The physical properties of amphipols underpin their ability to stabilize the native folds of membrane proteins without disrupting membranes. Amphipol delivery of the S2-E protein to pre-formed planar bilayers results in spontaneous membrane integration and formation of viroporin ion channels. Amphipol delivery of the S2-E protein to human cells results in membrane integration followed by retrograde trafficking to a perinuclear location immediate adjacent to the endoplasmic reticulum-to-Golgi intermediate compartment (ERGIC) and the Golgi, which are the sites of coronaviruses replication. Delivery of exogenous S2-E to cells enables chemical biological approaches for future studies of S2-E function in pathogenesis and for possible development of "Trojan Horse" anti-viral therapies. This work also establishes a paradigm for amphipol-mediated delivery of membrane proteins to cells.

Track: Therapeutics and Antibodies
Session: Measuring Forces of Biological Systems

ABS# 144 | Virtually Identifying Novel Tuberculosis InhA Inhibitors

Shravya Yarlagadda[1], Sarah Mayberry[1], Madison Tran[1], Sandy Bakheet[1], Josh Beckham[1]
[1]*University of Texas at Austin (TX, United States)*

This project involved virtual screening to identify novel inhibitors of the enoyl-acyl carrier protein reductase(InhA) in the bacterium Mycobacterium tuberculosis - the causative agent of Tuberculosis(TB). The prevalence of drug-resistant TB, along with the lengthy current treatment regimens, increases the need for novel drugs with diverse chemical scaffolds to reduce this disease burden. InhA plays a crucial role in the biosynthesis of mycolic acids, which are essential for bacterial wall formation. Thus, inhibiting InhA prevents proper cell wall formation, eventually leading to cell death, making InhA an effective target for therapeutic intervention. This project utilized molecular docking programs, such as GOLD, to virtually screen novel ligand libraries against the apo version of InhA(PDB:4TRM). The calculated GOLD scores were used to identify potential inhibitors as defined by their docking scores relative to a set of positive and negative controls. The control ligands, including Isoniazid, were prepared prior to docking in LigPrep (or OpenBabel) to generate low energy three-dimensional conformations with protonation states at physiological pH. Then, HitFinder9, Novacore, ZincpH7, Fragment, CNS, MayBridge, KINA, and ION large libraries were screened, resulting in a total of ~480,000 ligands screened. Positive control docking resulted in conformations similar to other current co-crystal structures. GOLD scores for positive controls ranged from 60-100. The HitFinder9, Novacore, and ZincpH7 library dockings resulted in ligands scoring in the same range, with multiple novel ligands scoring between 90 and 100. The Fragment and CNS libraries resulted in most ligand scoring between 50 and 80. The Maybridge, KINA, and ION libraries resulted in most ligands scoring between the 70 and 90 range. Novel ligands from the HitFinder9, Novacore, and ZincpH7 libraries scored on par with the positive controls. These ligands can be later analyzed with in vitro methods and could potentially be developed into drug compounds. Such high-scoring ligands give promising beginnings.

Track: Proteins in Cells

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 145 | Enabling Structural Studies of Metastable Proteins within Cellular Milieu via Dynamic Nuclear Polarization NMR

Whitney Costello[1], Yiling Xiao[1], Carla Madrid[1], Kendra Frederick[1]
[1]*University of Texas Southwestern Medical Center (TX, USA)*

This study aims to bridge the gap between atomic models of metastable proteins in isolation and in cellular milieu. Most biophysical techniques have limitations in either sample composition or resolution. One technique, Nuclear Magnetic Resonance (NMR) is not limited by sample composition or resolution, but is limited by sensitivity. However, the development of Dynamic Nuclear Polarization (DNP) NMR eliminates this sensitivity barrier. Using DNP NMR, we observe sensitivity enhancements of up to 90x on protein within cellular milieu at endogenous concentrations. Now, experiments that normally take over 30 years can be performed in a few hours.Recent work on a metastable protein, Sup35, assembled in cellular lysates demonstrates that the biological environment can and does have a dramatic effect on protein structure. We seek to harness sensitivity gains from DNP NMR to determine structures of Sup35 within cellular milieu. Working at physiological concentrations imposes a need for specificity from the biological environment. By using pulse sequences probing 15N-13C bonds, we show specificity to the two arginines of Sup35NM within cellular milieu. However, the SNR of these two arginines is low. Showing, even with DNP NMR, we push the boundary of necessary sensitivity. So, we optimized sample preparation for maximum sensitivity for DNP NMR on cellular milieu. DNP sensitivity enhancement depends on sample composition. DNP NMR is performed at 100 K, and requires sample glassing, biradical, and protonation for optimal DNP enhancement. Traditionally, a purified protein sample uses a matrix of 60:30:10, d8-glyercol:D2O:H2O with 10 mM biradical. We found in the presence of the cellular milieu high DNP enhancements maintain with lower cryoprotectant (15%) and biradical concentrations (5 mM). We also found deuteration was unnecessary. In conclusion, we propose a matrix of 15:0:85 d8-glyercol:D2O:H2O with 5 mM biradical to probe proteins with maximal sensitivity enhancement at physiological concentrations within cellular milieu.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 147 | The Penn State Protein Ladder System for inexpensive protein molecular weight markers

Ryan Santilli[1], John Williamson[1], Yoshitaka Shibata[1], Rosalie Sowers[1], Andrew Fleischman[1], Song Tan[1]
[1]*Penn State University (PA, USA)*

We have created the Penn State Protein Ladder system to produce protein molecular weight markers easily and inexpensively (less than a penny a lane). The system includes plasmids which express 10, 15, 20, 30, 40, 50, 60, 80 and

100 kD proteins in E. coli. Each protein migrates appropriately on SDS-PAGE gels, is expressed at very high levels (10-50 milligrams per liter of culture), is easy to purify via histidine tags and can be detected directly on Western blots via engineered immunoglobulin binding domains. We have also constructed plasmids to express 150 and 250 kD proteins. For more efficient production, we have created two polycistronic expression vectors which coexpress the 10, 30, 50, 100 kD proteins or the 20, 40, 60, 80, kD proteins. 50 ml of culture is sufficient to produce 20,000 lanes of individual ladder protein or 3,750 lanes of each set of coexpressed ladder proteins. These Penn State Protein Ladder expression plasmids also constitute useful reagents for teaching laboratories to demonstrate recombinant expression in E. coli and affinity protein purification, and to research laboratories desiring positive controls for recombinant protein expression and purification.

Track: Intrinsically Disordered Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 150 | Functional Role of Protein Disorder in Eukaryotic Gene Regulation

Rama Reddy Goluguri[1,2,3], Mourad Sadqi[1,2,3], Suhani Nagpal[1,2], Victor Muñoz[1,2,3]
[1]University of California, Merced, [2]Centre for cellular and Biomolecular Machines, [3]Department of Bioengineering, School of Engineering (California, United States)

Gene regulation requires efficient binding of transcriptional factors (TF) to their target DNA site. It is known that such an efficient binding to target sequence is achieved by a combination of 3D diffusion and 1D diffusion along the DNA by means of non-specific binding. The protein needs to scan the DNA by binding non-specifically, and quickly locks into the specific binding site once it's encountered. The mechanism by which a single domain protein alternates rapidly between these two binding-modes is however poorly understood. To address these questions, we studied the binding of Drosophila Engrailed homeodomain (EngHD) to the lambda phage DNA using optical tweezers coupled to confocal microscopy. EngHD folds ultrafast and the folding dynamics are consistent with a downhill folding scenario. We created two different mutant variants of EngHD: one that binds DNA tighter and other that makes EngHD partially unfolded. We observed that the EngHD-DNA complex is very dynamic, even for the variant that binds strongly to DNA. The partially unfolded variant was seen to diffuse very rapidly on DNA. The downhill folding mechanism of EngHD allows it to sample partially unfolded, search competent conformations under native-like conditions inside the cell and allows for efficient locking of the native conformation into the binding site upon encountering it. Our experimental results indicate a functional role for the intrinsically disordered regions present in many eukaryotic TFs and why they utilize folding coupled binding mechanism to regulate gene expression.

Track: Therapeutics and Antibodies
Session: Designer Proteins Through Genetic Code Expansion

ABS# 152 | Engineering EFNB2 as an Orthogonal Receptor Therapeutic for Nipah Virus

Krishna Narayanan[1], Erik Procko[1]
[1]University of Illinois, Urbana-Champaign (Illinois, United States of America)

Nipah virus (NiV) is a zoonotic virus from the Henipavirus genus. Bats act as a natural animal reservoir, with frequent crossover events to domestic animals and humans in South and Southeast Asia over the past decades. NiV is highly pathogenic with high morbidity and mortality, and currently there are no approved therapeutics or vaccines that specifically target the virus. To aid the development of therapeutics against NiV, as well as further our understanding of the structural basis for infection, we have engineered ephrin B2 (EFNB2), the host receptor for the viral attachment glycoprotein (NiV-G), into a soluble decoy receptor with enhanced specificity for the virus. EFNB2 is a ligand of ephrin type-B receptor 2 (EphB2) that regulates multiple developmental processes, and so to leverage EFNB2 as a possible neutralizing agent that is safe and efficacious in vivo, mutations were identified through deep mutagenesis that enhance specific binding to NiV-G over EphB2. The mutations highlight how different EFNB2 conformations are selected by NiV-G versus the human interaction partner. One mutant in particular, EFNB2-D59Q, demonstrated wildtype-like binding to NiV-G and substantially diminished binding to EphB2. EFNB2-D59Q also demonstrated pan-specificity to the attachment glycoproteins of other Henipaviruses, specifically Hendra virus, Cedar virus, and Ghana virus. However, due to the promiscuous nature of ephrin-Eph receptor interactions, EFNB2-D59Q was found to have high residual binding to EphB3 and EphB4, potentially causing off-target effects in vivo that may require additional modifications to increase specificity further. Overall, we demonstrate how a promiscuous cell entry receptor can be engineered as a soluble decoy with improved specificity for a viral target.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 155 | Hydrogen Exchange Mass Spectrometry Reveals Structural Features of BtuB Plug Domain's Allosteric Destabilization Upon Ligand Binding

Adam Zmyslowski[1], Isabelle Gagnon[1], Tobin Sosnick[1]
[1]*University of Chicago (IL, United States)*

During metabolite transport, TonB dependent transporters (TBDTs) found in the outer membranes of gram-negative bacteria are hypothesized to partially unfold their N-terminal "plug" domain. The 130 aa plug normally is folded inside a 470 aa, 22-strand β barrel, and must undergo significant rearrangement to allow passage of large substrates such as vitamin B12 (~1.4 kDa) in the case of BtuB, a well studied TBDT. Previous studies have suggested that binding of B12 to the extracellular face of BtuB leads to stronger binding between the TonBox found on BtuB's periplasm-facing N-terminus and the C-terminal domain of TonB, a periplasm-spanning intergral inner membrane protein. Our hydrogen exchange (HX) and limited proteolysis experiments performed on BtuB in a native-like preparation of minimally treated outer membranes show that the N-terminal region of BtuB's plug (extending 20-40 residues past the TonBox) is less stable than the plug's C-terminal half, even in the absence of B12. Upon addition of substrate the ~20 residues immediately C-terminal to the TonBox have accelerated HX rates, suggesting a destabilization of this region. In addition, we find stabilization in some, but not all, regions near the B12 binding site. Interestingly, regions of slowed and accelerated HX are not structurally contiguous, with the connecting secondary structural elements showing minimal differences in HX upon ligand binding. Experiments with TonB CTD are ongoing in order to determine whether the addition of TonB to the BtuB:B12 complex is necessary and/or sufficient for population of a putative pore-forming transport intermediate.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 156 | Conformational states guiding the monomer-fibril transition of α-Synuclein

Archi Saurabh[1], N. Prakash Prabhu[1]
[1]*Department of Biotechnology & Bioinformatics, School of Life Sciences, University of Hyderabad, Hyderabad – 500 046, India (Telangana, India)*

α-Synuclein (α-Syn), an intrinsically disordered protein, shows significant conformational plasticity and adapts different structures at different conditions. Conversion of native monomers of α-syn, mostly consists of α-helix, to fibril assembly with cross β-structures is the primary cause of Parkinson's disease. We investigated the major intermediate conformations involved during this transition using molecular dynamics simulations. A terminal-chain from the fibril structure of α-syn (PDB id: 2N0A) was dissociated away by steered molecular dynamics (SMD), and umbrella sampling (US) was performed on the conformations selected at regular distance-intervals. Further, the conformational sampling of native monomer (PDB id: 1XQ8) and a single chain of fibril structure was studied by replica exchange molecular dynamics simulation. The conformations sampled from SMD/US and REMD were analysed to find out the probable intermediate states in the fibrillation pathway. The analysis showed that monomeric α-syn forms a compact structure by the interaction between NAC region and C-terminal proceeded with the disruption of helix2 region. This could be the first stage of aggregation-initiation. From the different conformational sampling, at least three different states with predominantly β-hairpin structures were detected. Mainly, formation of β-hairpins covering the residues 51-57 and 69-77 or 77-86 were more noticeable. These hairpins, formed out of the conformational plasticity of central region of helix2, were stabilized by hydrogen bonds. In addition, another conformation with β-hairpin formed by the interaction of C-terminals of helix1 and helix 2 covering the residues 31-43 and 82-89 was identified. This β-hairpin underwent further conformational changes to form stable interchain cross β-structure which was facilitated by the interchain hydrogen bonding interactions and salt bridges, particularly between E46-K80 and K96-D98. These structural features also explain the experimentally observed changes in fibril progression due to amino acid mutations.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 157 | Urea as a Protein LID Opener: Overcoming Substrate Inhibition in Adenylate Kinase

David Scheerer[1], Dorit Levy[1], Inbal Riven[1], Hisham Mazal[1], Gilad Haran[1]
[1]*Weizmann Institute of Science (Rehovot, Israel)*

Enzymes accelerate vital chemical reactions by multiple orders of magnitude. Many of them harness large-scale

motions of domains and subunits to promote their activity. Studying structural dynamics is hence essential in order to decipher how protein machines function. Combined with H2MM, a photon-by-photon hidden Markov model analysis, single-molecule FRET studies on the enzyme adenylate kinase (AK) recently revealed very fast domain motions, two orders of magnitude faster than the enzyme's turnover.1 It was proposed AK may use numerous jumps between its open and closed states in order to find a relative orientation of its substrates that is optimal for catalysis.In the present work, we studied how the open/closed ratio mediates the activity of the enzyme. We find that high concentrations of the substrate AMP decrease the rate of LID-domain opening and disturb the balance between the two conformations. This effect is reflected in inhibition of enzymatic activity by high AMP levels. We manipulated the strength of substrate inhibition using several single-site mutations and could correlate the degree of substrate inhibition to changes in the open/closed ratio.Interestingly, we also found that low concentrations of urea partially revert the inhibitory impact of high AMP concentrations. Although urea commonly has been used as a protein denaturant, here, it actually enhances the catalytic activity of AK. Urea favors the open conformation of AK and can accordingly help to reestablish the optimal balance between domain opening and closing. However, in the absence of inhibitory concentrations of AMP, further promotion of the open state by urea is detrimental for turnover. This suggests a delicate balance between domain closure dynamics and substrate binding, which might not be a unique feature of AK, and is likely employed by a multitude of enzymes to regulate their activity.1. Aviram, H. Y. et al., PNAS 2018, 115, 3243-3248.

Track: Computational Modeling/Simulation
Session: Protein Evolution, Design and Selection

ABS# 158 | TopoBuilder, a generic framework for layered de novo protein design

Zander Harteveld[1], Jaume Bonet[1], Stéphane Rosset[1], Che Yang[1], Fabian Sesterhenn[1], Bruno Correia[1]
[1]*EPFL (Vaud, Switzerland)*

Fast and controlled searches for custom-shaped proteins through the vast fold space outside the currently limited repertoire of classified protein structures require efficient structural parametrizations as well as realistic decompression algorithms thereof. Here, we developed an algorithm—the TopoBuilder de novo design framework—that, fully automatically generates synthetic proteins with naturalistic features from a simple string parametrization describing the overall geometry of a particular protein fold. Our framework eliminates the need of manually formulating fold-specific rules via an iterative, data-driven approach that extracts simple geometrical parameters from sub-parts of known protein folds and uses these topological restraints to guide the overall construction and design process. We validate our framework by experimentally characterizing several 'topobuilded' design sequences and found that some designs fold to monomeric stable structures with circular dichroism spectra consistent with the predicted models. Hence, the TopoBuilder de novo design framework should be broadly useful for the generation synthetic proteins with customizable geometries, automated tracing of fundamental protein architectonic principles and lays the foundations for systematic, large-scale protein fold space searches.

Track: Design/engineering
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 159 | Multisensory Proteins - Making Proteins Accessible through Art

Erica Tandori[1], Stu Favilla[2]
[1]*Monash University,* [2]*Swinburne University (Victoria, Australia)*

Techniques such as X ray crystallography and microscopes have enabled us to see protein structures and interactions that are invisible to the naked eye. However. for people with Low Vision or Blindness, even these powerful tools are not enough. For them, the world of molecular biology remains out of sight and out of reach, contributing to a poor representation of blind and low vision students amongst STEM cohorts at university level, and far too few career opportunities within science generally.Although innovative projects such as NASA's 3D Tactile Hubble images and WonderDome UK, are enabling people with vision disability to understand astronomy, there is very little available to make molecular biology more accessible. How can the micro-molecular world of viruses and proteins be better communicated to a blind and low vision audience?Through our research into accessibility for diverse needs audiences, we are exploring new ways to make molecular biology accessible, equitable and inclusive for all through exhibitions and outreach programs. This poster-presentation reports on three years of projects utilizing multi-sensory approaches, including; tactile artworks, multisensory interactions, Arduino LED, data projection,

novel video displays, ASMR audio design, and AI, that enable people to touch, feel, smell, hear and interact with proteins big enough to hold or even climb inside!Our presentation includes explorations of multisensory proteins, molecules, viruses and microbes that are accessible to Low Vision, Blind, Hearing Impaired, Diverse Needs individuals and audiences of all ages and abilities alike.

Track: Chemical Biology
Session: Allostery & Dynamics in Protein Function

ABS# 160 | Zn(II) binding properties of human metallothioneins – Investigation of three classes of Zn(II) affinities

Sylwia Siadul[1], Karolina Szaro[1], Józef Ba Tran[1], Agnieszka Drozd[1], Artur Krężel[1]
[1]*Department of Chemical Biology, Faculty of Biotechnology, University of Wrocław, 50-137 Wrocław, Poland (Wrocław, Poland)*

Human metallothioneins (MTs) are small Cys-rich proteins involved in Zn(II) and Cu(I) homeostasis. MTs family consists of four main isoforms and their subisoforms named MT1-MT4. Human MTs contain 19-21 Cys residues that let them bind up to 7 Zn(II) in two separate a- and β-domain. Amino acid changes among isoforms are poorly understood and investigating the impact of these divergences could bring more information about the functions of MTs. Here, we present results that show how various human MTs isoforms bind Zn(II) with different affinities. The cDNA of particular MTs isoform was cloned into pTYB21 vector. The plasmid was transformed into BL21(DE3) E. coli and subsequently produced in E. coli using IMPACT system. SEC purification in 10 mM HCl let us obtain thionein (apo-MT) that was reconstituted with Zn(II) ions to obtain MT (holo-MT). All purified MTs isoforms concentrations were measured with Ellman's reagent (DTNB) and verified for a number of bound Zn(II) by PAR assay. To determine dissociation constants of 7 Zn(II) ions in isoform, two kinds of titrations with fluorescent ZnAF-2F probe were conducted (1). Titration of apo-MT with ZnAF-2F let us determine tight binding sites with binding constants Kd = 10-11.5-10-12.5 M. Weak binding sites (Kd = 10-7-10-9 M) were determined via competition of holo-MT with ZnAF-2F. Affinities for Zn(II) binding sites with medium affinity (Kd = 10-9.5-10-11 M) were determined through titration of MTs isoforms with ZnAF-2F and fitted by in-house Python script based on mass-balance principle. All of the obtained results shed new light on the necessity of such diversity between human MTs isoforms and give further directions for better understanding of their functions. Financial support by the National Science Center of Poland (grant OPUS 2018/31/B/NZ1/00567) is gratefully acknowledged.1) Krężel, A.; Maret, W. J. Am. Chem. Soc. 2007, 129, 10911.

Track: Chemical Biology
Session: Allostery & Dynamics in Protein Function

ABS# 161 | Zn(II)-binding properties of metallothioneins from various domains of life

Karolina Szaro[1], Sylwia Siadul[1], Kinga Jurczak[1], Artur Krężel[1]
[1]*Department of Chemical Biology, Faculty of Biotechnology, University of Wrocław, Joliot-Curie 14a, 50-383 Wrocław, Poland (Wroclaw, Polska)*

Metallothioneins (MTs) are small cysteine-rich (15-35%) proteins responsible for binding essential and toxic metal ions. They are widely spread through almost all life domains and play a crucial role in metal and redox homeostasis, transport of metal ions across cellular compartments, regulation of various metal-dependent pathways, and detoxification [1]. Although mammalian MTs are the most known, little is known about the functions of MT proteins from lower organisms and other than animal kingdoms or domains. One of the significant roles of MTs is metal ions buffering in cells to keep them at the proper concentrations. For these properties, metal affinities in MTs must differ between particular sites to increase buffering capacity. To shed more light on these properties, we obtained MTs from three phyla - Animal, Plant, and Bacteria, using a bacterial expression system with the Intein Mediated Purification with an Affinity Chitin-binding Tag system (IMPACT) without any additional amino acid residues [2]. The proteins were purified by size exclusion chromatography in 10 mM HCl to obtain apo-forms and reconstituted with ZnSO4 to get Zn(II)-saturated MTs. The measurements with DTNB and PAR allowed to determined metal-to-protein content. The use of ultrasensitive zinc probe ZnAF-2 allowed us to determine Zn(II) binding constants, which, similar to human MT2 differ by several orders of magnitude between each other. The molecular basis for affinity differentiation and its consequences are discussed. Our findings demonstrate that the tight and weak Zn(II)-binding sites appear in each of the study groups of organisms, indicating the Zn(II) buffering mechanism's importance. Financial support by the National Science

Centre of Poland (grant 2018/31/B/NZ1/00567) is gratefully acknowledged. References1. A. Ziller et al., Metallomics 2018, 10, 1549.2. A. Krężel, W. Maret, J. Am. Chem. Soc. 2007, 129, 10911.

Track: Systems Biology
Session: Measuring Forces of Biological Systems

ABS# 162 | Mapping transcriptome data on protein-protein interaction networks of Inflammatory Bowel Diseases reveals disease-specific sub-networks

Sefika Feyza Maden[1], Saliha Ece Acuner[1]
[1]*Istanbul Medeniyet University (Istanbul, Turkey)*

Inflammatory bowel disease (IBD) is the common name for chronic disorders associated with the inflammation of the gastrointestinal tract. Crohn's disease(CD) and ulcerative colitis(UC) are the two distinct types of IBD. While involvement in ulcerative colitis is limited to the colon, Crohn's disease may involve the whole gastrointestinal tract. Although these two disorders differ in macroscopic inflammation patterns, they share various molecular pathogenesis yet the diagnosis can remain unclear and it is important to reveal the molecular signatures of them in the network level. To this aim, we determine the sub-networks specific to UC and CD by mapping transcriptome data to protein-protein interaction networks and perform the functional enrichment analysis. Tumor protein 63 (TP63) is the most connected gene in UC-specific sub-network. It is in the same family as the tumor proteins p53 and p73, which have been studied for risk associated with both colorectal cancer and IBD. Amyloid-beta precursor protein (APP) is one of the most connected genes in CD-specific sub-network. CD-specific APP gene, which was also shown to have an important role in the pathogenesis of Alzheimer's disease (AD), suggests that some similar genetic factors may be effective between these two diseases. Functional enrichment analysis shows that UC-specific sub-network is mostly important in the regulation of the activity of cancer suppressor TP53 and literature shows that increased p53 protein expression or the development of dysplasia can be used as a biomarker in the diagnosis of UC-associated colon cancer. On the other hand, CD-specific sub-network is mainly involved in the formation of fibrosis in the gut. Overall, these findings reveal the differences between IBD subtypes at the molecular level and can facilitate diagnosis for UC and CD as well as provide potential molecular targets that are specific to disease subtypes.

Track: Evolution
Session: Protein Evolution, Design and Selection

ABS# 163 | Lost & Found. Indel-mediated cofactor specificity switch from NAD to SAM

Saacnicteh Toledo Patino[1]
[1]*OIST (Okinawa, Japan)*

Nearly 50% of protein catalysis requires the assistance of coenzymes to drive reactions that are otherwise unattainable. Hence, a better understanding on how these nanomachines have evolved to efficiently use their cognate coenzymes, not only provides valuable information about their sophisticated chemistry, but also crucial mechanistic constraints for enzyme engineering. An example of such coenzymes are S-adenosylmethionine (SAM) and nicotinamide adenine dinucleotide (NAD), which are utilized to catalyze (mainly) methylation and redox reactions, respectively. Despite their different chemistry outcomes, SAM- and NAD- dependent Rossmann domains have been hypothesized to be related due to their local structural resemblance. The present work sustains this hypothesis, demonstrating that cofactor-binding function can be switched from NAD to SAM on the oxidoreductase scaffold. Herein, we assessed sequence-based Hidden Markov Models and structural alignment tools, finding that their homologous region differs in an insertion of mostly three amino acids, which is only present in NAD-binding domains. Anticipating that this indel may have mediated cofactor specificity, we removed it from a NAD-binding scaffold, resulting in loss of NAD-binding, while acquiring SAM-binding functionality. To the best of our knowledge, this is the first example of cofactor-usage divergence, where the cofactor that is switched performs a distinct chemistry (redox Vs. methyl transfer). This interesting case illustrates how proteins may be able to modify their original chemistry by introducing a few mutations and adopting different cofactor specificities.

Track: Protein Interactions and Assemblies
Session: Measuring Forces of Biological Systems

ABS# 164 | Surface Specific Binding of Different Alginate Oligosaccharides to Beta-Lactoglobulin

Mikkel Madsen[1], Peter Westh[1], Günther Peters[1], Finn l. Aachmann[2], Bithe B. Kragelund[3], Birte Svensson[1]
[1]*Technical University of Denmark,* [2]*Technical University of Norway,* [3]*Copenhagen University (Capital, Denmark)*

β-Lactoglobulin (β-Lg) is able to bind a large variety of molecules of different affinities and functions. Binding of small organic compounds mostly occurs in a hydrophobic cavity, formed by an eight-stranded β-barrel, referred to as the calyx, but can also happen to the surface. Lately formation of networks between β-Lg and polymers, such as hydrocolloids, have come into focus1. Anionic polysaccharides are thought to bind to the surface of β-Lg through electrostatic interactions, leading to distinct particle formation. Structural details have been described in a few cases, where fragments of polysaccharides were found to bind at positive patches of the β-Lg surface2,3.In the present study, binding of alginate oligosaccharides (AOS of 4, 5 or 6 sugars) consisting of only mannuronic, guluronic acid residues or a mixture thereof was described, using NMR chemical shift perturbation and saturation transfer difference, ITC and molecular docking. Even though charge-charge interactions were observed, the binding sites also included hydrophobic patches. Notably, the number of binding sites varied from 2 to 5 depending on AOS length, and longer AOS did not prefer positive patches over shorter AOS, but extended the binding surface to include polar and hydrophobic side chains to connect short AOS binding sites. The work is funded by the Novo Nordisk Foundation (NNF). NMR was supported by SBiNLab, UCPH and NOBIPOL, NTNU.(1) Madsen et al. Impact of Alginate Mannuronic-Guluronic Acid Contents and PH on Protein Binding Capacity and Complex Size. Biomacromolecules 2020 22, 649-60. https://doi.org/10.1021/acs.biomac.0c01485. (2) Stender et al. Alginate Trisaccharide Binding Sites on the Surface of β-Lactoglobulin Identified by NMR Spectroscopy: Implications for Molecular Network Formation. ACS Omega 2019, 4, 6165–74. https://doi.org/10.1021/acsomega.8b03532. (3) Birch et al. Binding Sites for Oligosaccharide Repeats from Lactic Acid Bacteria Exopolysaccharides on Bovine β-Lactoglobulin Identified by NMR Spectroscopy. ACS Omega 2021, acsomega.1c00060. https://doi.org/10.1021/acsomega.1c00060.

Track: Protein Interactions and Assemblies

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 165 | Metal binding of a potential zinc finger nuclease

Bálint Hajdu[1], Béla Gyurcsik[1]

[1]*Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, Szeged, Hungary H-6720 (Csongrád, Magyarország)*

Zinc finger proteins with modular assembly can specifically target various selected DNA sequences [1]. In nature, they control the function of genes, but they also can be turned into specific DNA manipulating agents. Previously we obtained a potentially specific nuclease by selectively hydrolyzing a zinc finger protein by nickel(II) ions [2]. The metal ion induced protein cleavage resulted in formation of an amino terminal copper(II)/nickel(II) binding – so called ATCUN – motif which can cleave DNA [3]. Since zinc fingers can only recognize their target DNA sequences in the form of zinc(II)-complex, it is essential – especially in the case of a potential nuclease – to determine the zinc-binding stability of the protein. Furthermore the competition reactions with other metal ions inside living organisms must be taken into account, as well.UV and CD spectroscopy, ESI mass spectrometry, isothermal titration calorimetry (ITC), and platereader fluorimetric measurements were applied in our research. We optimized CD and ITC titration methods to analyze the zinc binding of a zinc finger nuclease consisting of three finger domains. We also investigated the competition effect of copper(II), silver(I), cadmium(II), mercury(II), and cobalt(II) ions for the zinc finger protein by fluorescence titrations. Further experiments were performed in the presence of specific or nonspecific DNA sequences to understand the impact of DNA during the competition reactions.Based on our results the zinc finger protein binds the zinc(II) ions strongly. Yet heavy metal ions may partially or completely replace the native metal ion. The presence of the DNA can stabilize the zinc finger protein.Acknowledgements: Financial support by the Hungarian National Research, Development and Innovation Office (GINOP-2.3.2-15-2016-00038 and K_16/120130).References:1. A. Klug; Annu. Rev. Biochem. (2010), 79, 213.2. A. B.-Ciesielska, et al. Metallomics, (2018), 10, 1089.3. C. Harford; B. Sarkar; Acc. Chem. Res. (1997), 30, 123.

Track: Proteins in Cells

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 166 | Alpha-Synuclein Translocates from Neurons to Glia and Impairs Glial Homeostasis in Drosophila

Yifan Zhang[1], Robert Fairman[1]

[1]*Haverford College, Department of Biology, Haverford, PA (PA - Pennsylvania, United States)*

α-synuclein is an intrinsically disordered, aggregation-prone protein that is involved in the pathogenesis of Parkinson's disease (PD). Emerging evidence suggests

that the transcellular propagation of α-synuclein exacerbates disease progression. However, the molecular mechanism and cellular consequences of α-synuclein transmission are not well-understood. Here, we report the development of a transgenic Drosophila model that phenomenologically mimics the intercellular transmission of α-synuclein and enables the investigation of α-synuclein transmission with high spatial and temporal resolution and with facile genetic and pharmaceutical perturbations. Fluorescently tagged α-synuclein is tissue-specifically expressed in a defined subset of cells, and their translocation into other cell populations and brain regions can be monitored with confocal fluorescence microscopy. We demonstrated that α-synuclein accumulates in brain regions distal to the expression site in a time-dependent manner. At the cellular level, we found that α-synuclein expressed in neurons propagates to synaptically connected neurons in the retrograde direction, consistent with previous observations in human and mouse models. Among known familial mutations, A30P mutant, which features a mild clinical manifestation, has a decreased rate of transmission compared to E46K and A53T mutants, both of which are associated with the early onset and rapid progression of PD in patients. In addition to neuron-to-neuron transmission, we observed the translocation of neuronally expressed α-synuclein into glial cells. Remarkably, neuronal α-synuclein causes extensive glial death and decreases the level of Draper, a glial surface receptor involved in phagocytosis and Wallerian degeneration. Together, our findings support the involvement of transcellular propagation in α-synuclein pathology. Our ongoing efforts focus on characterizing the cellular consequences of α-synuclein transmission on the donor and recipient cells and adapting this model for large-scale genetic screens to identify molecular modulators of the transmission process.

Track: Dynamics and Allostery

Session: Allostery & Dynamics in Protein Function

ABS# 168 | Differences in conformational dynamics between a virus polyprotein and its fully processed counterparts help to expand the functional proteome

David Boehr[1]
[1]*Pennsylvania State University (PA, USA)*

Viral genomes must encode processes to regulate the host cell's machinery to allow for the efficient replication and packaging of new virus particles. These processes are encoded by relatively small genomes, and so viruses face a formidable information storage problem. Picornaviruses are positive-strand RNA viruses, and first produce a large polyprotein that must then be cleaved to generate functional components. Proteolytic precursors and fully mature forms of viral proteins often have different functions. The picornavirus 3CD protein exemplifies this concept. The 3C protein is a protease and has RNA-binding capabilities, and the 3D protein is an RNA-dependent RNA polymerase (RdRp). The 3CD protein has unique protease and RNA-binding abilities relative to 3C and is devoid of RdRp activity. How these functional changes in 3CD arise is poorly explained by its X-ray crystal structure, which suggests that the 3C and 3D domains are merely tethered together by a linker (i.e. 'beads-on-a-string' type model), and the domains do not interact. Differences in conformational dynamics may be one explanation for how 3CD and its processed products are differentiated in their functions. Indeed, we show using NMR relaxation methodologies that there are key differences in conformational dynamics in these proteins across multiple timescales. Our studies also indicate that extension of the C-terminus of 3C by only a few residues dramatically changes function, including inducing 3CD-like binding abilities for RNA and phosphoinositide lipids. We identify a potential network of interactions that transmits information from the C-terminus to the protease active site and RNA/lipid binding sites in 3C. In general, we propose that the conformational dynamics of precursor and processed forms of viral proteins regulate the specific function of these molecules during the viral replication cycle to expand the functional proteome beyond the limited storage capacity available within small viral genomes.

Track: Chaperones

Session: Targeted Protein Degradation

ABS# 170 | ATAD1 Regulates Apoptosis by Extracting BIM from the Outer Mitochondrial Membrane

Matthew Wohlever[1], Heidi Fresenius[1], Jake Winter[2], Jared Rutter[2]
[1]*University of Toledo,* [2]*University of Utah (OH, United States)*

Evasion of apoptosis is a hallmark of cancer and reactivation of apoptosis is a promising strategy for cancer therapy. Key regulators of apoptosis include the BCL2 family of tail anchored membrane proteins. While there have been significant studies on how BCL2 family proteins are targeted to the outer mitochondrial membrane (OMM), the removal of these proteins from the OMM remains understudied. ATAD1 is a recently discovered

AAA+ ATPase that maintains mitochondrial proteostasis by removing tail anchored proteins from the OMM. Here, we report the discovery of ATAD1 as a new negative apoptotic regulator that modulates the mitochondrial concentration of BCL2 family proteins. Using a combination of in vitro reconstitution and cell biology experiments, we demonstrate that ATAD1 directly removes the pro-apoptotic protein BIM from the OMM. Loss of ATAD1 leads to accumulation of BIM on the OMM and renders cancer cells sensitive to proteotoxic stress. ATAD1 is only 40 kb away from the potent tumor suppressor gene PTEN and is co-deleted with PTEN in many cancers. Thus, ATAD1 null cancer cells have a pro-apoptotic vulnerability which can potentially be exploited in cancer treatment.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 171 | Improved chromatin biology analysis and CRISPR-based gene repression with MethTraps

Gianluca Veggiani[1], Sachdev Sidhu[1], Gregory Martyn[1], Rodrigo Villaseñor[2], Tuncay Baubec T[2]

[1]University of Toronto, [2]University of Zurich (Ontario, Canada)

Protein methylation is an important post-translational modification that plays a crucial part in regulating cellular functions, and whose misregulation 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. However, their application is limited by their modest affinity.By combining the power of phage display and rational engineering we developed a general method for enhancing the affinity of all chromodomains of the human Cbx protein family without affecting their binding specificity. Our strategy allowed us to improve the affinity of chromodomains >50 times, enabling the development of MethTraps, powerful probes for proteomics, genome-wide binding analysis, and live cell imaging.We showed that MethTraps can be successfully used to investigate protein interaction networks on chromatin and isolate nucleosome bivalently modified with the repressive H3K27me3 and the activating H3K4me3 marks. We demonstrated that MethTraps can also outperform antibodies in ChIP-seq experiments, thus confirming their utility in investigating chromatin biology.Furthermore, we combined MethTraps with transcriptional repressors and showed that their enhanced affinity enables the development of highly potent CRISPRi repressors for specific and potent gene silencing.Our results highlight the power of MethTraps to analyze protein interaction networks on chromatin and represent a modular platform for efficient gene silencing.

Track: Protein Interactions and Assemblies

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 172 | Ligand-dependent interactions between phosphatidylcholine transfer protein and PPARδ: Therapeutic implications for the metabolic syndrome.

Samuel Druzak[1], Matteo Tardelli[2], Suzanne Mays[1], Michael Cato[1], Matthew Tillman[1], David Cohen[2], Eric Ortlund[1]

[1]Emory university, School of medicine, Department of Biochemistry, [2]Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medical College (GA, United States)

Obesity is an epidemic in the United States that predisposes to the development of the metabolic syndrome (MS). Peroxisome proliferator-activated receptors (PPARs) are an attractive therapeutic target to treat various aspects of MS. We have shown that PPARδ activation is tuned, in part, through an interaction with fatty acid binding protein five (FABP5). FABP5 is a ligand chaperone that modulates PPARδ activity; however, FABP5 only binds a subset of reported PPAR ligands. To find other candidate lipid transport proteins (LTPs), we performed a protein complementation assay between LTPs and PPARs. We uncovered a novel interaction between PPARδ and phosphatidylcholine transfer protein (PC-TP). In vitro characterization of complex formation showed that the interaction between PC-TP and PPARδ is robust and selective, as PC-TP did not interact with other PPAR isoforms. Functional characterization in cells suggest an inhibitory role for PC-TP in modulating PPARδ transactivation. Through altering cellular availability to nutrients, knocking down enzymes involved in the biosynthetic pathway of PCs, and mutating residues implicated in PC-TP PC binding and transfer, we show evidence of ligand mediated complex formation with PPARδ. Whereas cell culture data suggest that full length PPARδ is necessary and sufficient for interacting with PC-TP, further in vitro characterization reveal that the interaction may require

additional cellular factors. The consequence of this interaction was underscored through characterization of newly generated liver-specific PC-TP knockout mice which when fed high fat diet, exhibits a phenotype similar to wild type mice subjected to PPARδ activation suggesting an important role for PC-TP in regulating PPARδ activity in vivo.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 173 | Probing the positional equilibrium of the carrier domain of pyruvate carboxylase

Amanda Laseke[1], Joshua H. Hakala[1], Martin St. Maurice[1]
[1]*Marquette University (WI, United States)*

Probing the positional equilibrium of the carrier domain of pyruvate carboxylase Amanda Laseke1, Joshua Hakala1, Martin St. Maurice1Marquette University, Department of Biological Sciences, Milwaukee, WI 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, with a large carrier domain translocation between distantly located catalytic domains facilitating the transfer of the reaction intermediate between two distinct 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 and allosteric effectors by monitoring changes in intrinsic tryptophan fluorescence. This approach contributes to a more detailed kinetic description of the carrier domain translocation range and regulation in PC by directly observing dynamic conformational changes.

Track: Computational Modeling/Simulation
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 174 | The Molecular Basis of Obligate Dimerization in the MS2 Capsid Assembly Unit

DIGVIJAY LALWANI[1], SHACHI GOSAVI[1]
[1]*National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru 560065, India (Karnataka, India)*

Coarse-grained computational models aim to reduce the complexity of large systems such as viral capsids, allowing for faster computation. The capsids of RNA viruses such as MS2 are great benchmarking systems for studying protein self-assembly because they are made almost entirely of multiple copies of a single coat protein (CP). Although CP is the minimal repeating unit, previous studies have indicated that the coat protein homodimer (CP2) is the capsid assembly unit. Here, we investigate the molecular basis of this CP2 obligate dimerization using coarse-grained structure-based models, which encode the protein native structure in their potential energy functions. We find that unlike monomeric proteins of similar size, CP populates a single partially folded ensemble, where the extent of "folded-ness" depends on the denaturing conditions. In contrast, CP2 folds similar to single-domain proteins populating only the folded and the unfolded ensembles separated by a substantial free energy barrier and no intermediates. This implies that for CP2, the folding and binding events occur simultaneously and unbinding of the two monomers requires partial unfolding of the dimer. We design topological and energetic mutants to identify the source of the dimer stability. Together our results show that while both protein topology and interchain interactions are independently sufficient for making the dimer kinetically stable, a combination of both make the dimer obligatory in nature.

Track: Proteins in Cells
Session: New Protein Post-Translational Modifications

ABS# 175 | An alternative photoacclimation response in cryptophyte algae through differential chromophorylation of their phycobiliproteins

Mina Yu[1], Leah Spangler[1], Gregory Scholes[1]
[1]*Princeton University Chemistry Department (Georgia, U.S.A.)*

Many photosynthetic microorganisms have demonstrated the ability to acclimate to restricted light. Some types of red algae and cyanobacteria are capable of acclimation to changes in the spectral quality of light (i.e. red, green, or blue light) through macromolecular rearrangements of their auxiliary light harvesting antennas made up of phycobiliproteins (PBPs). A related class of microalgae, cryptophytes, also possess PBPs, but, unlike in red algae and cyanobacteria, these do not possess macromolecular structure and are instead dispersed in the thylakoid lumen. Because of this difference, cryptophytes were assumed to be incapable of acclimating to different spectral qualities of incident light. However, recent work has found a similar type of photoacclimation in cryptophytes that results in shifts in the absorbance peaks of the PBPs in response to light restricted in spectral quality. Here, we reproduce this photoacclimation response in two species of cryptophyte algae, P. sulcata and H. pacifica, and identify the origin of this response at the level of their individual PBPs. After these algae were grown in the restricted light conditions, both demonstrated shifts in the region of their absorbance spectra associated with their PBPs. These shifts were preserved in the absorbance spectra of the isolated PBPs, indicating that the origin of the photoacclimation response is on the protein level. Structural investigations of the acclimated PBPs demonstrated no change in primary or tertiary structure, and analysis of the separated α and β subunit components of the acclimated PBPs revealed the change to be located on the β subunit. The fact that a stable absorption shift response occurred in the absence of a structural change in the protein suggests that cryptophytes are capable of acclimating through differential chromophorylation—modifications of individual chromophores—of the β subunit to changes in the spectral quality of available light.

Track: Therapeutics and Antibodies

Session: Protein Evolution, Design and Selection

ABS# 176 | A Database of Coronavirus Protein Structures Enables Insights into SARS-CoV-2 Antibody Recognition and Escape

Rui Yin[1,2], Johnathan Guest[1,2], Ghazaleh Taherzadeh[1,2], Ragul Gowthaman[1,2], Ipsa Mittra[1,2], Jane Quackenbush[1,2], Brian Pierce[1,2]

[1]University of Maryland Institute for Bioscience and Biotechnology Research, Rockville, MD 20850, USA, [2]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA (MD, United States)

To enable structure-based design of therapeutics and vaccines against SARS-CoV-2 and other coronaviruses, we developed CoV3D, a database and resource for coronavirus protein structures. Updated on a weekly basis, CoV3D provides users with comprehensive sets of structures of coronavirus proteins and their complexes with antibodies, receptors, and small molecules, that collectively reveal a map of virus vulnerabilities. Integrated molecular viewers allow users to visualize structures of the spike glycoprotein and spike-antibody complexes, as well as spike sequence variability and known polymorphisms. With a nonredundant set of experimentally determined antibody-receptor binding domain (RBD) structures collected in our CoV3D database, we performed a comprehensive mapping of antibody recognition of the SARS-CoV-2 spike RBD, which is the target of most neutralizing antibodies. The structure-based mapping of antibody footprints on the RBD and unsupervised clustering led to the identification of four major antibody groups based on their recognition signatures. These antibody-spike complexes were assessed for key energetic features using computational alanine mutagenesis to identify shared and distinct binding hotspots on the RBD. The structure-based antibody clusters were also assessed both for residue conservation with SARS-CoV-1, and predicted effects of individual RBD substitutions from circulating SARS-CoV-2 variants, showing substantial differences between groups of RBD-targeting antibodies. These structural features and clusters can serve as a reference for rational vaccine design and therapeutic efforts, and updated antibody cluster information based on this analysis is available to the community on the CoV3D site: https://cov3d.ibbr.umd.edu/antibody_classification.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 177 | The Biophysical basis of protein domain compatibility

Daniel Schmidt[1], Willow Coyote-Maestas[1], David Nedrud[1], Antonio Suma[2], Yungui He[1], Kenneth A. Matreyek[3], Douglas M. Fowler[4], Vincenzo Carnevale[2], Chad L. Myers[1]

[1]University of Minnesota, [2]Temple University, [3]Case Western Reserve University School of Medicine, [4]University of Washington (Minnesota, United States)

Protein domains are the basic units of protein structure, function, and evolution. While genomic mechanisms and adaptive dynamics that drive domain gain in multi-domain proteins are extensively studied, much less is known about

the biophysical constraints of domain compatibility. Understanding the biophysical mechanisms that govern the combination of protein domains into viable multi-domain proteins is of fundamental interest in protein science and essential for advancing synthetic biology and biomedical engineering. To broadly explore domain recombination space for a global picture of protein domain compatibility as it applies to protein evolution and rational design, we use a high-throughput biochemical assay to determine the surface-expression fitness effect of inserting hundreds of domains into all positions of the Inward Rectifier K+ channel Kir2.1. We then apply machine learning on this massive dataset (>300,000 insertion variants) to derive a quantitative biophysical model and protein design rules for domain recombination. We find that insertional fitness depends on nonlinear interactions between specific biophysical properties of inserted motifs and the recipient protein, which adds a new dimension to the rational design of fusion proteins. Our data suggest that genomic and biophysical mechanisms act in concert to favor gain of protein domains at protein termini, which may explain the preferential gain of larger structured domain in metazoan evolution. Unbiased clustering of insertion fitness reveals a hierarchical organization of Kir2.1 structure into classes, and we provide a mechanistic model that relates these classes to ion channel structural elements important for stable folding or conformational dynamics required for function, respectively. This hierarchical organization generalizes to several ion channel families, which suggests that insertional profiling may be a useful high-throughput coarse-grain method to functionally annotate proteins.

Track: Therapeutics and Antibodies

Session: Protein Evolution, Design and Selection

ABS# 178 | Characterisation of Bluetongue Virus VP7

Samantha Gildenhuys[1], Bonnie Leigh Russell[1]
[1]*University of South Africa (Gauteng, South Africa)*

Bluetongue virus is an Orbivirus, which causes great economic losses due to its symptomatic occurrence in sheep as well as asymptomatic occurrence in other domestic animals and wild ruminants. New subunit based vaccines could allow vaccinated animals to be distinguished from infected animals as well as reduce the number and complexity of vaccine doses easing the economic costs. The virion consists of three protein layers, enclosing the double stranded segmented RNA genome. The middle protein layer is composed of viral protein 7 (VP7) which has a highly conserved sequence across serotypes and is the major serogroup reactive antigen. Sequence analysis resulted in a consensus VP7 sequence displaying conserved epitope regions which was then overexpressed using a bacterial expression system. Following expression VP7 was solubilised and then purified using nickel affinity chromatography. The pure protein is highly heat resistant maintaining secondary structure in the presence of 5M urea up to 42°C. The tertiary structure as monitored by intrinsic tryptophan fluorescence indicates complete denaturation is only possible with 6M guanidinium chloride. Glycerol and sodium chloride, common vaccine additives, also have a protective effect on the protein. VP7 therefore would be a highly stable components suitable for a subunit vaccine formulation.

Track: Dynamics and Allostery

Session: Allostery & Dynamics in Protein Function

ABS# 179 | Fast Conformational Dynamics in GroEL Revealed by Single-molecule FRET

Demian Liebermann[1], Gilad Haran[1], Amnon Horovitz[1]
[1]*Weizmann institute of Science (Rehovot, Israel)*

Many biological processes are executed by molecular machines, which efficiently convert chemical energy into mechanical motion and vice versa. In order to maintain function in steady-state and to tune the activity according to the cellular environment, the conformational motions of a machine's domains have to occur in a cooperative and synchronized manner. This is possible due to allosteric signaling pathways, embedded in the protein's architecture. A well-studied example for such a machine is the tetradecameric chaperonin GroEL, which operates under the consumption of ATP to promote the folding of substrate proteins to their native form. Surprisingly, while much is known about GroEL's structure and function with respect to its many substrates and co-factors, a detailed dynamic description of its internal allosteric conformational changes and their typical time scales is still missing. We report here the first single-molecule FRET experiments on the conformational dynamics of GroEL. FRET efficiency histograms depict how a GroEL subunit samples its conformational space in the presence of various substrates, such as nucleotides and its co-chaperon GroES. Interestingly, the histograms indicate conformational transitions that are fast on the measurement time (miliseconds). Indeed, analysis of the single-molecule data using a photon-by-photon hidden Markov modeling algorithm, H2MM, yields conformational transition rates ranging from 100-5000 s-1, even though the catalytic cycle of GroEL takes place on the timescale of seconds. We aim to understand the significance of these fast conformational motions for GroEL's

function. For that, we are targeting specific steps in the cycle by measuring variants that lack certain functional aspects, such as ATP hydrolysis or cooperative interactions between subunits. Our experiments allow us to describe synchronized motions and reveal the timescales of allosteric signal propagation within GroEL, with general implications for the operation principles of molecular machines.

Track: Design/engineering
Session: Allostery & Dynamics in Protein Function

ABS# 180 | Porous Protein Crystal Scaffolds for Peroxidase Pixel Detectors

Lauren Beatty[1], Alec Jones[1], Christopher Snow[1]
[1]*Colorado State University (CO, USA)*

In recent years, demand for point of care detection devices for a variety of viruses has grown.While myriad technical strategies are in development to fill this role, we are interested incapitalizing on the organized nanostructure, high concentration, and long-term shelf stabilitybenefits associated with crosslinked protein crystals. In particular, we are working with acrosslinked porous protein crystal scaffold that can host functional macromolecular guests. Here,the functional domains are hemin and a G-quadruplex DNA sequence reported to bind heme andcatalyze a peroxidase reaction. We have observed both the G-quadruplex and hemin loading andadsorbing within the porous protein crystal interior. To assess peroxidase function, we are usingtetramethylbenzidine (TMB), a color change peroxidase substrate, to generate a colorimetricreadout. Specifically, the insoluble product of the peroxidase reaction is observed topreferentially accumulate within the host crystal, resulting in a dramatic color change within 60minutes from transparent to opaque dark blue. With further optimization we will determine if wecan discover a regime where the color change is dependent upon the addition of G-quadruplex.In that case, such crystals could serve as the readout component within integrated paper-basedvirus detection devices.

Track: Proteins in Cells
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 181 | Optimizing DNP-Assisted NMR in Living Cells

Yiling Xiao[1], Rupam Ghosh[1], Jaka Kragelj[1], Whitney Costello[1], Carla Madrid[1], Kendra Frederick[1]
[1]*UT Southwestern Medical Center (TX, United States)*

Insights from protein structural biology are sometimes limited because bringing the purified sample out of the cellular settings may alter its molecular structure. This is a particular concern for biomolecules with environmentally-sensitive conformations, such as proteins that can adopt more than one stable conformation or proteins that contain regions of intrinsic disorder. In such cases, it may be difficult, if not impossible to fully reconstitute the biologically relevant conformation with a purified sample. Yet, there is increasing evidence of the importance of these regions for biological activity. Nuclear Magnetic Resonance (NMR) spectroscopy has exquisite specificity for NMR active nuclei, which are non-perturbative probes that offer the possibility of atomic-level information, which is ideal for in-cell structural biology. Further, the addition of dynamic nuclear polarization (DNP) to NMR can increase the sensitivity of experiments to the level that allows observation of an intrinsic disordered protein at its endogenous concentration (micromolar or lower) in cell within reasonable experimental times.However, the DNP-assisted NMR, like all techniques imposes constraints on the sample. Sample preparation can significantly impact both the sensitivity of the technique and the cellular viability. Here, we describe several approaches, including the choice of cell freezing media, the cautious sample handling to avoid temperature fluctuation, and the means of delivering the DNP enhancing agent into the cell, that synergistically lead to high gains in experimental sensitivity while sustaining cellular viability throughout the experiment. These approaches will enable detection of proteins at their endogenous concentrations in viable cells, permitting correlation of structure with cellular phenotype.

Track: Structure (X-Ray/NMR/EM)
Session: Allostery & Dynamics in Protein Function

ABS# 182 | Structural Characterization of Functionally Important Chloride Binding Sites in Vibrio splendidus Alkaline Phosphatase using X-ray Crystallography

Sigurbjörn Markússon[1], Jens G. Hjörleifsson[1], Petri Kursula[2], Bjarni Ásgeirsson[1]
[1]*Science Institute, University of Iceland, Reykjavík, Iceland,* [2]*Faculty of Biochemistry and Molecular Medicine, University of Oulu, Finland (Seltjarnarnes, Iceland)*

The periplasmic space of Gram-negative bacteria is a concentrated oxidizing gel-like matrix that may constitute up to 40% of the total cell volume. It is the location of over

60 known proteins of several types, including hydrolytic enzymes (phosphatases, proteases, and endonucleases). The outer membrane is relatively permeable and, therefore, the periplasm is highly exposed to fluctuations in the external environment. This provides a challenging environment for protein folding, function and stabilization in the absence of ATP. In marine bacteria, the salt and pH conditions are the same as on the outside, ≈ 0.55 M chloride, ≈ 0.47 M sodium and pH 7.6-8.4. We found that the alkaline phosphatase from Vibrio splendidus (VAP) is dependent on a high chloride concentration for function and stability. The catalytic turnover of the enzyme was increased up to 3-fold in high sodium chloride concentrations (≥1 M) and this was highly dependent on pH in the range pH 7-10. In order to locate the specific chloride binding sites, the protein was co-crystallised with varying concentrations of chloride or bromide ions. Two sites were localised utilising the anomalous scattering of the halogens. One site was peripheral, bridging an unstructured portion of the protein to its central fold, and likely providing rigidity and global thermal stability. The second site was located in the vicinity of the active site and possibly serves the role of stabilizing the product-releasing conformer of the substrate binding Arg129, leading to a faster rate-limiting step and an increased turnover rate. Chloride was also shown to relieve the inhibition of VAP by HEPES, a sulfonic acid buffer that binds in the same site in the crystals of the enzyme.

Track: Proteins in Cells

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 183 | Characterization of S-nitrosoglutathione Reductase (GSNOR) in Saccharomyces cerevisiae using Fluorescence Live Cell Imaging

Sara Aljoudi[1], Bulent Mutus[1]
[1]*University of Windsor (Ontario, Canada)*

S-nitrosoglutathione reductase (GSNOR) is a potent endogenous enzyme that can specifically catalyze the NADH-dependent reduction of S-nitrosoglutathione (GSNO) to hydroxylamine, thereby regulating the intracellular S-nitrosothiol (SNO) levels. GSNO is an intracellular SNO and a source of bioavailable nitric oxide. To date, the dysregulation of GSNOR expression is implicated in the etiology of several human diseases, including cystic fibrosis, asthma, lung cancer, and neuronal dysfunction [1]. Recently, we have introduced a novel live-cell probe, O-aminobenzoyl-S-nitrosoglutathione (OAGSNO), which is a specific, fluorogenic, pseudo- substrate for the enzyme GSNOR. [1,2]. This cell-permeable probe allows for the visualization of GSNO entry into cells, which can be directly monitored using an inverted epifluorescence microscope. Consequently, this study aims to determine the in-cell kinetic behaviour of GSNOR and understand GSNO mode of entry into yeast Saccharomyces cerevisiae using this fluorogenic probe. In preliminary studies, we observed that methyl-glutathione and GSNO are inhibitors of OAGSNO metabolism in yeast, indicating they are utilizing the same mode of entry. Additionally, blocking free thiols on yeast cells using Ellman's reagent, DTNB, is observed to inhibit OAGSNO entry into cells. S-(p-azidophenacyl)-glutathione (PAPA-GS) is also shown to inhibit the enzyme GNSOR in a concentration-dependent manner. These observations suggest one of the following: 1) An essential thiol is present on the GSNO carrier, 2) GSNOR is membrane associated with a surface exposed essential thiol, or 3) GSNOR associates with a membrane redox-sensitive regulatory protein.[1] Free Rad. Biol. Med. 2017; [2] Methods. 2019 ;168:29-34Acknowledgements: Natural Sciences and Engineering Research Council (NSERC).

Track: Therapeutics and Antibodies

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 184 | Quantifying Protective Effects of Engineered Porous Protein Crystals on Adsorbed Guest RNA

Mahmoud Masri[1], Christopher Snow[1], Alec Jones[1]
[1]*Colorado State University (Colorado, United States)*

Functional RNA molecules constitute a new class of therapeutics of incredible promise, as exemplified by the Moderna and Pfizer SARS-CoV-2 vaccines. One ongoing challenge for RNA therapeutics is the need to protect RNA from environmental challenges. Engineered porous protein crystals can adsorb nucleic acids with very high affinity. The porous protein crystals in question are composed of an isoprenoid binding protein, CJ, originally derived from Camphlobacter jejuni gene cj0420. The crystals feature a hexagonal array of 13 nm diameter nanopores. Here, we sought to evaluate the possibility of using host protein crystals for the transport of guest RNA. Strong adsorption of a model RNA was confirmed by confocal microscopy. We hypothesized that the host crystal may confer some stabilization to challenging conditions, since crosslinked protein crystals can be highly resistant to extreme pH and hydrolase attack. We therefore sought to test for the extent of hydrolysis that occurs to crystal-adsorbed RNA (relative to

RNA in solution) due to exposure to RNase and low pH. To quantify RNA hydrolysis, we used denaturing gel electrophoresis and devised a RT-qPCR protocol to assess the ratio of full-length RNA to RNA fragments.

Track: Protein Interactions and Assemblies
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 185 | Purification and Characterization of NFI-BD

Zeyad H. A. Nafaee[1], Béla Gyurcsik[2], Nagata Kyosuke[3]
[1]University of Szeged, Dom ter 7, 6720 Szeged Hungary, University of Babylon, Iraq, [2]University of Szeged, Dom ter 7, 6720 Szeged Hungary, [3]University of Tsukuba, Japan (Csungrad, Hungary)

The nuclear factor I (NFI) family of site-specific DNA binding proteins plays a role in both transcription and adenovirus (Ad) DNA replication. The studied NFI [1] consists of N-terminal DNA binding domain (NFI-BD), and a transcription activation part at the C-terminus. NFI-BD can be a metallonuclease candidate for gene therapy. Based on this purpose, this work covers the study on the purification, DNA and metal ion binding to get more information about its and properties.The purification of this protein is a challenging task. High quantity of solubilized fraction, elution from the resin, sensitivity to room temperature, and tag cleavage are the most difficult steps in the NFI-BD expression and purification processes. In our strategy the expression conditions of GST-tagged NFI-BD (1-240 amino acids) is optimized using Rosetta E.coli strain, low concentration of inducer and long incubation time at low temperature in comparison with strain BL21 (DE) that produced high overexpressed insoluble target protein. To purify this fusion protein we explore the strong binding to the glutathione resin, which is reflected by the high concentration of glutathione solution needed to elute the target protein.By gel electrophoresis mobility assay we showed that the GST-tagged NFI-BD specifically binds to its recognition site (located at the 5' end of the Ad5 genome). The solution structure of NFI-BD was studied by circular dichroism spectroscopy showing a good agreement with the predicted secondary structure. The effect of metal ion coordination is of peculiar interest, since the protein has several heavy metal binding cysteine and histidine amino acids. We studied the effect of metal ions on the secondary structure and DNA binding. The results suggest that metal ions may play role in both DNA binding and potential cleavage of specific DNA sequence.[1] K. Nagata, R.A. Guggenheimer, J. Hurwitz; PNAS (USA), 1983, 80, 6177.

Track: Protein Interactions and Assemblies
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 186 | Pin1 interactions with substrates and drugs probed by 19F NMR

Xiao-Ru Chen[1], Tatyana I. Igumenova[1]
[1]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas (Texas, United States)

Pin1 is a peptidyl-prolyl isomerase that catalyzes the cis-trans isomerization of pThr/pSer-Pro bonds in its target proteins. Moreover, Pin1 regulates the activities of proteins involved in cancer initiation and progression, and Pin1 is overexpressed in various cancers. Thus, development of Pin1 inhibitors is a focus for both pharmaceutical companies and academic research groups. One known problem identified in high-throughput screening experiments applied to Pin1 is the high percentage of false-positive readouts. Direct validation of Pin1-inhibitor interactions therefore becomes essential. In this work, we demonstrate that 19F NMR spectroscopy can be used as a facile tool to detect Pin1 interactions with substrates and inhibitors. We have successfully introduced 19F probes into the WW domain of Pin1 that is responsible for substrate recruitment, and into the isomerase domain that is responsible for catalysis. Both probes showed readily quantifiable response to binding peptide substrates and inhibitors in the form of 19F chemical shift perturbations. The information about the affinity of Pin1-ligand interactions can be extracted from the analysis of the 19F-based affinity curves. Overall, the ease of sample preparation, the high sensitivity of 19F experiments and hence moderate sample requirements, and the ability to detect Pin1-ligand interactions in the domain specific manner make our approach highly suitable for both direct target validation and identification of novel Pin1 substrates.

Track: Folding
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 187 | WW Domain for in vivo FRET Labeling

Edward Knab[1], Caitlin Davis[1]
[1]Yale University (Connecticut, United States)

Computational studies have predicted that molecular sticking and crowding can either enhance or impede the

kinetics and stability of small ultrafast folding WW domain proteins in vivo. WW domain proteins are twisted triple-stranded β-sheet peptides composed of 30-40 amino acids, containing two highly conserved tryptophan residues. This protein family is commonly studied in vitro because its small size and fast folding times can be modeled with all-atom resolution. Complementary experiments are necessary to test computational predications. Our experimental approach is to use FRET to probe WW domain stability and kinetics inside living cells and in vitro via FRET. Therefore, it is crucial to understand how FRET labels affect the protein being studied, especially for a small peptide like the WW domain. We will test two FRET labeling strategies: the first using fluorescent proteins, the FRET pair mTurquoise2 and mNeonGreen, and the second using organic dye labels, ATTO 488 and ATTO 594. In vitro measurements of the labeled and unlabeled WW domain will be compared to determine which of the two pairs provides the smallest impact to stability and folding kinetics. Our hypothesis is that the ATTO FRET pair will provide the smallest perturbation, as their size relative to the protein is small. We predict that fluorescent protein FRET pairs may still be able to measure WW domain stability, but that the large fluorophores will slow diffusion rates in cells. This work will provide a basis for understanding which quantitative questions each FRET labeling strategy can answer, so that the best strategy is use to characterize protein behavior in the cellular environment.

Track: Protein Interactions and Assemblies
Session: Measuring Forces of Biological Systems

ABS# 188 | Influence of the Type of Phospholipids on the Membrane Binding of the S100A16 Protein in the Presence of Calcium

Francis Noël[1], Xiaolin Yan[1], Stefan W. Vetter[2], Élodie Boisselier[1]
[1]Department of ophthalmology, Faculty of medicine, Saint-Sacrement Hospital, Laval University, Quebec City, Quebec, Canada, [2]School of pharmacy, North Dakota State University, USA (Québec, Canada)

Maintaining the structural and functional integrity of membranes is essential for proper cells function. A recent proteomic study suggests that S100A16 protein participates to the maintain of the membrane integrity in the rod outer segment (ROS) of the photoreceptors in the eye. The protein S100A16, recently discovered, is one of the S100 family proteins for which no protein and membrane interaction has yet been identified. Furthermore, maintain of the membrane integrity is a calcium sensitive process. The polar headgroup composition of the human ROS is 32.5% ofphosphatidylcholine, 37.6% of phosphatidylethanolamine and 12.1% of phosphatidylserine. In addition, the polyunsaturated chains constitute between 30-60% of the total lipid fraction in humans. The cholesterol concentration of the discs varies from 5 to 30% between base and summit of ROS.The main objective consists of studying the membrane interactions of the S100A16 protein to better understand its function in maintaining membrane integrity. Specific objectives are: i) to achieve the purification of this protein, ii) to gather information on its membrane interactions, and iii) to study the influence of calcium on these interactions. Langmuir monolayer model combined with surface tensiometry allows mimicking the composition of cell membranes and performing the membrane binding study.The S100A16 protein was obtained with a purity greater than 99% and its saturating concentration is 0.5 μM. Preliminary results obtained show that S100A16 preferentially interacts with saturated phospholipids with short acyl chains and zwitterionic polar head group in presence of calcium ions.In conclusion, these preferential lipid interactions suggest that S100A16 would have a preference for binding to the plasma membrane in the outer segment of photoreceptors, rather than to membrane disks in the presence of calcium ions.

Track: Bioinformatics
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 189 | Molecular assembly of NDH-1 quinone oxidoreductase from Moringa oleífera.

Iván Corral-Guerrero[1], Monica Valdez- Solana[1], Claudia Domínguez-Avitia[2], Miguel Aguilera-Ortiz[2], Alfredo Téllez-Valencia[2], Erick Sierra-Campos[1]
[1]Principal, [2]Colaborator (Durango, México)

Moringa oleifera is a tree with different therapeutic properties, such as anticancer, antidiabetic, great antioxidant potential, and others. It is a plant resistant to hot climates, drought conditions, fast growing, with little demand for soil nutrients, which can be easily cultivated in arid and tropical areas. One of the adaptive mechanisms of plants under unfavorable conditions, such as fluctuations in the amount of light captured by photosystems due to the effect of the organism's grow region, is the cyclical transport of electrons mediated by the NDH-1 quinone oxidoreductase complex in which this study focuses on. In M. oleifera, only the transmembrane region has been studied without characterizing the cytosolic part, and in none of these has a three-dimensional structure been developed, we, using

free access bioinformatic tools such as Phyre2 and ClusPro 2.0, propose by means of molecular assembly a three-dimensional model of the NDH-1 quinone oxidoreductase complex for M. oleifera. Using Phyre2, the search for structural homologues was carried out for all the subunits reported in the genome and that are not yet characterized in this plant. There were 6 subunits of the cytosolic portion (J, I, M, N, S and O) and 7 subunits in the membrane portion (A, B, C, D, E, F and G). In addition, a three-dimensional model was made with all the subunits found by molecular coupling with ClusPro 2.0 and HDOCK server. Although we do not know the scope of our results, we consider that this study can help to understand the metabolic relationship between the activity of the complex, with the resistance and easy development of M. oleifera, in the face of adverse conditions.

Track: Computational Modeling/Simulation
Session: Protein Evolution, Design and Selection

ABS# 190 | Building blocks of protein structures

Tatjana Skrbic[1], Jayanth Banavar[1], Achille Giacometti[2], Trinh Xuan Hoang[3], Amos Maritan[4], George Rose[5]
[1]*University of Oregon,* [2]*University of Venice,* [3]*Institute of Physics, Vietnam Academy of Science and Technology,* [4]*University of Padua,* [5]*Johns Hopkins University (Oregon, USA)*

Proteins, the amazing molecular machines of life, are complex with myriad degrees of freedom. Linus Pauling launched the field of molecular biology by developing the principles of quantum chemistry and applying them to predict the structures of the modular building blocks, helices and strands assembled into sheets, of protein structures. Now, seventy years later, we present a first principles prediction of these same geometries with no chemistry and hence no hydrogen bonding, and no adjustable parameters. Our predictions arise from just one constructive hypothesis that the dominant folding mechanism of a protein is the drive to maximize its self-interaction, thereby attaining a space-filling folded state. One of our results is that discreteness, which underpins quantum mechanics, is also essential for life. Our results are in good accord with experimental data on more than four thousand protein structures and they underscore the consilience in the fit of chemistry and biology to the dictates of mathematics and physics. Our work has consequences for the energy landscape of proteins and the role of evolution in shaping sequences and functionalities.References:[1] T. Škrbić, A. Maritan, A. Giacometti, G. D. Rose and J. R. Banavar, Phys. Rev. E (2021) – in press[2] T. Škrbić, A. Maritan, A. Giacometti, G. D. Rose and J. R. Banavar, bioRxiv (2021), doi: https://doi.org/10.1101/2020.11.10.375105

Track: Synthetic Biology
Session: Protein Evolution, Design and Selection

ABS# 191 | A de novo ATPase from combinatorial design

Michael Wang[1], Michael Hecht[1]
[1]*Princeton University (NJ, USA)*

Our understanding of biological chemistry is shaped by the observation that all life comes from other life. As Pasteur put it, omne vivum ex vivo. This has had profound impacts on the sequences and structures we see in natural proteins, even those that perform key biochemical reactions. For example, very limited sequence diversity is associated with ATP binding and hydrolysis, which fuels many life-essential reactions. Does this limited diversity indicate that only certain privileged sequences and structures can accomplish ATP hydrolysis? Or alternatively, does the similarity of natural ATPases simply reflect evolution from common ancestry?A key step in approaching this question is expanding our biochemical vocabulary with new functional sequences and structures that did not arise from common ancestry. De novo proteins that perform life-essential reactions enable new structure-function relationships to be explored, beyond what is found in nature. This helps us test evolutionary hypotheses and contributes non-natural enzyme building blocks for synthetic biology applications. Here we describe an enzyme designed completely de novo that hydrolyzes ATP. This protein was designed to lack β-sheet structure and is competitively inhibited by magnesium, two traits that are unlike natural ATPases.

Track: Therapeutics and Antibodies
Session: Allostery & Dynamics in Protein Function

ABS# 192 | Autoantibodies in traumatic spinal cord injury – global players in neuronal regeneration?

Annika Guntermann[1,2,3], Romana Höftberger[4,5], Marcel A. Kopp[6,7,8], Katrin Marcus[1,2,3], Jan M. Schwab[9,10,11,12], Caroline May[1,2,3]
[1]*Functional Proteomics,* [2]*Medizinisches Proteom-Center,* [3]*Ruhr University Bochum,* [4]*Division of Neuropathology and Neurochemistry,* [5]*Medical University Vienna ,* [6]*Clinical and Experimental Spinal Cord Injury Research (Neuroparaplegiology),* [7]*(NW, Germany)*

Background:Preliminary data indicate that autoantibodies are apparently crucial players in the pathology of spinal cord injury (SCI). Since the blood-spinal cord barrier is consequently damaged, proteins of the central nervous system are released into the blood. These proteins trigger an immune response including the formation of autoantibodies. However, the extent of this autoimmune response and thus the impact on SCI rehabilitation is unknown. Scientific question / aim:Objective of this study was the identification and characterization of autoantibodies that occur over time following SCI. In this way, the following questions should be answered:Do autoantibody profiles change during injury progression?Are there perhaps similarities between patients?What influence do secondary clinical manifestations have on autoantibody formation? Methods: Twenty-four patients with an acute traumatic SCI were hospitalized and voluntarily consented to this ethically approved study. Their blood was drawn at both an early (week 1) as well as a subacute (week 12) phase post-injury. Screening of patients' blood serum for SCI specific autoantibodies was performed using the protein microarray technology. Results:To investigate if the functional and neurological state after SCI rehabilitation correlates with distinct underlying immunological patterns, autoantibody profiles were compared. As a first result, 116 different autoantibody targets with a significantly increased fluorescence intensity signal could be detected (p-value ≤ 0.05). Based on the matched target autoantigens, several ion channels, cytokines, and in particular a glycogen metabolism protein appear to be involved in the pathophysiology of SCI. Moreover, similar autoantibodies for patients could be observed. Evaluation of the relationship between autoantibody profiles and clinical data is still in progress. Conclusion:The study presented here is currently ongoing to gain further insight into the detailed pathophysiological role of the identified autoantibody targets. Some of these autoantigens may be beneficial targets for a prospective immunomodulatory treatment of SCI.

Track: Folding

Session: Allostery & Dynamics in Protein Function

ABS# 193 | Residue substitutions alter locally the connectivity of the C-terminus of the prion protein from species resistant to prion diseases

Patricia Soto[1], Garrett Gloeb[1], Steffany Nguyen[1], Hannah Brockman[1], Alyssa Thallemer[1]
[1]*Creighton University (NE, US)*

Prion diseases are fatal neurodegenerative disorders in mammals, including humans. The molecular level hallmark of prion diseases is the presence of "prions", agents composed of the misfolded and aggregated form of the prion protein. Although prion diseases are infectious across hosts and species, not all hosts and species show the same level of susceptibility to the disease. Evidence indicates that residue substitutions in the host-encoded native form of the prion protein, PrPC, render disease susceptibility. Residue substitutions are found on the C-terminus of PrPC, a globularly folded domain that shows two β-sheets and three α-helices. However, how residue substitutions modulate PrPC structural dynamics towards pathological misfolding is unknown. Deciphering such mechanism will identify potential druggable hot spots to stabilize PrPC and prevent conversion. Using structural bioinformatics techniques, we implemented a comparative study of the C-terminus of PrPC across species each displaying a distinct degree of susceptibility to prion diseases. Our analysis indicates that residue substitutions in structures with high resistance to prion diseases (1) decrease the connectivity density between the loops that flank the β-sheet 2, and (2) increase the connectivity between the loop preceding β-sheet 2 and α-helix 3. Our results support evidence from the literature on the role of the loops and α-helix 3 in prion disease susceptibility and transmission barrier.

Track: Structure (X-Ray/NMR/EM)

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 194 | Investigation of the Structure and the Structural Interactions of Scc4 from Chlamydia trachomatis

Thilini Oshadhi Senarath Ukwaththage[1], Marco Tonelli[2], Megan Macnaughtan[1]
[1]*Louisiana State University,* [2]*NMRFAM, University of Wisconsin-Madison (Louisiana, USA)*

Chlamydia trachomatis (CT) is the most reported sexually transmitted bacterial disease in the world with 90 million infections annually. Over 18 serovars, CT can cause trachoma, genital infections, and lymphogranuloma venereum to both humans and animals. Due to the inevitable antibiotic resistance and lack of commercially available vaccines, the investigation of novel drug candidates for CT is important. The specific chlamydia chaperone 4 (Scc4) is recognized as a significant virulence target in the CT developmental cycle with dual functions. Scc4 can function as a type III secretion system chaperone (T3SS) by interacting with Scc1 and binds with CopN, an essential effector for virulence. As a transcription factor,

Scc4 also modulates transcription in CT by interacting with σ66 region 4 and the β subunit of RNA polymerase. In this study, the 3D structure determination of Scc4 was performed using solution NMR spectroscopy. A novel technique was developed to purify high yields of tag-free Scc4 with better NMR characteristics to use in triple resonance experiments. The backbone and sidechain resonances were assigned with the completion of 89% of the amino acids in Scc4 and the protein secondary structure was determined. Using these assignments and the nuclear Overhauser experiments, distance and dihedral angles constraints were calculated. Currently, the final structure refinements are conducting to obtain the complete 3D structure of Scc4. With the Scc4 structure, small molecular inhibitors will be identified with molecular docking with fragment libraries. The interactions of Scc4 with Scc1 were investigated using NMR spectroscopy with chain selectively labeled Scc1:Scc4 protein complexes. The Scc4 showed total structural rearrangement upon binding with Scc1 which may be important to function as a T3SS chaperone. Further investigations will be conducted to determine the importance of these structural differences and the protein-binding interface.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 195 | Development of porous protein nanocrystals as a delivery vector for DNA and RNA

Alec Jones[1], Mahmoud Masri[1], Christopher Snow[1]
[1]*Colorado State University (Colorado, 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 plasmid DNA and small interfering RNA. Furthermore, we have demonstrated that CJ micro and nanocrystals are able to deliver functional plasmid and transfect cells in vitro. Currently, we are attempting to quantify the extent of guest RNA hydrolysis within CJ nanocrystals across various pH conditions, and following RNase exposure. Furthermore, we have demonstrated that CJ nanocrystals are capable of adsorbing functional Nanoluciferase, and display chemiluminescent activity following exposure to substrate. Future work will investigate the pharmacokinetics of Nanoluciferase-labeled CJ nanocrystals in mice via IVIS imaging, following oral, intravenous, and intramuscular administration.

Track: Computational Modeling/Simulation
Session: Protein Evolution, Design and Selection

ABS# 196 | Modelling of Photo-Controlled Protein Binding

Paul Maximilian Meiering Reed[1], Jaewan Jang[1], Jakeb Reis[1], Sameer Al-Abdul-Wahid[2], Karl Z. Demmans[3], Maruti Uppalapati[4], G. Andrew Woolley[1]
[1]*University of Toronto Department of Chemistry,* [2] *University of Guelph NMR Center,* [3]*University of Toronto CSICOMP Facility,* [4]*University of Saskatchewan Department of Pathology and Laboratory Medicine (Ontario, Canada)*

Cyanobacteriochromes can respond to a wide range of light wavelengths, however at present there are a limited number of synthetic biological tools that have made use of this class of proteins. Using phage display methods, we have developed proteins capable of selectively binding a cyanobacteriochrome (GAF) domain (all2699-GAF3) under light irradiation with the goal of expanding the optogenetic toolset. At present, structural characterization of the GAF domain in question is limited and the binding sites are unclear. To improve understanding of the system, a combination of NMR spectroscopy, homology modelling, and molecular dynamics was used to suggest a likely binding site for one of the proteins capable of selectively binding the GAF domain under light irradiation. The nature of the binding site suggests a general mechanism by which the light state is selectively bound, and knowledge of this may be useful in further rational design of the system.

Track: Peptides
Session: Protein Evolution, Design and Selection

ABS# 197 | Water-Soluble Porphyrin-Peptide Constructs for Light-Harvesting and Energy Transfer

Brett J. Mozarsky[1], Karin S. Åkerfeldt[1]
[1]*Haverford College (NY, United States)*

Porphyrins are macrocyclic, aromatic chromophores capable of harvesting solar energy. Through noncovalent π-stacking interactions, porphyrins orient themselves into favorable assemblies to transfer energy via exciton coupling. Such extensive supramolecular architectures may serve as organic semiconductors in photovoltaic devices. Our aim is to design water-soluble porphyrin-based light-harvesting agents that may function at biological interfaces. In addition, the porphyrins are incorporated into a peptide scaffold that guides them into predictable, organized structures. Here we report porphyrin-peptide constructs consisting of two protoporphyrin IX (PPIX) chromophores covalently attached via an amide linkage to two lysine side chains present on a decamer peptide. Three iterations of peptide scaffolds were evaluated. The initial construct, AcNH-NAKASAKSAY-CONH2, was modified to incorporate one (N1E) or two (S5E+S8E) glutamic acid residues to increase its polarity. The unmodified construct is soluble in 2,2,2-trifluoroethanol and forms a precipitate over time. In contrast, PPIX-N1E and PPIX-S5E+S8E demonstrate increased solubility in water. UV-visible spectroscopy confirms the presence of PPIX in solution. CD spectroscopy of all three constructs indicates that individual porphyrin-peptide monomers form an α-helix, but that those monomers refrain from further self-assembly. PPIX-N1E and PPIX-S5E+S8E represent novel conjugates that may serve as building blocks for water-soluble, self-assembling energy transfer systems.

Track: Design/engineering

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 198 | A general computational method for disassembling protein quaternary structure

Frances Chu[1], James Bowman[1], Jared Adolf-Bryfogle[1], Christopher Bahl[1]
[1]*Institute for Protein Innovation (MA, United States)*

The ability of proteins to oligomerize is critically important for a wide variety of biological functions. Thus, the ability to accurately and precisely ablate protein quaternary protein structure while conserving protein tertiary structure facilitates interrogation of the biological function of oligomeric proteins. Here, we describe a general method for disassembling protein quaternary structure. We accomplish this by increasing the solubility of protein-protein interfaces using the Rosetta computational protein design software in silico, followed by protein characterization in vitro.Our method uses a protein as an input structure, detects residues at the oligomeric interface, and uses Rosetta's FastDesign protocol to mutate these residues to polar amino acids commonly found on protein surfaces. Consequently, we solubilize the interface and disrupt existing interactions. The mutations are evaluated based on their calculated change in binding energy compared to the wild-type (ΔΔGbind), as well as their impact on the stability of the protomer in isolation (ΔGmutation). The designs with the worst ΔΔGbind and best ΔGmutation are chosen for further characterization. To validate our approach, we converted the dimeric stem cell factor (SCF) to a soluble monomer. SCF is the ligand of the receptor tyrosine kinase c-KIT, whose dimerization induces signaling. Thus, a monomeric version of SCF has the potential to serve as therapeutic by inhibiting c-KIT activity. The designed monomeric SCF variants were produced using the Expi293 expression system and subsequently purified. Preliminary results from size exclusion chromatography indicate a difference in elution time between the designs and wild-type SCF, suggesting a difference in oligomeric state. Further characterization experiments will be performed to confirm the oligomeric status and the designs' retained binding to c-KIT. In the future, this method can be used to create inhibitors from a variety of natural ligands, as well as better understand the role of quaternary structure in protein function.

Track: Proteins in Cells

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 199 | High-resolution mapping of functional regions of essential bacterial proteins using dominant negative inhibitory fragments

Andrew Savinov[1], Andres Fernandez[2], Stanley Fields[1]
[1]*Department of Genome Sciences, University of Washington,* [2]*Colorado State University (WA, United States)*

Polypeptide fragments derived from full-length proteins can act as dominant negative inhibitors, often by forming inactive complexes with the parent protein or one of its interaction partners. We developed an approach to screen for such inhibitory protein fragments in a systematic, high-throughput manner in E. coli. To conduct these screens, we perform growth selections on bacteria carrying a library of fragments derived from genes of interest under conditions in which these genes are important for growth, and use high-throughput sequencing to identify

inhibitory fragments based on their depletion during selection. We applied this approach to a diverse set of E. coli proteins that includes the model enzyme dihydrofolate reductase (DHFR) and essential proteins involved in processes such as DNA replication and cell division. Assays on comprehensive fragment libraries derived from these proteins revealed potent dominant negative inhibitors. Further analyses provided a mapping of inhibitory fragments to their parent protein sequences, revealing distinct inhibitory regions which are involved in key interactions. In many cases, inhibitory fragments mapped to structural elements critical for protein complex formation, suggesting a traditional dominant negative mechanism. For instance, we discovered many inhibitory fragments mapping to reciprocal contact sites between FtsZ monomers in the context of filament formation. Other inhibitory regions suggest that some fragments may interfere with the folding of the protein of origin. For example, screening of the monomeric enzyme DHFR revealed an inhibitory region mapping to the same portion of the sequence as a fragment previously demonstrated to inhibit refolding in vitro. Our approach has the potential to functionally map important regions of many proteins, even those without known or stable structures, and to provide a window into the principles underlying protein-protein interactions and protein folding in living cells.

Track: Enzymology

Session: Protein Evolution, Design and Selection

ABS# 200 | **Resistance-Guided Mining of Bacterial Genotoxins Defines a Family of DNA Glycosylases**

Noah Bradley[1], Katherine Wahl[1], Jacob Steenwyk[1], Plamen Christov[1], Lauren Washburn[2], Coran Watanabe[2], Antonis Rokas[1], Brandt Eichman[1]
[1]Vanderbilt University, [2]Texas A & M (TN, United States)

Interstrand DNA crosslinks (ICLs) are covalent linkages between opposing strands of DNA and are a highly toxic form of DNA damage that inhibit processes requiring DNA unwinding, such as replication and transcription. Streptomyces AlkZ and E. coli YcaQ belong to a recently discovered family of bacterial DNA glycosylases that repair ICLs via base excision repair (BER). Streptomyces AlkZ is found in the biosynthetic gene cluster (BGC) of azinomycin B—a potent antimicrobial/antitumor agent—and provides self-resistance to the natural product. In contrast, E. coli YcaQ, which we propose to rename AlkX, is not associated with a metabolic gene cluster and excises a broad range of ICL substrates. AlkX and AlkZ are found a wide range of antibiotic-producing and pathogenic bacteria. However, their phylogenetic relationship and genomic distribution relative to BGCs is unknown. Here, we determine the phylogenetic relationship and BGC proximity of approximately 900 AlkX/AlkZ homologs from 435 Streptomyces species. Among the differences between these two subfamilies, AlkZ homologs are plastic in their genomic location and copy number, whereas AlkX is highly conserved sequence-wise and by genetic neighborhood and copy number. Using a resistance-based genome mining approach, 11 AlkZ homologs were identified in known BGCs, and 68 in uncharacterized BGCs; no AlkX homologs were in proximity to a cluster. We validated our method by characterizing HedH4 as an AlkZ homolog in the hedamycin BGC which uniquely excises hedamycin-DNA adducts and provides cross-resistance for E. coli. This work defines the roles of AlkX/AlkZ-mediated base excision repair in antimicrobial resistance to a broad range of natural products, and serves as a proof-of-principle for the resistance genome mining approach for targeted discovery of previously uncharacterized genotoxins.

Track: Proteostasis and quality control

Session: Targeted Protein Degradation

ABS# 201 | **Derlin rhomboid pseudoproteases employ substrate engagement and lipid distortion function for ERADicating multi-spanning membrane substrates**

Sonya Neal[1]
[1]University of California San Diego (CA, United States)

Nearly one-third of proteins are initially targeted to the endoplasmic reticulum (ER) membrane where they are correctly folded, assembled, and then delivered to their final cellular destinations. In order to prevent the accumulation of misfolded membrane proteins, ER associated degradation (ERAD) moves these clients from the ER membrane to the cytosol; a process known as retrotranslocation. Our recent work in S. cerevisiae has revealed a derlin rhomboid pseudoprotease, Dfm1, is involved in the retrotranslocation of ubiquitinated ERAD membrane substrates. We have sought to understand the mechanism associated with Dfm1's actions and found that Dfm1's conserved rhomboid residues are critical for membrane protein retrotranslocation. Specifically, we identified several retrotranslocation-deficient Loop

1 mutants that display impaired binding to membrane substrates. Furthermore, Dfm1 has retained the lipid thinning functions of its rhomboid protease predecessors to facilitate in the removal of ER membrane substrates. We find this substrate engagement and lipid thinning feature is conserved in its human homolog, Derlin-1. Utilizing interaction studies and molecular dynamic simulations, this work reveals that rhomboid pseudoprotease derlins employ novel mechanisms of substrate engagement and lipid thinning for catalyzing extraction of multi-spanning membrane substrates.

Track: Intrinsically Disordered Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 202 | Characterizing the binding mechanisms of castration-resistant prostate cancer therapeutics to the disordered N-terminal domain of the androgen receptor

Jiaqi Zhu[1], Paul Robustelli[1]
[1]*Dartmouth Chemistry (NH, United States of America)*

Castration-resistant prostate cancer (CRPC) is a lethal condition suffered by ~35% of prostate cancer patients. These patients cannot be cured by surgery or radiotherapy and inevitably become refractory to androgen deprivation treatments. The discovery of small molecules that target the intrinsically disordered N-terminal transactivation domain of the Androgen Receptor provides a promising avenue for the treatment of CRPC, as this interaction may circumvent the most common CRPC drug-resistance adaptions. Two such compounds, EPI-002 and EPI-7170, have shown efficacy in CRPC animal models, and EPI-002 entered human trials in 2015. NMR measurements have localized the interactions of EPI compounds to a transiently helical region of the disordered transactivation unit 5 (Tau-5) domain of the Androgen Receptor, but no structural or mechanistic rationale exists to explain their inhibition mechanisms or the increased potency of EPI-7170 in CRPC animal models. We have utilized all-atom molecular dynamics simulations with state-of-the-art force fields and enhanced sampling strategies to simulate the disordered binding mechanisms of these compounds to Tau-5. We observe that both compounds induce the formation of collapsed helical molten-globule conformations that remain highly dynamic in their bound states. We find that EPI-7170 has a higher affinity to Tau-5 and that the Tau-5:EPI-7170 bound ensemble is more compact and contains substantially more helical content than the Tau-5:EPI-002 bound ensemble. We identify a dynamic network of intermolecular interactions in the EPI-7170 bound ensemble that stabilize collapsed helical conformations and sequester molecular recognition elements of Tau-5 more effectively than EPI-002. Our results suggest inhibition mechanisms for these compounds and potential avenues for developing more potent androgen receptor inhibitors for the treatment of CRPC.

Track: Proteins in Cells
Session: Targeted Protein Degradation

ABS# 203 | A conserved acetylation switch enables pharmacological control of tubby-like protein stability

Evan Kerek[1], Kevin Yoon[2], Shu Luo[3], Jerry Chen[1], Robert Valencia[1], Olivier Julien[3], Andrew Waskiewicz[2], Basil Hubbard[1]
[1]*Pharmacology: University of Alberta,* [2]*Biological Sciences: University of Alberta,* [3]*Biochemistry: University of Alberta (Alberta, Canada)*

Tubby-like proteins (TULPs) are characterized by a conserved C-terminal domain that binds phosphoinositides. Collectively, mammalian TULPs 1-4 play essential roles in intracellular transport, cell differentiation, signaling, and motility. However, little is known about how the function of these proteins are regulated in cells. Here, we present the protein–protein interaction network of TULP3, a protein that is responsible for the trafficking of G-protein-coupled receptors to cilia and whose aberrant expression is associated with severe developmental disorders and polycystic kidney disease. We identify several protein interaction networks linked to TULP3 that include enzymes involved in acetylation and ubiquitination. We show that acetylation of two conserved lysine residues on TULP3 by p300 increases TULP3 protein levels and that deacetylation of these sites by HDAC1 does the opposite. Moreover, we show that one of these sites is ubiquitinated in the absence of acetylation and that acetylation inversely correlates with ubiquitination of TULP3. This mechanism is evidently conserved across species and is active in zebrafish during development as a TULP3 knockdown results in development delay and apoptosis. Finally, we identify this same regulatory acetylation switch in TULP1, TULP2, and TULP4 and demonstrate that the stability of these proteins is regulated similarly by this switch. This study unveils a signaling pathway that links nuclear enzymes

to ciliary membrane receptors via TULP3, describes a mechanism for the regulation of all tubby-like proteins, and explores how to exploit it pharmacologically with various drug molecules.

Track: Protein Interactions and Assemblies
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 204 | Development of Probes to Dissect Med25 Protein Protein Interaction Networks

Estefania Martinez Valdivia[1], Olivia Pattelli[1], Matthew Beyersdorf[1], Anna Mapp[1]
[1]*University of Michigan Ann Arbor (MI, United States)*

Defects in transcriptional regulation are prominent in human disease. Protein-protein interactions (PPIs) between transcriptional components including activators and coactivators play a protagonist role in transcription initiation, and dysregulation of their PPI network can profoundly alter gene expression. Activators bind to the genome to promote expression at specific genes. Coactivators use their activator binding domains to establish a bridge of communication between activators and the general transcription machinery. Thus, coactivator•activator PPIs are potential targets to modulate gene expression for mechanistic insight and therapeutic development. Several lines of evidence demonstrate that coactivator Med25, a subunit of Mediator, is a promising target for therapeutic intervention in cancer, given that, through the complexes it forms with activators, it controls genes involved in cell migration and differentiation. A critical need to establish the functional role of Med25•activator network in these processes, is a synthetic molecule that selectively engages Med25 in vitro and in cells. The focus of this work is the development of a chemical probe based on natural product lipopeptide previously discovered by the Mapp Lab, and its use to uncover the functional role(s) of the Med25•activator network in the context of triple-negative breast cancer. We studied the structure-activity relationship of this inhibitor through the synthesis of various structural analogs and their subsequent characterization with in vitro binding assays and protein thermal shift assays. Additionally, the mode of binding of potent and selective analogs was determined using 1H13C HSQC. We identified lipopeptide residues important for binding Med25 and for the selective disruption of Med25•activator PPIs, over other coactivator•activator complexes. Together, the studies provide information on how structure is related to the inhibitory activity of this lipopeptide inhibitor. Additionally, they inform the development of a chemical probe for the characterization of dysregulated Med25•activator PPI networks in cancer.

Track: Chemical Biology
Session: Allostery & Dynamics in Protein Function

ABS# 205 | Development of a tetrazine-cyclized peptide library

Ariane Borges[1]
[1]*Clark University (MA, USA)*

Protein-protein interactions (PPI) are essential for cell signaling pathways. Mutations on proteins involved in PPI are hallmarks of different cancers, hence there is an increasing interest in inhibiting protein-protein interactions between oncogenic proteins. K-Ras is part of the Ras family of proteins and it gets activated by SOS; K-Ras is involved in cell signaling pathways that control cell growth, proliferation, and apoptosis. It is commonly mutated in different cancers, and one of the approaches to inhibit K-Ras is by inhibiting its interaction with SOS.We aim to use Chemical Epitope Targeting technology to inhibit the interaction between an active form of K-Ras, KRas(G12V), with its GEF, SOS. Here we describe the initial step to developing a tetrazine-cyclized peptide library that will be used to screen for inhibition of KRAS-Sos interaction. Additionally, we describe the development and optimization of tetrazine modified Sos-helix peptides that will be used to determine optimal Inverse Electron Demand Diels-Alder (IEDDA) cycloaddition reaction conditions between alkyne modified KRas and tetrazine modified Sos-peptides.To generate the tetrazine-cyclized peptide library, peptides were first synthesized via standard solid-phase synthesis using Fmoc-protected amino acids on Rink and Sieber amide resin. We examined the effectiveness of cysteine side chain deprotection of different protecting groups such as 4-Monomethoxytrityl (Mmt), Trityl (Trt), 4-Methyltrityl (Mtt), and Acetamidomethyl (Acm), and the Mmt group yielded the best results. The peptides containing Mmt protecting group were deprotected using a modified protocol and cyclized using dichlorotetrazine; the results showed tetrazine-cyclized peptides were the main product. Lastly, we are currently optimizing the synthesis and cyclization of SOS-helix peptides that will be used for determining IEDDA cycloaddition reaction conditions between the tetrazine peptide and alkyne modified KRas.

Track: Folding

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 207 | RNase A Folding in Response to Hypoxia

Brooke Ramsey[1], Caitlin Davis[1]
[1]*Yale University (CT, United States of America)*

Hypoxic conditions, decreased availability of oxygen, disrupt glycosylation and disulfide bonding, processes that are necessary for correct protein folding and secretion. In particular, oxygen is critical as a terminal electron acceptor for disulfide bond formation in oxidative protein folding. Here we measure the stability and folding of ribonuclease A (RNase A) Förster resonance energy transfer (FRET) constructs in vitro and in eukaryotic cells. There are eight cysteine residues in RNase A that form four disulfide bonds in the native protein. Despite being a highly-used test protein for folding studies, RNase A still has a number of properties that are unresolved, including the full characterization of its oxidative folding pathway in cells. Our hypothesis is that folding of RNase A is oxygen dependent. First, we design a FRET reporter on protein folding in vitro that minimally perturbs RNase A's stability and enzymatic activity. Then, we compare the folding of RNase A in cells under normal conditions and oxidative stress. cobalt chloride simulates hypoxic conditions. This work may inform therapeutic interventions to stall or promote protein folding in the context of cancer, diabetes, or neurodegenerative disease.

Track: Structure (X-Ray/NMR/EM)

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 208 | Structural characterization of the catalytic cycle of the chaperone Get3 from a human pathogen

Michelle Fry[1], Vladimira Najdrova[2], Shyam Saladi[1], Ailiena Maggiolo[1], Vanessa Mechem[1], Pavel Dolezal[2,3], William Clemons, Jr.[1]
[1]*Caltech,* [2]*Charles University,* [3]*BIOCEV (CA, USA)*

The correct targeting and insertion of membrane proteins is crucial for the maintenance of cellular homeostasis. A certain class of membrane proteins, tail-anchored (TA) proteins, accounting for 2% of the eukaryotic genome, are targeted post-translationally. One pathway responsible for delivering and inserting these proteins to the ER membrane is the Guided Entry of TA proteins (GET) pathway. Recently, it has been observed that Get3 is conserved throughout the eukaryotic tree and several GET components have been identified in the human pathogen Giardia lamblia. Here we present structures of GlGet3 in different nucleotide states using single particle cryo-electron microscopy (cryo-EM) and x-ray crystallography. These structures of Get3, the first from a protozoan, reflect key states in the ATPase cycle in the nucleotide free (apo), pre-hydrolysis, and post-hydrolysis conformations. This provides the first comprehensive characterization of Get3 from a single organism. This structural picture illustrates the conformational changes in Get3 responsible for successful targeting of TA proteins to the ER membrane.

Track: Protein Interactions and Assemblies

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 209 | Forward Osmosis desalination with a biocompatible, biofriendly protein hydrogel

Mansi Malhotra[1], Jingwen Ding[1], Challa Kumar[1]
[1]*University of Connecticut (CT, United States)*

Forward osmosis is an emerging technique to purify the water with high salinity where reverse osmosis is inept or expensive for the treatment. However, there are certain intricacies in draw solutions which limit its application. Here, we propose a facile method to synthesize protein thermal hydrogel. The 3D framework of protein, when thermally treated, forms a highly porous hydrogel which can absorb water resulting in easy and inexpensive water production. The hydrogels could absorb the mass of water as high as 4 times the mass of the hydrogel in 3% NaCl solution, making them highly efficient in addition to being biocompatible and biofriendly. Water regeneration from these protein hydrogels can be easily achieved by mechanical compression. These results prove that protein hydrogels can be used as a potential material for extracting pure water from saline water in an effortless but highly efficient approach.

Track: Proteomics

Session: Measuring Forces of Biological Systems

ABS# 214 | Analysis of the lake trout heart and blood proteome using evolutionary proteomics

Shelby Alwine[1], Emma Dupree[1], Bernard Crimmins[2], Thomas Holsen[1], Costel Darie[1]
[1]*Clarkson University,* [2]*AEACS, New Kensington, PA (New York, United States)*

Salvelinus namaycush (lake trout) is a top-predator fish in the Great Lakesregion. The Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) useslake trout as bioindicators of the presence of persistent, bioaccumulative and toxic(PBT) chemicals. Elevated levels of PBTs can cause changes in transcribed genes,translated mRNAs, proteins produced, and post-translational modifications of theseproteins in aquatic species. Though lake trout is used as a bioindicator of PBTs, there iscurrently no well-developed proteome database for the species that has been monitoredfor five decades. In this study, heart and blood samples from lake trout were analyzedby SDS-PAGE, followed by in-gel trypsin digestion and analysis by nanoLC-MS/MS.The data was searched against different NCBI and UniProtKB databases in MascotDaemon and the output was analyzed by Scaffold 4.3 software. Databases used includeActinopterygii, Salmonidae, Salvelinus, as well as the highly studied speciesOncorhynchus mykiss and Danio rerio. Using these better developed protein databases,we were able to identify many novel proteins for the lake trout, as well as exploreevolutionary relationships for the lake trout species. This ongoing project will potentiallylead to a more developed, comprehensive proteome database for lake trout that can beused for further proteomic studies on the effects of legacy PBT chemicals in the GreatLakes ecosystem.

Track: Structure (X-Ray/NMR/EM)

Session: Designer Proteins Through Genetic Code Expansion

ABS# 215 | Metal Ion Binding Motifs in Cholinesterases

Joel Sussman[1], Israel Silman[1], Valery Shnyrov[2], Yacov Ashani[1], Esther Roth[1], Anne Nicolas[1], Lev Weiner[1]
[1]Weizmann Institute of Science, [2]Universidad de Salamanca (Central Region, Israel)

The three divalent cations Ca+2, Mg+2 and Mn+2, all substantially protect Torpedo californica acetylcholinesterase (TcAChE) from thermal inactivation. Electron paramagnetic resonance revealed one high-affinity binding site for Mn+2, and several much weaker sites. Differential scanning calorimetry showed a single irreversible thermal transition. All three cations raise both the transition temperature and activation energy, with the transition becoming more cooperative. The crystal structures of the Ca+2 and Mg+2 TcAChE complexes revealed a principal binding site that, in both cases, consists of four aspartates (a 4D motif), within which the divalent ion is embedded, together with several waters.1 It makes direct contact with two of the aspartates, and indirect contact, via waters, with the other two. Zebrafish AChE also contains the 4D motif; it, too, is stabilized by divalent metal ions. Overall, the motif has been identified in 31 AChE sequences and 28 butyrylcholinesterase (BChE) sequences. The ASSAM server2 retrieved 200 other proteins that display the 4D motif, in many of which it is occupied by a divalent cation. It is a very versatile motif, since, even though tightly conserved in terms of rmsd values, it can contain from one to as many as three divalent metal ions, together with a variable number of waters. This novel motif, which binds primarily divalent metal ions, is shared by a broad repertoire of proteins. Further investigation of AChEs and BChEs revealed several in which only three of the four residues of the 4D motif are retained. They thus contain a 3D motif. For equine serum BChE we have already demonstrated stabilization by the three divalent ions. The ASSAM server revealed a large number of crystal structures displaying 3D motifs, many of which contain a divalent metal ion. 1. Silman et al. [2021] Protein Sci. (Online).2. Nadzirin et al. [2012] NAR 40:W380-W386.

Track: Therapeutics and Antibodies

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 216 | Optimizing Purification of Outer Membrane Vesicles for Protein-Based Vaccine Development

Tyler Pugeda[1], Milena Dinu[1], Lea Michel[1], Timothy Chapman[2], Michael Pichichero[2]
[1]Rochester Institute of Technology, [2]Rochester General Hospital (California, United States)

Outer membrane vesicles (OMVs) produced by Gram-negative bacteria, such as Nontypeable Haemophilus influenzae (NTHi), are spherical, membrane-enclosed entities that contain periplasmic and outer membrane proteins, as well as other cellular contents. Bacteria often produce OMVs under stressful environmental conditions as a defense mechanism and to remove misfolded outer membrane proteins that could compromise the integrity of the bacterial cell. Due to their potentially rich antigenic content, OMVs are a relatively new target in the field of vaccinology. The objective of this work is to optimize the yield of purified NTHi OMVs with the overarching goal of using OMVs in a vaccine formulation to protect against NTHi infection. We employed several strategies to increase the yield and purity of OMVs, including the use of ion exchange column

chromatography and ultracentrifugation. To quantify and analyze the OMV samples, we used a standard Bradford assay, gel electrophoresis, immunoblotting, nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM). Preliminary results suggest that ion exchange chromatography allows for rigorous purification, but greatly lowers the overall yield of OMVs. Based on this work, we propose the need for a better isolation method that allows for more efficient purification of the OMVs, ideally into subpopulations based on size.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 217 | Fusion to the TELSAM Protein Polymer Dramatically Improves the Success Rate and Speed of Target Protein Crystallization by Stabilizing Weak Crystal Contacts

James Moody[1], Supeshala Sarath Nawarathnage[1], Sara Soleimani[1], Moriah Longhurst[1], Braydan Bezzant[1], Diana Ramirez[2], Parag Gajjar[1], Derick Bunn[1], Cameron Stewart[1], Tobin Smith[1], Maria Pedroza[1], Seth Brown[1], Tzanko Duokov
[1]Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah, United States of America, [2]Department of Natural Sciences, California State University Chico, Chico, California, United States of America, [3]Macromolecular Crystallo (Utah, United States)

We investigate the requirements for TELSAM protein polymers to reliably form well-diffracting crystals when fused to target proteins. We tested various numbers of target proteins fused per turn of the TELSAM helical polymer, both semi-rigid and flexible TELSAM–target connections, and a variety of target proteins. The time to crystal appearance versus the target protein alone was quantified, as was the diffraction quality of the resulting crystals. We determined in multiple cases that fusion to TELSAM accelerates the rate of crystal formation by as much as 27-fold versus the target protein alone. While crystals could be obtained by fusing either 2, 3, or 6 copies of the target protein per turn of the 6-fold helical TELSAM polymer, fusions of 2 or 3 copies frequently result in datasets that are difficult to phase, while fusion of 6 copies invariably gives datasets that can be readily phased and refined. We determined that adjacent polymers need not make direct contact to form well-diffracting crystals of TELSAM–target protein fusions, being separated by at least 40 Å in some cases. Flexible linkers between TELSAM polymers and target proteins do not appear to impair crystal quality, as the target proteins are able to find low-energy binding modes against the polymers. In some cases, TELSAM is seen to stabilize extremely weak inter-target protein crystal contacts, likely through an immense avidity effect. In cases where the target protein's structure is not known, the TELSAM polymer is able to solve the X-ray phases, allowing the target protein to be built de novo into the electron density. We conclude that TELSAM is a powerful crystallization chaperone meriting further investigation.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 218 | Varying The Linker Composition to Improve ScFv Antibodies

Michael Ryan-Simkins[1], Thomas Magliery[1]
[1]The Ohio State University (OH, United States)

Antibodies are important tools in scientific research and medicine, accounting for roughly a quarter of all drugs approved by the FDA over the last five years. The variable fragment is the minimum binding fragment for antibodies of human origin, containing the two variable domains which originate from separate polypeptide chains. When expressed recombinantly, the two domains are commonly fused together with an artificial linker to create a single-chain variable fragment (scFV) which greatly increases the overall stability of the construct. The length of this non-native linker governs the possible oligomeric states that the scFV can assume (monomer, dimer, trimer, etc.) because the spatial distance that the artificial linker must span is quite long, about 35 Angstrom. Previous work from several groups identified the transition to monomer to be around 12 amino acids using glycine and serine linkers. As a result (GGGGS)3 and (GGGGS)4 linkers are commonly used to generate novel monomeric scFVs. We recently demonstrated that the oligomeric distributions of scFVs constructed with (GGGGS)N- linkers are much more heterogeneous than previously thought due to artifacts in the purification process. However, one of the linkers we tested, the 205C linker which is primarily composed of charged residues, had the highest proportion of monomer, suggesting that varying the composition could yield a more homogeneous monomeric scFV. To investigate how linker composition affects the biophysical properties of scFVs we constructed, purified, and characterized a rational library of 40 different scFV constructs with a variety of linkers. Our work demonstrates that when monomeric scFVs are desired the linker should be at least 20 amino acids in length, and scFV dimers with higher stability and homogeneity can be generated with linkers that have higher conformational rigidity.

Track: Enzymology
Session: Allostery & Dynamics in Protein Function

ABS# 219 | Association Between Human Paraoxonase 2 Protein and Efficacy of Acetylcholinesterase Inhibitor Drugs Used Against Alzheimer's Disease

Fauzia Parween[1], Md. Summon Hossain[1], Kshetrapal Singh[2], Rinkoo Devi Gupta[3]
[1]Ph.D. scholar, South Asian University, New Delhi, [2]Defence Research and Development Establishment (DRDO), Gwalior, [3]Faculty of Life Sciences and Biotechnology, South Asian University, New Delhi-110021, India (Delhi, India)

Paraoxonase 2 (PON2) belongs to the paraoxonase gene family and is expressed in all the tissues including the brain. Changes in PON2 activities are associated with a number of pathophysiological conditions such as atherosclerosis and cardiovascular disease. Since cholinergic deficiency is closely linked with Alzheimer's disease (AD) progression, acetylcholinesterase inhibitors (AChEIs) are the treatment of choice for patients with AD. However, there is heterogenous response to these drugs and mostly the subjects do not respond to the treatment. Gene polymorphism, simultaneous occurrence of two or more discontinuous alleles in a population, could be the possible factor for this. Hence, we hypothesized that PON2 and its polymorphic forms may be hydrolyzing the AChEIs differently, and thus, different patients respond differently. To investigate this, two AChEIs, donepezil hydrochloride (DHC) and pyridostigmine bromide (PB), were selected. Human PON2 wildtype gene and four mutants, two catalytic sites (H115W and K192Q) and two polymorphic sites (A148G and S311C) were cloned, recombinantly expressed and purified for in vitro analysis. Enzyme activity and AChE activity were measured to quantitate amount of DHC and PB hydrolyzed by the wildtype and the mutant proteins. Herein, PON2 esterase activity and AChE inhibitor efficiency was found to be inversely related. A significant difference in enzyme activity of the catalytic site mutants was observed as compared to the wildtype, and subsequent AChE activity showed that esterase activity of PON2 is responsible for hydrolysis of DHC and PB. PON2 polymorphic site mutants, 148G and 311C, showed increased esterase activity; therefore, could be the reason for ineffectiveness of the drugs. Thus, our data suggested that esterase activity of PON2 was mainly responsible for hydrolysis of AChEI, DHC and PB, and that might be responsible for variation in individual response to AChEI therapy.

Track: Design/engineering
Session: Designer Proteins Through Genetic Code Expansion

ABS# 220 | Modification of Insulin Lispro by Incorporation of Non-canonical Proline Residues

Stephanie Breunig[1], Alex Chapman[1], Aiden Aceves[1], Jeanne LeBon[2], Janine Quijano[2], Hsun Teresa Ku[2], Steven Mayo[1], David Tirrell[1]
[1]California Institute of Technology, [2]City of Hope (CA, United States)

Recombinant insulin is a life-saving therapeutic in the treatment of diabetes mellitus. A number of FDA-approved insulin variants that enable improved control of blood glucose have been achieved through standard mutagenesis approaches. For instance, the fast-acting insulin lispro contains two point mutations (ProB28Lys and LysB29Pro) that destabilize insulin oligomers, leading to faster absorption. However, even fast-acting insulins are delayed when compared to the insulin-action profile of a healthy pancreas, and insulins undergo irreversible denaturation upon storage. Here we replace ProB29 of insulin lispro with a set of non-canonical proline analogs by residue-specific replacement in Escherichia coli. We find that lispro variants exhibit altered hexamer dissociation rates and fibrillation lag times in vitro, while maintaining their ability to reduce blood glucose in diabetic mice. In particular, we find that the presence of a 4S-fluoro or hydroxy substituent at ProB29 accelerates hexamer dissociation, while 4R- or 4,4-di-fluorination delays fibril formation. These results demonstrate how subtle molecular changes through non-canonical amino acid mutagenesis can be used to engineer therapeutically-relevant properties of protein drugs.

Track: Structure (X-Ray/NMR/EM)
Session: Targeted Protein Degradation

ABS# 221 | Finding a Way Out of the Labyrinth: Degrader-Induced Ternary Complex Modelling

Scott Eron[1]
[1]C4 Therapeutics (MA, United States)

With the exponential growth in the development of targeted protein degraders come significant challenges for the structural biology and computational modelling communities. Numerous examples now exist in the literature of the exquisite SAR possible through modifications of these molecules and this has driven a need to generate atomic level ternary complex information to assist degrader design and elucidate

mechanism of action. Here we will present our approach combining biophysical and computational methods to generate weighted models to support medicinal chemistry campaigns.

Track: Chemical Biology

Session: Protein Structures Through the Lens of Machine Learning

ABS# 222 | A CRYPTIC K48 UBIQUITIN CHAIN-SPECIFIC SITE ON THE PROTEASOME-ASSOCIATED DEUBIQUITINASE UCH37/UCHL5 IS REQUIRED FOR DEGRADATION

Jiale Du[1], Sandor Babik[1], Yanfeng Li[1], Eric Strieter[1]
[1]*UMass-Amherst (Massachusetts, United States)*

Deubiquitinases (DUBs) regulate myriad biological processes by removing ubiquitin modifications from proteins and disassembling ubiquitin chains. These fundamentally distinct activities are thought to require a single S1 ubiquitin binding site that steers the C-terminus into the active site for cleavage. In our effort to understand how the DUB UCH37/UCHL5 exclusively binds K48 chains and cleaves K48 branchpoints, we discovered the presence of multiple S1 sites that contribute to distinct functions. Using a multi-faceted approach, including hydrogen-deuterium exchange mass spectrometry (HDX-MS), chemical cross-linking, nuclear magnetic resonance (NMR), small angle X-ray scattering (SAXS), and molecular dynamics (MD) simulations, we uncovered cryptic K48-specific binding sites located on the opposite face of UCH37 relative to the canonical S1 site. Using targeted mutagenesis, we found that the canonical S1 site is dispensable for chain editing activity and the regulation of proteasomal degradation, but the backside containing the cryptic S1 and S1′ sites are required. Tandem mass tagging-based proteomics further reveals unique sets of proteins whose abundance is differentially regulated by the frontside and backside of UCH37. These findings are significant as they suggest that DUBs may use distinct surfaces for distinct activities.

Track: Design/engineering

Session: Protein Structures Through the Lens of Machine Learning

ABS# 223 | RamaNet: Computational De Novo Helical Protein Backbone Design Using a Long Short-Term Memory Generative Neural Network

Sari Sabban[1], Mikhail Markovsky[2]
[1]*King Abdulaziz University,* [2]*North Caucasian Federal Scientific Center of Horticulture, Viticulture, Wine-making (Makka, Saudi Arabia)*

The ability to perform de novo protein design will allow researchers to expand the variety of available proteins. By designing synthetic structures computationally, they can utilise more structures than those available in the Protein Data Bank, design structures that are not found in nature, or direct the design of proteins to acquire a specific desired structure. In this project, we decided to test whether it is possible to perform de novo design of the backbone of helical protein structures using machine learning's long short-term memory (LSTM) architecture. The LSTM model used only the φ and ψ angles of each residue from an augmented dataset of only helical protein structures that we developed. The network's generated helical backbone structures were adequate, but the loops were not perfect resulting in an open conformation, post generation these structures were brought together using RosettaRelax then evaluated using a filter that we developed to filter out the non-ideal structures and keep the adequate structures. The results were successful in developing a logical, rigid, compact, helical protein backbone topology. One structure out of 25 generated structures had the lowest RMSD value of 4.37 when superimposed with every structure within the dataset, indicating that it was truly generated by the network and not copied from within the dataset. This project was a proof of concept that showed it is possible to generate a novel helical backbone topology using an LSTM neural network architecture using only the φ and ψ angles as features.

Track: Synthetic Biology

Session: Designer Proteins Through Genetic Code Expansion

ABS# 224 | Strategies for quantifying and enhancing genetic code manipulation in yeast

James Van Deventer[1], Jessica Stieglitz[1], Kelly Potts[1], Matthew Zackin[1], Ming Lei[1]
[1]*Tufts University (MA, USA)*

Noncanonical amino acids (ncAAs) facilitate a diverse range of applications in protein science and engineering ranging from dissecting key protein functions to engineering proteins with more "druglike" properties. Genetically encoding ncAAs in proteins produced within cells and in vitro provides the basis for preparing ncAA-containing proteins that would be difficult or impossible

to produce via chemical synthesis. However, our understanding of the performance of these systems remains limited, especially from a quantitative perspective. Here, we present our efforts to (1) establish tools to measure ncAA incorporation in Saccharomyces cerevisiae in response to the amber stop codon; and (2) use these tools to enhance ncAA incorporation efficiency and fidelity in yeast. We have identified a series of dual reporter systems that support flow cytometry-based measurements of ncAA incorporation efficiency and fidelity. These reporters enable quantitative measurements for a diverse range of orthogonal translation systems (OTSs) and for a wide range of yeast strains. We surveyed a number of previously described OTSs in yeast and determined that these systems typically support ncAA incorporation at approximately 15-20% of wild-type protein translation events. Many of these OTSs also exhibit polyspecificity (incorporation of several ncAAs). Ongoing work in the laboratory seeks to use these reporter systems to identify OTSs with improved or altered ncAA incorporation. We have also initiated studies in which we use our reporters to screen a broad range of yeast strains for phenotypes of enhanced ncAA incorporation. Our early findings demonstrate the feasibility of this approach using single-gene knockout strains with known alterations in stop codon readthrough. The measurement tools we have developed form the basis for evolving yeast to better accommodate alternative genetic codes.

Track: Protein Interactions and Assemblies

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 225 | Signalosome nucleation enables binary innate immune proinflammatory responses

Alejandro Rodriguez Gama[1], Tayla Miller[1], Jeffrey Lange[1], Shriram Venkatesan[1], Randal Halfmann[1]
[1]*Stowers Institute for Medical Research, Kansas City, MO (Missouri, United States)*

Pathogen sensing by innate immune cells results in binary all-or-none proinflammatory responses. These responses are coupled to the formation of polymeric protein self-assemblies known as signalosomes. However, whether signalosome formation is directly responsible for binary signaling remains unclear. We have previously found that the formation of certain signalosomes involves a rate-limiting nucleation step. Here, we evaluated the existence and functional consequences of a hypothetical nucleation barrier associated with the assembly of the CARD9-Bcl10-MALT1 (CBM) signalosome, which activates transcription factor NF-κB upon fungal pathogen sensing. We employed Distributed Amphifluoric FRET (DAmFRET), a recently developed flow cytometry-based method that determines the frequency of protein nucleation in cells using flow cytometry. We determined that CARD9 and Bcl10 form nucleation-limited polymers with saturating concentrations similar to those from in vitro studies. We then dissected the nucleation steps required for signalosome formation. Co-expression of artificially oligomerized CARD9 robustly nucleated Bcl10 to its polymeric form. Remarkably, CARD9 oligomers containing mutations that impair antifungal signaling in humans were unable to nucleate Bcl10, suggesting that the nucleating activity of CARD9 is essential for signaling. To address whether the nucleation-limited polymerization of Bcl10 causes binary activation of NF-κB, we introduced a single-cell fluorescent transcriptional reporter of NF-κB activity into the DAmFRET assay. This approach revealed that cells containing Bcl10 assemblies strongly induced NF-κB activation, while cells expressing the same level of Bcl10 in its pre-assembled monomeric state did not. We further showed that rational point mutations that increase or decrease the nucleation barrier for Bcl10 correspondingly impaired or enhanced, respectively, NF-κB activation. Our results indicate a critical role of nucleation barriers in governing the functional outcome of signalosomes. This line of inquiry could uncover a fundamental molecular mechanism of innate immune signaling, which will inform our understanding of the causes of autoinflammatory diseases.

Track: Protein Interactions and Assemblies

Session: Allostery & Dynamics in Protein Function

ABS# 226 | Elucidating the Interactome of the Lytic Transglycosylase RlpA of Pseudomonas aeruginosa

Luis Avila[1], Stefania De Benedetti[1], Rhona Feltzer[1], Matthew Champion[1], Shahriar Mobashery[1]
[1]*University of Notre Dame (Indiana, United States of America)*

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogenic bacterium that has been given a threat level of "serious" by the Centers for Disease Control and Prevention. Many antibiotics that treat P. aeruginosa infections target the cell wall. Inhibition of cell-wall synthesis by β-lactam antibiotics results in bacterial lysis. However, drug-resistant P. aeruginosa strains have acquired the genes necessary to allow for the detection of β-lactams, which initiates a signaling pathway that

culminates in the production of the AmpC β-lactamase, an antibiotic-resistance enzyme. This enzyme hydrolytically degrades the β-lactam antibiotics. This pathway utilizes the cell-wall recycling process in its signaling, which mobilizes the organism for the antibiotic-resistance phenotype. Lytic transglycosylases (LTs) are enzymes that facilitate cell-wall recycling by catalyzing the cleavage of the cell-wall peptidoglycan. Rare lipoprotein A (RlpA) is one of the 11 LTs of P. aeruginosa and little is known of their interactome, the complex assembly of other proteins that interact with it. We have identified the binding partners of RlpA using a pull-down approach, followed by proteomics analysis. We have cloned and purified the putative recombinant partner proteins detected in our mass spectrometry assay and have explored binary and tertiary interactions with RlpA using Microscale Thermophoresis (MST) to obtain dissociation constants (KD). Additionally, we have assessed the rate constants of association (kon) and of dissociation (koff) for the formation of binary complexes between RlpA and the binding partners by Surface Plasmon Resonance (SPR). Currently, we are attempting a chemical cross-linking mass spectrometry platform to study the RlpA interactome within live P. aeruginosa cells. Elucidating the interactome of the LTs, and specifically of RlpA, is important in understanding the bacterial physiology of resistance and in aiding drug-discovery efforts.

Track: Folding

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 227 | Dependence of Proline Isomerization on the Kinetics of Folding of Anthrax Lethal Factor

Wyel Halimeh[1], James Bann[1,2]
[1]*Wichita State University,* [2]*Associate Professor (KS, United States)*

The death that can come within a few days after exposure to the anthrax toxin from Bacillus anthracis is due to the presence and effects of anthrax lethal factor (LF). LF is a zinc metalloproteinase whose function depends upon the translocation of LF from the endosome of a host cell into the cytosol, where it cleaves mitogen-activated protein kinase kinases and disrupting cell signaling pathways. The translocation requires LF to unfold as it transits through the narrow channel of the pore, formed by the anthrax protective antigen (PA). Unfolding of LF has been shown to be pH-dependent, but little is known regarding the kinetics of unfolding and refolding of LF. Specifically, refolding of LF must occur fairly rapidly within the cell cytosol to prevent degradation of the protein by cellular proteases. The N-terminal PA binding domain of LF, LFN, has a single cis-proline residue (Pro166), and we hypothesized that this cis proline must isomerize to trans during the unfolding and translocation process and that refolding would be slow, and perhaps dependent on cellular prolyl isomerases for refolding. To this end, we have performed a detailed kinetic refolding/unfolding study of LFN from urea solutions. Our preliminary experiments indicate that LFN refolding occurs rapidly (within 1 second), suggesting that Pro166 does not isomerize to an appreciable extent in the unfolded state, or if it does isomerize, that isomerization back to cis is a fast process. The implications of these experiments on the mechanism of anthrax toxin lethality will be discussed.

Track: Proteomics

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 228 | Proteomic discovery of densely encoded human genes that encode regulatory polypeptides

Jessie Mohsen[1], Sarah Slavoff[1]
[1]*Yale University (CT, USA)*

Proteogenomic technologies have revealed as many as ten thousand small open reading frames (smORFs) that are translated into polypeptides and small proteins in human cells. smORFs previously escaped annotated due to their short length (<100 codons), non-AUG start codons, and, in some cases, co-encoding with annotated protein coding sequences. While many smORF polypeptides have now been demonstrated to bind to and regulate intracellular proteins including the mRNA decapping complex, the non-homologous end joining machinery, and histone modifications, the vast majority of smORFs remain uncharacterized, leaving open the question of their contributions to human (patho) physiology. My research group leverages quantitative chemoproteomics combined with deep sequencing to discover new smORFs overlapping annotated protein coding sequences. We further apply interactomics, phosphoproteomics and cell-based assays to demonstrate that these overlapping smORFs encode novel regulators of functional proteins in human cells. First, we describe a smORF-encoded polypeptide overlapping human ribosomal protein L36, alt-RPL36. Phosphorylated alt-RPL36 binds to and inhibits the phospholipid transporter TMEM24, decreasing transport of phosphatidyl inositol

from the endoplasmic reticulum to the plasma membrane and, consequently, reducing downstream PI3K/Akt/mTOR signaling and cell growth. We further report chemoproteomic identification and characterization of an overlapping smORF polypeptide co-encoded with an mRNA splicing factor that associates with factors required for ribosome biogenesis to downregulate this critical process. Taken together, these studies support the emerging model that smORF-encoded polypeptides bind to and regulate cellular proteins and complexes. More generally, our results show that overlapping smORFs can function independently from the annotated proteins with which they are co-encoded, but nonetheless regulate broadly related cellular processes – protein translation and cell growth, in the case of alt-RPL36, and mRNA processing vs. rRNA biogenesis, in the latter case. Further exploration of the class of overlapping smORFs may therefore reveal how complex human genes encode multiple regulatory inputs into cellular pathways.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 229 | Ensemble-based enzyme design can recapitulate the effects of laboratory directed evolution in silico

Rojo V. Rakotoharisoa[1], Aron Broom[1], Michael C. Thompson[2,3], Niayesh Zarifi[1], Erin Nguyen[1], Nurzhan Mukhametzhanov[1], Lin Liu[2], James S. Fraser[2], Roberto A. Chica[1]
[1]Department of Chemistry and Biomolecular Sciences, University of Ottawa, 10 Marie Curie, Ottawa, ON K1N 6N5, Canada, [2]Department of Bioengineering, and Therapeutic Science, University of California, San Francisco, San Francisco, CA 94158, USA, [3](Ontario, Canada)

The creation of artificial enzymes is a key objective of computational protein design. Although de novo enzymes have been successfully designed, these exhibit low catalytic efficiencies, requiring directed evolution to improve activity. Here, we use room-temperature X-ray crystallography to study changes in the conformational ensemble during evolution of the designed Kemp eliminase HG3 (kcat/KM 146 M−1s−1). We observe that catalytic residues are increasingly rigidified, the active site becomes better pre-organized, and its entrance is widened. Based on these observations, we engineer HG4, an efficient biocatalyst (kcat/KM 103,000 M−1s−1) containing key first and second-shell mutations found during evolution. HG4 structures reveal that its active site is pre-organized and rigidified for efficient catalysis. Our results show how directed evolution circumvents challenges inherent to enzyme design by shifting conformational ensembles to favor catalytically productive sub-states, and suggest improvements to the design methodology that incorporate ensemble modeling of crystallographic data.

Track: Structure (X-Ray/NMR/EM)
Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 230 | Structural analysis of Legionella pneumophila Dot/Icm type IV secretion system core complex

Clarissa Durie[1], Michael Sheedlo[2], Michele Swanson[1], D. Borden Lacy[2], Melanie Ohi[1]
[1]University of Michigan, [2]Vanderbilt University (Michigan, United States of America)

One important way bacterial pathogens establish infections is by transporting effector proteins into a host cell across multiple membranes. Bacteria have evolved elaborate strategies to accomplish this, including the Type IV Secretion System (T4SS). The Legionella pneumophila Dot/Icm T4SS translocates hundreds of effector proteins and is essential for pathogenesis, leading to the potentially fatal pneumonia Legionnaires' Disease. The components used by bacteria to move virulence factors across membrane have been thoroughly catalogued, but our mechanistic understanding of how these components fit together and move substrate lags behind. Using biochemistry, genetics, and cryo-electron microscopy, I have isolated the Dot/Icm T4SS core complex and determined its macromolecular structure, revealing distinctive structural characteristics and previously unknown components.

Track: Evolution
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 231 | Using metagenomics to investigate the evolutionary origin of highly trimethoprim resistant type B dihydrofolate reductases

Claudèle Lemay-St-Denis[1,2,3], Zakaria Jemouai[1], Lorea Alejaldre[1,2,3], Christian Baron[1], Janine N. Copp[4], Joelle N. Pelletier[1,2,3]
[1]Université de Montréal, [2]PROTEO, [3]Center in Green Chemistry and Catalysis, [4]University of British Columbia (QC, Canada)

Type B dihydrofolate reductases (DfrBs) were first identified in the 1970s, following the introduction of the synthetic antimicrobial trimethoprim that inhibits the chromosomal dihydrofolate reductase. To this day, ten DfrBs have been identified. Their active form is the homotetramer of an SH3-like domain. DfrBs are distinguished from most enzymes in the fact that their single, central active site requires distinct contribution from each of the four identical protomers, creating an evolutionary conundrum. Among other particularities, no change in secondary structure is observed upon heating up to 95°C. DfrBs share no similarities with any characterized protein, begging the question: what evolutionary path did the SH3-like domain follow to emerge in pathogenic bacteria where they procure high resistance to a recently introduced antibiotic?We address this question by identifying and characterizing putative proteins identified in metagenomic databases that share low sequence homology to DfrBs. Our preliminary metagenomic search identified 89 distant homologs to DfrBs. Among the first five distant homologs we synthesized, four displayed the same antibiotic resistance phenotype as DfrBs. Characterization of one distant homolog, predicted by I-TASSER to include a DfrB-like SH3 domain, showed the same kinetic constants for reduction of dihydrofolate as DfrBs. Furthermore, its multimerization pattern is consistent with formation of a homotetrameric, central active site, like the DfrBs.The information we continue to gather from our metagenomic search and characterization will allow us to explore the evolutionary path taken by DfrBs during their evolution. This research will deepen our knowledge on the evolution of antimicrobial resistance mechanisms, a major issue in modern medicine.

Track: Structure (X-Ray/NMR/EM)

Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 232 | Structural basis of proofreading in oxidized PheRS

Pooja Srinivas[1], Rodney Tollerson[2], Mike Ibba[3], Christine Dunham[1]

[1]Emory University, [2]California Institute of Technology, [3]Chapman University (Georgia, USA)

High fidelity during protein synthesis is accomplished by a key enzyme class called aminoacyl-tRNA synthetase (aaRS). These enzymes ligate an amino acid to a cognate tRNA and have proofreading and editing capabilities that ensures high fidelity. PheRS ligates a phenylalanine to a tRNAPhe over the chemically similar tyrosine, which differs from phenylalanine by a single hydroxyl group. In bacteria that undergo exposure to oxidative stress such as Salmonella enterica serovar Typhimurium, tyrosine levels increase due to phenylalanine oxidation. Additionally, several residues are oxidized in PheRS that were found to contribute to a hyperactive editing PheRS, despite these residues not being directly implicated in PheRS activity. Hyperaccurate PheRS displays enhanced editing activity against mischarged Tyr-tRNAPhe in response to oxidative stress and overall secondary structural changes. Here, we solve a 3.6 Å cryo-electron microscopy structure of the oxidized S. Typhimurium PheRS. Our findings visualize how changes caused by oxidation results in widespread structural rearrangements in the catalytic domains. Residues in the PheRS β-subunit editing domain undergo conformational changes that alter the Tyr-tRNAPhe binding surface. Further, oxidized PheRS has both an expanded editing domain and a phenylalanyl-adenylate binding pocket, which may contribute to the hyperaccurate editing and increased aminoacylation rates observed. Taken as a whole, these results help explain why the oxidized PheRS displays hyperaccurate editing activity and provides increased understanding on the survival of S. Typhimurium during human infection.

Track: Folding

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 233 | Fluorescence-Anisotropy Decays and Microscale-Volume Viscometry Reveal the Cotranslational Compaction of Ribosome-bound Nascent Proteins

Xi Chen[1], Rachel Hutchinson[1], Ningkun Zhou[1], Silvia Cavagnero[1]

[1]University of Wisconsin - Madison (WI, United States)

While protein folding is a fundamental process of life, protein folding in the cell, especially when the nascent chain emerges from the ribosomal tunnel, is not well characterized. We explore the co-translational compaction and dynamics of ribosome-bound nascent chains (RNCs) using a novel approach that combines fluorescence anisotropy decay with microscale-volume viscometry. Using an E. coli cell-free system, we studied the model protein sperm whale apomyoglobin (apoMb). Previous studies using fluorescence anisotropy decay demonstrated that apoMb RNCs form a compact structure, but this work alone could not determine the size of the compact region. By combining fluorescence anisotropy with

microscale-volume viscometry, we can determine the size of compact nascent-chain subdomains. Our results indicate that the independently-tumbling compact domain of apoMb RNCs contains 57-83 amino acids. Additionally, the two native C-terminal helices (denoted as G and H), which are important for burying the nonpolar residues in the native structures, are not contained in the compact region. This result indicates that these C-terminal helices are only available for folding into the native structure post-translationally. We propose that the G-helix residues may interact with the ribosome co-translationally, since they are unable to sufficiently stabilize the nascent chain until the H-helix residues become available for intramolecular folding. Based on a square-well-potential model depicting local motions across a cone, the compact nascent-chain subdomains are highly spatially constrained within a cone semi-angle of 15 degrees. This spatial confinement shows the highly geometrically constrained environment of apoMb RNCs within the ribosome. Overall, we demonstrate that integrating fluorescence anisotropy decay and microscale-volume viscometry is a powerful approach that enables identification of the size of independently-tumbling compact regions of nascent proteins. Additionally, this method requires only a single fluorophore and does not require unnatural stalling sequences, and can be applied to any complex biomolecule with an independently-tumbling region.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 234 | Regulation of MKP7 Functionality via Novel Allosteric Site

Shanelle Shillingford[1]
[1]*Yale University (CT, United States)*

The dual-specificity phosphatases responsible for the downregulation of the mitogen-activated protein kinases (MAPKs), are designated as the MAPK phosphatases (MKPs). MAPKs are a group of serine/threonine kinases that play important roles in cell proliferation, death, and migration. Due to their involvement in key cell functions, aberrant signaling of the MAPKs are associated with a wide range of diseases. Therefore, MKP inactivation of these kinases is vital for maintaining cellular homeostasis. Catalytic activity of the MKPs is critically dependent upon a conserved active-site cysteine, neighboring residues, and MKP-MAPK interactions. These properties work in concert to facilitate MAPK dephosphorylation. We have demonstrated that MKP5 is regulated through a novel allosteric site suggesting additional regulatory mechanisms of catalysis exist amongst the MKPs. Here, we sought to determine whether the highly similar MKP family member, MKP7, is also regulated by an allosteric mechanism at a conserved site in its phosphatase domain. We found that mutation of tyrosine 271 (Y271) within the comparable allosteric site of MKP7 is required for its catalytic activity. Overexpression of MKP7 in cells results in p38 MAPK and JNK inhibition. In contrast, MKP7 Y271 mutants failed to dephosphorylate either p38 MAPK or JNK, demonstrating that the allosteric site is critical for MAPK dephosphorylation in cells. We noted that MKP7's binding efficiency to JNK1/2 and p38 MAPK are significantly reduced when MKP7 Y271 is mutated. Consistent with reduced MAPK binding, a greater accumulation of nuclear p38 MAPK and JNK was observed when MKP7 Y271 mutant is expressed in cells as compared with wild-type MKP7, which sequesters p38 MAPK/JNK in the cytoplasm. Collectively, these results provide insight into the regulatory mechanisms of MKP7 catalysis and interactions with the MAPKs. Furthermore, these data support the generality of the MKP allosteric site and provides a basis for small molecule targeting of MKP7.

Track: Proteomics
Session: Targeted Protein Degradation

ABS# 235 | Characterization of the Complex Interactome of the ATP-binding ClpC1 Chaperone Protein in Mycolicibacterium smegmatis by shotgun proteomics

Emmanuel Ogbonna[1], Karl Schmitz[1]
[1]*University of Delaware (Delaware, United States)*

Tuberculosis remains a leading cause of worldwide infectious mortality and one of the top ten leading causes of death overall. While advances in public health have contributed to a reduction in tuberculosis cases, the prevalence of multidrug-resistant Mycobacterium tuberculosis (Mtb) (MDR-TB) infections has created an urgent need to exploit novel drug targets. One such target is the ClpC1P1P2 protease, which degrades folded cytosolic proteins through the cooperation of the ATP-dependent unfoldase ClpC1 and the ClpP1P2 peptidase. Both protease components are strictly essential for Mtb viability and are validated therapeutic targets. However, efforts to develop anti-Mtb compounds are constrained by a limited understanding of Clp protease function and

essentiality. Thus, it is crucial to identify physiological substrates and pathways regulated by this protease. In this study, we identify cellular proteins that interact with the ClpC1 unfoldase in Mycolicibacterium smegmatis (Msm), a nonpathogenic surrogate for Mtb. Using a FLAG-tagged ClpC1 variant with mutations within the Walker B motifs, candidate ClpC1 interaction partners were captured by co-immunoprecipitation and identified by mass spectrometry-based proteomics (LC-MS/MS). Notably, our work reveals a novel proteolytic substrate, 5-oxoprolinase, which is recognized by ClpC1P1P2 via an N-terminal degradation sequence. Our findings suggest that the mycobacterial ClpC1P1P2 protease plays a role in the regulation of arginine and proline metabolism.

Track: Structure (X-Ray/NMR/EM)
Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 237 | Structural basis of long-range to short-range synaptic transition in NHEJ

Siyu Chen[1,2], Linda Lee[3,4], Tasmin Naila[5,6,7], Susan Fishbain[1], Annie Wang[1], Alan Tomkinson[5,6,7], Susan Lees-Miller[3,4], Yuan He[1,2,8,9]
[1]Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA, [2]Interdisciplinary Biological Sciences Program, Northwestern University, Evanston, IL, USA, [3]Department of Biochemistry and Molecular Biology, University of Calgary (IL, United States)

DNA double-strand breaks (DSBs) are a highly cytotoxic form of DNA damage and the incorrect repair of DSBs is linked to carcinogenesis. The conserved error-prone non-homologous end joining (NHEJ) pathway has a key role in determining the effects of DSB-inducing agents that are used to treat cancer as well as the generation of the diversity in antibodies and T cell receptors. Here we applied single-particle cryo-electron microscopy to visualize two key DNA–protein complexes that are formed by human NHEJ factors. The Ku70/80 heterodimer (Ku), the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), DNA ligase IV (LigIV), XRCC4 and XLF form a long-range synaptic complex, in which the DNA ends are held approximately 115 Å apart. Two DNA end-bound subcomplexes comprising Ku and DNA-PKcs are linked by interactions between the DNA-PKcs subunits and a scaffold comprising LigIV, XRCC4, XLF, XRCC4 and LigIV. The relative orientation of the DNA-PKcs molecules suggests a mechanism for autophosphorylation in trans, which leads to the dissociation of DNA-PKcs and the transition into the short-range synaptic complex. Within this complex, the Ku-bound DNA ends are aligned for processing and ligation by the XLF-anchored scaffold, and a single catalytic domain of LigIV is stably associated with a nick between the two Ku molecules, which suggests that the joining of both strands of a DSB involves both LigIV molecules.

Track: Folding
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 238 | Evolution of Metamorphic Protein Folding

Acacia Dishman[1], Robert Tyler[1], Jamie Fox[1], Andrew Kleist[1], Kenneth Prehoda[2], Madan Babu[3], Francis Peterson[1], Brian Volkman[1]
[1]Medical College of Wisconsin, [2]University of Oregon, [3]St. Jude Children's Research Hospital (Wisconsin, USA)

Metamorphic proteins reversibly interconvert between multiple, distinct three-dimensional structures, defying the traditional protein folding paradigm in which each amino acid sequence adopts a single folded structure. The objective of this study was to understand how metamorphic protein folding arises during evolution using XCL1, a human immune protein, as a model system. XCL1 interconverts between two different folds with distinct functions: the canonical chemokine fold, and an alternate all-beta fold. Using nuclear magnetic resonance and bioinformatics, we found that XCL1 evolved from an ancestor that adopts only the chemokine structure. Metamorphic folding then arose in XCL1 about 150 million years ago. The oldest metamorphic XCL1 ancestor predominantly populates the chemokine structure, and the alternate fold is only about 10% populated. A more recent XCL1 ancestor, however, populates the alternate fold 90%, and the chemokine fold 10%. Modern day XCL1 occupies both folds in equal proportion. This suggests that metamorphic folding may be a molecular phenotype that has been selected for in XCL1 during evolution. We also found that for metamorphosis to evolve in XCL1, amino acid substitutions occurred which introduced three sets of changes: changes in molecular strain and flexibility, changes at the dimer interface of the alternate fold, and changes in the intramolecular residue-residue contact networks in the chemokine fold. Reversion of a single amino acid mutation at the dimer interface of the alternate fold eliminates metamorphosis, suggesting that mutations at this dimer interface are necessary but not

sufficient to introduce metamorphic folding in XCL1. These principles for encoding metamorphosis can be used to guide the design of metamorphic proteins in the laboratory for use as components of molecular motors, biosensors, and switchable therapeutics.

Track: Structure (X-Ray/NMR/EM)
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 239 | Structural Basis for NEC Coat Formation During HSV-1 Nuclear Egress

Elizabeth Draganova[1], Jiayan Zhang[2], Hong Zhou[2], Ekaterina Heldwein[1]
[1]Tufts University School of Medicine, [2]University of California, Los Angeles (MA, United States)

Herpesviruses infect a majority of the human population, establishing latent infections that can periodically reactivate. Viral reactivation can lead to severe disease states, including encephalitis and cancers, particularly in the immunocompromised, yet there is no universal cure. Therefore, it is critical to understand how these viruses replicate to prevent and treat infections. An essential step in herpesvirus replication, termed nuclear egress, involves transporting newly formed capsids from the nucleus to the cytoplasm for maturation. As these capsids are too large to pass through nuclear pores, they undergo an unusual budding process through the inner nuclear membrane (INM). This process is mediated by the viral nuclear egress complex (NEC), which deforms the INM around the capsid upon egress. Our lab has shown that the NEC buds synthetic liposomes in the absence of infection or other proteins through the formation of hexagonal arrays, establishing it as a complete budding machine. However, how this process occurs during infection is unclear as an entirely hexagonal NEC lattice would be incapable of forming a spherical coat around exiting capsids. Here, by structure-guided truncations, confocal microscopy, and cryoelectron tomography, we show that binding of the capsid protein UL25 promotes the formation of NEC pentagons rather than hexagons. We hypothesize that during nuclear budding, binding of UL25 situated at the pentagonal capsid vertices to the NEC at the INM promotes the formation of NEC pentagons that would anchor the NEC coat to the capsid. Incorporating NEC pentagons at the points of contact with the vertices would also promote the assembly of a curved hexagonal NEC coat around the capsid, leading to the productive egress of capsids.

Track: Structure (X-Ray/NMR/EM)
Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 240 | Structure of Escherichia coli Respiratory Complex I Reconstituted into Lipid Nanodiscs Reveals an Uncoupled Conformation

Piotr Kolata[1,2], Rouslan Efremov[1,2]
[1]Center for Structural Biology, Vlaams Instituut voor Biotechnologie, Brussels, Belgium, [2]Structural Biology Brussels, Department of Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium (Brussels, Belgium)

Respiratory complex I is a multi-subunit membrane protein complex that reversibly couples NADH oxidation and ubiquinone reduction with proton translocation against trans-membrane potential. Complex I from Escherichia coli is among the best functionally characterized complexes, but its structure remains unknown, hindering further mechanistic studies to understand the enzyme coupling mechanism. Here we describe the single particle cryo-electron microscopy (cryo-EM) structure of the entire catalytically active E. coli complex I reconstituted into lipid nanodiscs. The structure of this mesophilic bacterial complex I displays highly dynamic connection between the peripheral and membrane domains. The peripheral domain assembly is stabilized by unique terminal extensions and an insertion loop. The membrane domain structure reveals novel dynamic features. Unusual conformation of the conserved interface between the cytoplasmic and membrane domains suggests an uncoupled conformation of the complex. Based on these structural data we suggest a new simple and testable coupling mechanism for the molecular machine.

Track: Folding
Session: Protein Evolution, Design and Selection

ABS# 241 | TMAO: protecting proteins from feeling the heat

Mayank M. Boob[1], Shahar Sukenik[2,3], Martin Gruebele[1,2,4,5,6,7], Taras Pogorelov[1,2,5,6]
[1]Center for Biophysics and Quantitative Biology UIUC, [2]Department of Chemistry UIUC, [3]Department of Chemistry and Chemical Biology UC Merced, [4]Department of Physics UIUC, [5]School of Chemical Sciences UIUC, [6]Beckman Institute for Advanced (IL, United States)

Osmolytes are ubiquitous in the cell and play an important role in controlling protein stability under stress. The natural osmolyte trimethylamine N-oxide (TMAO) is used by marine animals to counteract the effect of pressure denaturation at large depths. The molecular mechanism of TMAO stabilization against pressure and urea denaturation has been extensively studied, but the effect of TMAO against high temperature has not been addressed. To delineate the effect of TMAO on folded and unfolded ensembles at different temperatures, we study a mutant of the well-characterized, fast-folding model protein B (PRB). We have carried out extensive, >190 ms in total, all-atom simulations of thermal folding/unfolding of PRB at multiple temperatures and concentrations of TMAO. The simulations captured folding and unfolding events and show increased stability of PRB in presence of TMAO. At higher TMAO concentration, intermediate ensembles are gradually more favored over the unfolded state. Quantifying TMAO-water interactions revealed that at a low concentration threshold, TMAO forms a shell near but not at the protein surface, disrupting the water network and increasing hydration of the protein to help stabilize it. Intriguingly, we found that there are intermittent interactions between TMAO and certain protein side chains with preferred TMAO orientations. Although previous studies have proposed such interactions, the long time scales we study here help to highlight the protein's sensitivity to the local environment, particularly hydration, and raise questions about how even transient interactions could couple protein stability to TMAO effects.

Track: Enzymology

Session: Protein Evolution, Design and Selection

ABS# 242 | Single-Step Replacement of an Unreactive C–H Bond by a C–S Bond Using Polysulfide as the Direct Sulfur Source in the Anaerobic Ergothioneine Biosynthesis

Ronghai Cheng[1], Lian Wu[2], Rui Lai[1], Chao Peng[2], Nathchar Naowarojna[1], Qiang Cui[1], Jiahai Zhou[1], Pinghua Liu[1]
[1]Boston University, [2]Shanghai Institute of Organic Chemistry (MA, United States)

Ergothioneine, a natural longevity vitamin and antioxidant, is a thiol-histidine derivative. Recently, two types of biosynthetic pathways were reported. In the aerobic ergothioneine biosyntheses, non-heme iron enzymes incorporate a sulfoxide into an sp2 C–H bond from trimethyl-histidine (hercynine) through oxidation reactions. In contrast, in the anaerobic ergothioneine biosynthetic pathway in a green-sulfur bacterium, Chlorobium limicola, a rhodanese domain containing protein (EanB), directly replaces this unreactive hercynine C–H bond with a C–S bond. Herein, we demonstrate that polysulfide (HSSnSR) is the direct sulfur source in EanB catalysis. After identifying EanB's substrates, X-ray crystallography of several intermediate states along with mass spectrometry results provide additional mechanistic details for this reaction. Further, quantum mechanics/molecular mechanics (QM/MM) calculations reveal that the protonation of Nπ of hercynine by Tyr353 with the assistance of Thr414 is a key activation step for the hercynine sp2 C–H bond in this trans-sulfuration reaction.

Track: Dynamics and Allostery

Session: Allostery & Dynamics in Protein Function

ABS# 243 | Biophysical Insights of Key Ligand Sensor of Ancestral Steroid Receptor 2

Sabab Hasan Khan[1], Anna C Hou[1], C. Denise Okafor[1]
[1]Pennsylvania State University (Pennsylvania, USA)

Steroid receptors (SRs) are hormone-activated nuclear transcription factors that regulate crucial biological processes of cells. Steroid hormone binding initiates multiple inter- and intradomain allosteric effects in transcriptional activation, including the recruitment of coregulator proteins to the activation function 2 (AF-2) surface on nuclear receptors. A poorly understood aspect of this regulation is how ligands with diverse structures signal between the ligand binding pocket and AF-2 surface and selectively alter it to modulate coregulator binding. Here we investigate hormone specificity in the ancestral steroid receptor 2 (ancSR2) which is transcriptionally activated by 3-ketosteroid hormones and unresponsive to estrogens. Previous work uncovered evolutionarily-conserved ligand sensing-residues in the binding pocket which also enables hormone discrimination. Specifically, the L42 (helix3)-M75 (helix5) interaction enables discrimination between 3-ketosteroids and estrogens. To further probe the role of these ligand sensors in mediating differential hormone responses, we have combined site-directed mutagenesis with biophysical methods, cell-based assays and molecular dynamics simulations, to determine how the modulation of this h3-h5 interaction affects hormone response in ancSR2. Our MD simulations reveal that M75 mutations modulate the status of the h3-h5 interaction, while secondary structure measurements reveal no

large changes in the structure. Differential ligand behavior is supported by hormone response in luciferase reporter assays. These studies will provide biophysical and structural insight as to how hormone specificity evolved in the steroid receptor family.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 244 | Investigating the Interaction between SARS-CoV-2 NSP15 and a Human E3 Ubiquitin Ligase Using In Silico Methods

Annika Viswesh[1], Soichi Wakatsuki[2]
[1]*Palo Alto High School (Palo Alto, CA, United States),* [2]*Department of Structural Biology, Stanford University (Stanford, CA, United States) (CA, USA)*

Patients with acute respiratory distress due to SARS-CoV-2 infection exhibit hyper-inflammatory response and Type 1 Interferon (IFN-1) deficiency. Recent studies indicate that SARS-CoV-2 NSP15 suppresses the immune response; however, this has not been investigated at a molecular level. RNF41, a human E3 ubiquitin ligase, controls inflammation and IFN-1 production by binding to MYD88 and TBK1 in the immune signaling pathways. We hypothesized that SARS-CoV-2 NSP15 binds to RNF41 and inhibits RNF41 from regulating the immune signaling pathways. Molecular docking of RNF41 C-terminal domain (CTD) to five NSP15 poses, MYD88, TBK1, and USP8, were each performed, and binding residues with distances <= 3 Å were measured. Previously unknown structure of RNF41 Zinc-finger domain (ZFD) was generated using homology modeling. Previously unknown active sites on RNF41 ZFD were determined by developing computational algorithms to explore ~170,000 structures in PDB with a structural alignment score of < 2 Å and having zinc finger motifs with complexes. The resulting sites were used to dock RNF41 ZFD to five NSP15 poses. Results showed that NSP15, TBK1, MYD88, and USP8 bound to the same residues of RNF41 CTD. NSP15 had the highest binding affinity to RNF41 CTD. RMSD plots of Molecular Dynamics (MD) simulations indicated that the RNF41-NSP15 complex reached stability at 7 ns. RMSD at equilibrium was ~0.51 nm. RMSF of 83% of the binding residues was lower than average fluctuations indicating high stability. The MD simulations indicate that the docked structure is stable. This confirmed our hypothesis that binding between RNF41 CTD and NSP15 could cause the immune system's disruption. Further, NSP15's binding sites were located > ~8 Å away from its catalytic site, indicating that NSP15's cleaving function could continue even when NSP15 binds to RNF41 CTD. These results set the direction for researching drugs to target SARS-CoV-2 NSP15's binding sites.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 245 | Allostery Through DNA Drives Phenotype Switching

Gabriel Rosenblum[1], Nadav Elad[2], Haim Rozenberg[1], Felix Wiggers[1], Jakub Jungwirth[3], Hagen Hofmann[1]
[1]*Department of Structural Biology, Weizmann Institute of Science,* [2]*Department of Chemical Research Support, Weizmann Institute of Science,* [3]*Department of Chemical and Biological Physics, Weizmann Institute of Science (Rehovot, Israel)*

Allostery is a pervasive principle to regulate protein function. Here, we show that DNA also transmits allosteric signals over long distances to boost the binding cooperativity of transcription factors. Phenotype switching in Bacillus subtilis requires an all-or-none promoter binding of multiple ComK proteins. Using single-molecule FRET, we find that ComK-binding at one promoter site increases affinity at a distant site. Cryo-EM structures of the complex between ComK and its promoter demonstrate that this coupling is due to mechanical forces that alter DNA curvature. Modifications of the spacer between sites tune cooperativity and show how to control allostery, which paves new ways to design the dynamic properties of genetic circuits.

Track: Design/engineering
Session: Diffraction Methods are Alive and Well

ABS# 247 | Modular and Expandable Protein-DNA Co-crystal Scaffolds to Assist in X-ray Diffraction of DNA-Binding Molecules

Abigail Ward[1], Ethan Shields[1], Sara Dmytriw[1], Ananya Vajapayajula[1], Dr. Christopher Snow[1]
[1]*Colorado State University (CO, United States)*

Isoreticular co-crystals (ICC) are a novel class of designed protein-DNA co-crystals. In each ICC, the

DNA stacks end-to-end. Furthermore, the crystal symmetry allows expansion of the DNA-DNA interaction without breaking protein-protein contacts, hence providing larger solvent channels for guest diffusion. Due to canonical base-pairing, the DNA inserted for the expansion is modular, providing an interchangeable DNA sequence for scaffold assisted X-ray diffraction studies. ICC design starts from known protein-DNA co-crystals forms. Candidate structures were narrowed down using custom Python scripts, PyMOL visualization, and experimental convenience factors to a working list of 20 candidate structures. To experimentally synthesize ICC scaffolds, molecular biology techniques were utilized: cloning, expression, purification and crystallization. To date, we have grown expanded ICC crystals using sitting and hanging drop vapor diffusion. The crystals grown have a specific binding sequence exposed to the pores suitable for capture of a cognate DNA-binding target protein. The research presented offers a new approach to scaffold assisted X-ray crystallography. In principle, macromolecules that fit within the engineered crystals and bind tightly to a specific DNA sequence may be revealed in the X-ray diffraction pattern of the co-crystal scaffold.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 248 | Chemical DNA ligation templated by a DNA-binding protein scaffold

Sara Dmytriw[1], Ananya Vajapayajula[1], Abigail Ward[1], Dr. Christopher Snow[1]

[1]*Colorado State University (CO, United States)*

Bioconjugation, or crosslinking, is a strategy to improve the structural integrity of biomolecular crystals. Here, we show unique bioconjugation techniques within two classes of co-crystals consisting of DNA-binding proteins and cognate DNA sequences. The protein in the crystal lines up the DNA ends and enables an abiotic ligation via chemical conjugation. To date, we have used 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), a water soluble carbodiimide, to ligate DNA stacked via these protein insulated interactions. In addition to EDC ligation, we have optimized crosslinking the co-crystals with formaldehyde. We hypothesize the formaldehyde crosslinking is producing covalent bonds between the DNA-binding protein and the DNA duplex. Amazingly, both conjugation methods have produced crystals with integrity at the macro- and nano-scale. The crystal shape and structure are visibly robust in extreme solution conditions, including water (no ions present). The co-crystals also maintain nano-scale integrity as they diffract with X-ray crystallography. The bioconjugation techniques presented can open applications for biomolecular crystals to previously unattainable uses due to crystal fragility.

Track: Evolution

Session: Protein Evolution, Design and Selection

ABS# 249 | Dissecting the Structural and Functional Differences of Eosinophil Ribonucleases Using Ancestral Sequence Reconstruction

Thi Thanh Quynh Tran[1], Chitra Narayanan[2], Andrea N. Loes[3], Timothy H. Click[4], Ngoc Thu Hang Pham[1], Myriam Létourneau[1], Michael J. Harms[3], Pratul K. Agarwal[4], Nicolas Doucet[1,5]

[1]*Centre Armand-Frappier Santé Biotechnologie, Institut national de la recherche scientifique (INRS), Université du Québec, 531 Boul. des Prairies, Laval, QC, H7V 1B7, Canada.,* [2]*Chemistry Department, New Jersey City University, 2039 Kennedy Blvd, Jer (Quebec, Canada)*

How distinct biological activities can evolutionarily arise from a conserved structural fold remains elusive. For instance, human pancreatic-type ribonucleases (RNases) share a common core structural fold that has evolved to preserve diverse biological functions such as angiogenesis, antiviral, antibacterial, antifungal activities, while also preserving ribonucleolytic activity. Ancestral Sequence Reconstruction (ASR) is a useful methodology to reveal the relationship between sequence and function in protein evolution. In this study, an ancestral sequence between two human eosinophil RNases (RNase 2 and 3) was generated based on a phylogenetic tree constructed from 26 different existing RNase sequences, using PhyML, PAML and Lazarus approaches. The ancestral RNase exhibits 91% and 72% sequence identity with human RNase 2 and RNase 3, respectively. Our results show that the ancestor conserves the typical RNase 2 and RNase 3 structural fold. We also show that it shares common properties with these modern RNases, including ribonucleolytic and antibacterial activities. To tease apart the evolutionary conservation between structural and functional dynamics observed in homologous RNase family members, X-ray crystallography and MD simulations of this ancestral RNase are currently underway. Results will offer insights into residue mutations and

evolutionary pathways governing protein structure and function, in addition to offering means to specifically target and modulate RNase activity in various pathological states.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 250 | Substrate discrimination via a proline switch in an allomorphic enzyme

F. Aaron Cruz Navarrete[1], Nicola J. Baxter[1,2], Anamaria Buzoianu[1], Henry P. Wood[1], Andrea M. Hounslow[1], Jonathan P. Waltho[1,2]
[1]University of Sheffield, [2]University of Manchester (SYK, United Kingdom)

The ability of an organism to adapt its metabolism to environmental changes and physiological requirements is critical for survival. Besides changing overall genetic expression, biochemical regulation, such as allostery and allokairy, also plays a crucial role in enabling enzyme activity modulation. Here we describe allomorphy, a hitherto unidentified post-translational control mechanism of enzyme activity operating in β-phosphoglucomutase (βPGM) from Lactococcus lactis. βPGM is a phosphoryl transfer enzyme that catalyses the isomerisation of β-glucose 1-phosphate and glucose 6-phosphate via a β-glucose 1,6-bisphosphate (βG16BP) intermediate. Through combined use of solution NMR spectroscopy, coupled-assay kinetics, site-directed mutagenesis and X-ray crystallography, two conformers with different activities, resulting from the isomerisation of proline 146, were characterised. In vivo phosphorylating agents, such as fructose 1,6-bisphosphate, generate phosphorylated forms of both conformers, leading to a lag phase in activity until the more active cis conformer dominates. In contrast, the βG16BP reaction intermediate couples the conformational switch and the phosphorylation step, resulting in the rapid generation of the more active phosphorylated species. Therefore, allomorphy relies on the existence of alternative pathways with different rate-limiting steps, where the observed catalytic rate depends on the capacity of an activator, acting as a substrate, to bias the enzyme population towards the fastest pathway. Moreover, allomorphy delivers important control with which L. lactis can reconcile two seemingly contradictory requirements: the need to maximise its responsiveness to changes in carbohydrate source and the need to minimise unproductive diversion of valuable metabolites.

Track: Synthetic Biology
Session: Allostery & Dynamics in Protein Function

ABS# 251 | Protein and substrate flexibility contribute to enzymatic specificity in human and bacterial methionine adenosyltransferases

Madhuri Gade[1], Andie Delaney[2], Li Lynn Tan[2], Mahakaran Sandhu[2], Jospeh S. Brock[2], Alejandro Villar-Briones[1], Colin J. Jackson[2], Paola Laurino[1]
[1]Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna-son, Kunigami, Okinawa 904-0495, Japan, [2]Research School of Chemistry, Australian National University, Canberra, 2601, Australia (Okinawa, Japan)

Protein conformational change can facilitate the binding of non-cognate substrates and underlie promiscuous activities. However, the contribution of substrate conformational dynamics to this process is comparatively poorly understood. Here we analyze human (hMAT2A) and Escherichia coli (eMAT) methionine adenosyltransferases that have identical active sites but different substrate specificity. In the promiscuous hMAT2A, non-cognate substrates bind in a stable conformation to allow catalysis. In contrast, non-cognate substrates rarely sample stable productive binding modes in eMAT owing to increased mobility of an active site loop. Different cellular concentrations of substrate likely drove the evolutionary divergence of substrate specificity in these orthologs. The observation of catalytic promiscuity in hMAT2A led to the detection of a new human metabolite, methyl thioguanosine, that is produced at an elevated level in a cancer cell line. This work establishes that identical active sites can result in different substrate specificity owing to the combined effects of both enzyme and substrate dynamics.

Track: Proteomics
Session: Protein Evolution, Design and Selection

ABS# 253 | Analysis of the lake trout brain proteome using evolutionary proteomics

Hannah Yorkey[1], Emmalyn Dupree[1], Bernard Crimmins[2], Thomas Holsen[1], Costel Darie[1]
[1]Clarkson University, [2]AEACS (NY, United States)

Salvelinus namaycush (lake trout) is a top-predator fish in the Great Lakes region and it is used as bioindicator of chemical stress, including the presence of persistent,

bioaccumulative and toxic (PBT) chemicals. Elevated levels of PBTs in a species can cause changes in transcribed genes, translated mRNAs, proteins produced, and posttranslational modifications of these proteins. Our lab has investigated liver, blood and heart proteomes of Great Lakes lake trout. In this study, brain samples from lake trout were analyzed by SDS-PAGE, followed by in-gel trypsin digestion and analysis using nanoLC-MS/MS. The data was searched against different NCBI and UniProtKB databases in Mascot Daemon and the output was analyzed by Scaffold 4.3 software. Databases used include Actinopterygii, Salmonidae, Salvelinus, as well as the highly studied species Oncorhynchus mykiss and Danio rerio. Using these well-developed protein databases, we were able to identify many novel proteins for the lake trout, as well as reveal interesting information about evolutionary relationships for the lake trout species.

Track: Protein Interactions and Assemblies

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 254 | 3D Interaction Homology: Evaluation of ASP, GLU and HIS Residue pKas in Diverse Local Environments Using Hydropathic Interaction Maps

Noah Herrington[1,2], Glen Kellogg[1,2]
[1]*Department of Medicinal Chemistry,* [2]*Institute for Structural Biology, Drug Discovery and Development (Virginia, United States)*

Our objective with this work is to showcase clustered, mapped electrostatic and hydrophobic environments surrounding aspartate, glutamate, and histidine residues in a 2703-protein structure dataset and a method for ionizable residue protonation state prediction. Using a standard Ramachandran plot, divided into an 8x8 "chess board" with letter-number coordinates based on phi (φ) and psi (ψ) angles, residues in our dataset were binned into "chess squares" by these backbone angles and further parsed by their chi (χ1) angles for ASP and HIS and (χ1 / χ2) angles for GLU, according to common conformations of the side chain. All binned and parsed residues were scored with their environment in protonated and deprotonated states in their protein structures using HINT, our validated scoring tool, to determine the most likely ionization state. HINT is designed to categorize and score interactions (ΔΔG values = 1 kcal/mol for ~500 HINT units) between a species and its environment by favorable and unfavorable hydrophobic and polar types. 3D maps describing the interaction environment of each residue were calculated and then clustered within each chess square and parse according to their 3D similarity using k-means clustering in the R programming language. These maps, which are backbone angle-dependent, are revealing of interaction types, loci and intensities surrounding each residue. Using a combination of the Henderson-Hasselbalch equation and the ideal gas law, a mathematical term was calculated in HINT units to simulate free energy change for each 1 ΔpH unit in the environment around individual residues, between pH 0 and 14. We reasonably predicted pKa values for residues with experimental data. Our average deviations of predicted values from experimental ones were ~0.52 for aspartate and ~0.60 for glutamate. A complex relationship between secondary structure, solvent accessibility and ionization state will be discussed.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 255 | Imaging cellular biochemistry using chemical-genetic tools

Alison Tebo[1]
[1]*HHMI - Janelia Research Campus (Virginia, United States)*

Fluorescence imaging has become an indispensable tool in cell and molecular biology. Fluorescent proteins such as GFP have revolutionized fluorescence microscopy, giving experimenters exquisite control over the localization and specificity of tagged constructs. However, these systems present certain drawbacks so alternative hybrid systems based on the interaction between a small molecule fluorophore and a protein have been developed. A way to avoid unspecific background in cells and achieve high imaging contrast is to use fluorescent probes that display no fluorescence until labeling occurs. Such probes are often called fluorogenic probes to highlight their ability to generate fluorescence upon reaction/interaction with their target. A new fluorogenic hybrid system called the Fluorescence-activation and Absorption Shifting Tag (FAST) was developed, which consists of a small protein tag that reversibly and dynamically binds a small molecule chromophore, activating its fluorescence. Hybrid chemical-genetic systems such as FAST represent unique opportunities to extend the utility of the system as the protein and fluorogen present two separate opportunities for engineering. I will present the development of

techniques to image multiple targets and to monitor protein-protein interactions.

Track: Design/engineering
Session: Designer Proteins Through Genetic Code Expansion

ABS# 256 | Effects of Global Incorporation of Non-Canonical Amino Acids on the Stability of Tannase and Pyrogallol Dioxygenase

Thomas Grant[1], Joshua Lister[1], Peter Adewale[2], Hanna Parker[1], Kelly Robinson[2], Tony St-Jacques[2], Matthew Loewen[3], Nediljko Budisa[4], Michele Loewen[1,2]
[1]University of Ottawa, [2]National Research Council of Canada, [3]University of Saskatchewan, [4]University of Manitoba (Ontario, Canada)

High levels of tannins reduce the palatability and nutritional value of animal feed, which excludes high-tannin pulse meals as cost-effective, protein-rich options for mono-gastric animals. We are developing a biocatalytic system incorporating tannase and pyrogallol dioxygenase (PDase) to degrade tannin to improve the value of high-tannin meals. The galloyl ester bond in hydrolysable tannins will be hydrolyzed by tannase to yield gallic acid, which will then be oxidatively cleaved by PDase to yield 4-carboxy-2-hydroxy-cis,cis-muconic acid. However, these enzymes currently do not display activity and stability suitable for tannin degradation in animal feed. Here, we propose a novel approach to enhance the stability and catalytic performance of tannase and PDase by global replacement of aliphatic, aromatic, and proline residues with isosteric non-canonical amino acids (ncAAs). This approach allows selective sequence modifications and specific control of the nature and position of side-chain exchanges within a target protein structure. We set out to perform sequence modifications of wild-type Lactobacillus Plantarum tannase and Rhodococcus opacus PDase that have been successfully expressed recombinantly in E. coli, using the pET31b+ vector. The reaction kinetics for both wild type tannase (Km = 0.42, Kcat = 6.0x10^8) and PDase (Km = 2.30, Kcat = 25.7) were determined following affinity and size exclusion purification. Moving forward, ncAAs will be introduced globally into L. Plantarum tannase and R. opacus PDase and stability assessed at a variety of temperatures (30-100 C°) and solvents such as DMSO, tert-butanol, N-hexane and hexadecane. In the longer term tannin degradation ability will be tested on faba bean meal, which is notably high in tannins. Improving the efficiency of tannase and of PDase through GCE methods should lead to a more stable biocatalyst for the removal of tannins from pulse meal and other crop fractions. This project is funded by NSERC Alliance Grant #550057-20 to MCL and MEL.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 257 | CowN decreases carbon monoxide inhibition of Mo-Nitrogenase through protein-protein interactions

Terrence Lee[1], Cedric Owens[1]
[1]Chapman University (CA, USA)

Biologically available nitrogen is vital for agriculture, however, most nitrogen exists as inert nitrogen gas. The industrial method to convert or "fix" nitrogen gas into biologically usable nitrogen in the form of ammonia is the Haber-Bosch Process, which operates under harsh conditions. Meanwhile, bacteria can also fix nitrogen gas into ammonia, but do so under ambient conditions. Bacteria such as Gluconacetobacter diazotrophicus rely on the enzyme Mo-nitrogenase to fix nitrogen. Studying Mo-nitrogenase opens up prospects for more sustainable ammonia production. However, Mo-nitrogenases are highly sensitive to inhibition by carbon monoxide (CO). Only small amounts of CO drastically reduce ammonia production. Yet, nitrogen-fixing organisms are still able to grow under CO when Mo-nitrogenase is expected to be inhibited. Mo-nitrogenase activity is maintained thanks to another protein, CowN. Neither CowN's structure nor its mechanism of protecting Mo-nitrogenase from CO have been studied in detail. This work explains how CowN protects Mo-Nitrogenase. Our results show that CowN increases the inhibition constant, Ki, of CO from 1.1 ± 0.3 x 10-4 atm to 6.2 ± 3.2 x 10-4 atm, meaning that CowN decreases the affinity of CO for Mo-nitrogenase. We then tested whether CowN and Mo-nitrogenases interact. The results from crosslinking and mass spectrometry experiments show that CowN forms protein-protein interactions with Mo-nitrogenase. Overall, this study reveals how CowN allows Mo-nitrogenase to tolerate CO in the environment. We speculate that CowN binds Mo-nitrogenase at the entrance of a proposed CO channel, thereby decreasing the ability of CO to reach the Mo-nitrogenase active site. Furthermore, this work sheds light on how protein-protein interactions are able to modulate Mo-nitrogenase activity by changing its reactivity or altering gas access to its active site.

Track: Folding
Session: Protein Evolution, Design and Selection

ABS# 258 | Stability Contributions of a Conserved Calcium-Binding Motif in the Catalytic Domain of a Prolyl Dipeptidase from L. helveticus

Jacob Pedigo[1], Katelyn Rose[1], Deanna Ojennus[1]
[1]*Whitworth University (Washington, United States)*

Recently, the structure for the Lactobacillus helveticus x-prolyl dipeptidyl aminopeptidase (PepX) was determined in which a bound calcium ion was observed in the catalytic domain. The amino acids interacting with the calcium ion match a typical DX(D/N)XDG calcium-binding motif sequence. Analysis of PepX sequences shows that the calcium-binding motif is strictly conserved among lactobacilli. However, enzyme activity is unaffected by the addition of ethylenediaminetetraacetic acid (EDTA) and the calcium-binding loop is distant from the active-site serine (27 Å); thus, it is hypothesized that calcium-binding by PepX evolved to stabilize the enzyme structure. Titrations with additional calcium or EDTA were performed in protein thermal shift assays to measure the effect calcium binding has on thermal stability. Denaturation of PepX suggests calcium binding only has modest effects on protein stability.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 259 | A Favorable Path to Domain Separation in the Orange Carotenoid Protein

Mahmoud Sharawy[1], Natalia Pigni[1], Eric May[1], José Gascón[1,2]
[1]*University of Connecticut,* [2]*Instituto de Ciencia y Tecnología de Alimentos Córdoba (Connecticut, United States)*

The Orange Carotenoid Protein (OCP) is responsible for nonphotochemical quenching (NPQ) in cyanobacteria, a defense mechanism against potentially damaging effects of excess light conditions. This soluble two-domain protein undergoes profound conformational changes upon photoactivation, involving translocation of the ketocarotenoid inside the cavity followed by domain separation. Domain separation is a critical step in the photocycle of OCP because it exposes the N-terminal domain (NTD) to perform quenching of the phycobilisomes. Many details regarding the mechanism and energetics of OCP domain separation remain unknown. In this work, we apply metadynamics to elucidate the protein rearrangements that lead to the active, domain-separated form of OCPR. We find that translocation of the ketocarotenoid canthaxanthin profoundly affects the energetic landscape and that domain separation only becomes favorable following translocation. We further explore, characterize, and validate the free energy surface (FES) using equilibrium simulations initiated at different states on the FES. Through pathway optimization methods, we characterize the most probable pathway to domain separation and reveal the barriers along that pathway. We find that the free energy barriers are relatively small (<5 kcal/mol), but the overall estimated kinetic rate is consistent with experimental measurements (>1 ms). Overall, our results provide detailed information on the requirement for canthaxanthin translocation to precede domain separation and an energetically feasible pathway to dissociation.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 261 | A novel homodimerization mechanism of LI-cadherin

Anna Yui[1], Jose Manuel Martinez Caaveiro[1,2], Daisuke Kuroda[1], Makoto Nakakido[1], Satoru Nagatoishi[1], Takahiro Maruno[3], Susumu Uchiyama[3], Kouhei Tsumoto[1]
[1]*The University of Tokyo,* [2]*Kyushu University,* [3]*Osaka University (Kanagawa, Japan)*

Liver Intestine-cadherin (LI-cadherin) is a membrane protein responsible for Ca2+-dependent cell adhesion. Since the expression of LI-cadherin is observed on various types of cancer cells, LI-cadherin is a promising target for cancer treatment and imaging. However, poor understanding of the molecular characteristics of LI-cadherin has hampered rational design of therapeutic molecules targeting this cadherin. Here, we aimed to describe the molecular characteristics of LI-cadherin using biochemical and computational techniques. Homodimerization is the fundamental event in cadherin-mediated cell adhesion. Notably, we obtained a crystal structure showing the novel homodimer architecture of LI-cadherin, which is different from that of any other cadherin reported so far. Cell aggregation assays and mutational analysis indicated that LI-cadherin-dependent cell adhesion is mediated by the formation of the homodimer. In addition, the crystal structure revealed the existence of a noncanonical Ca2+-free linker connecting extracellular cadherin (EC) repeats 2 and 3. In most

cases, three calcium ions bind to the linker between each EC repeat and contribute to the rigidity of the extracellular region. Molecular dynamics simulations of LI-cadherin monomer showed that the Ca2+-free linker is highly flexible whereas canonical Ca2+-bound linkers are not. Our data suggested that the flexibility of the Ca2+-free linker may be one of the reasons why LI-cadherin forms the unique homodimer. Collectively, our findings made a breakthrough in the understanding of the molecular characteristics of LI-cadherin and may assist the development of therapeutic molecules targeting LI-cadherin.

Track: Transcription/translation/post-translational modifications

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 262 | A Large-Scale Human Biopsy Analysis Approach for Inference of eIF4F Protein Complex Composition and Interactions under Physiological Conditions

Su Wu[1], Gerhard Wagner[1]
[1]*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School (MA, USA)*

Eukaryotic translation initiation complex (eIF4F) – considered a potential therapeutic target by cancer researchers – regulates translation by either "cap-dependent" translation initiation (CDTI) (relying on protein interaction among eIF4E, eIF4G1 and eIF4A1) or "cap-independent" translation initiation (CITI) (with eIF4G1 and eIF4A1) mechanisms. Understanding the prevalence of eIF4F subunit interactions, and the clinical relevance of initiation mechanisms, has so far required cell culture and animal model experiments, which are often complicated by varying physiological conditions or cellular states. We demonstrate that analysis of large (n > 10,000) public datasets mitigates this complication and provides an overarching understanding of functional and regulatory effects that are driven by eIF4F protein interactions across tumor types.Our computational assessments of public protein and mRNA data, from human healthy and tumor biopsies, include: copy number comparison; survival analysis; quantification of subunit abundance and stoichiometry; principal component analysis / correlation analysis of gene expression; and mRNA/protein correlations of eIF4F subunits.We found that most tumors frequently gain gene copies of, and elevate expression of, EIF4G1. EIF4G1 expression correlates more closely with poor survival than other initiation-related factors. In healthy tissues, CDTI drives strong correlation of EIF4G1, EIF4A1 and EIF4E with house-keeping genes. However, tumors exhibit altered stoichiometry: EIF4G1 and EIF4A1 correlate with cancer-preferred genes that are uncorrelated with EIF4E, implying partial use of CITI. We found evidence of altered biochemical interactions among eIF4F subunits that are mechanistically linked to eIF4G1 phosphorylation.Our work indicates that lung adenocarcinoma might apply CDTI or CITI as needed to sustain optimal cellular functions, through eIF4G1's adaptable biochemical interactions with eIF4F subunits. Our findings in general demonstrate the clinical relevance of interactions among eIF4F subunits, offer a means of ranking susceptibility of cancer types to eIF4F inhibition, and highlight the value of computational analysis to guide biochemical and protein research efforts.

Track: Enzymology

Session: Measuring Forces of Biological Systems

ABS# 263 | Regulation of the Nonreceptor Tyrosine Kinase Tnk1

Sultan Ahmed[1], Todd Miller[1]
[1]*Dept. of Physiology and Biophysics, Stony Brook University (NY, NY)*

Human Tnk1 (thirty-eight-negative kinase 1) is a member of the Ack family of tyrosine kinases. Depending on the cell context, Tnk1 has been reported to act either as a tumor suppressor or as an oncogenic kinase. Recent studies suggest a pro-proliferative role of Tnk1 in Hodgkin's Lymphoma and pancreatic cancer. Currently, little is known about how the structure of Tnk1 affects its function. Tnk1 contains a tyrosine kinase catalytic domain, an SH3 domain, and a C-terminal region of unknown function. To establish an in vitro assay system, we expressed and purified Tnk1 ΔCT (lacking the C-terminus) from Sf9 insect cells using a recombinant baculovirus. We screened synthetic peptides containing a variety of kinase recognition motifs and found that the best substrate was derived from the Wiskott-Aldrich Syndrome Protein (WASP).A fusion Tnk1 protein (Tnk1-Fusion) previously identified in a Hodgkin's Lymphoma cell line contains an unrelated sequence (from the C17ORF61 gene) fused to the C-terminus. To test the importance of the C-terminal region, we expressed wild-type Tnk1, Tnk1-Fusion, and two C-terminally truncated forms of Tnk1(Tnk1-ΔCT and Tnk1-465) in human embryonic kidney (HEK) 293T cells. Western blotting experiments showed that

expression of Tnk1-Fusion or the Tnk1-465 truncation led to increased tyrosine phosphorylation of cellular substrates, including AKT and STAT3. The fusion sequence was dispensable for the increased phosphorylation. We performed immunoprecipitation reactions and in vitro kinase assays with the WASP peptide. Removal of the C-terminus did not result in overall activation of Tnk1, suggesting that the truncation did not simply remove an autoinhibitory element. We hypothesize that the C-terminus of Tnk1 plays an important role in the subcellular localization of the kinase, and that its removal leads to increased phosphorylation of cellular substrates.

Track: Chemical Biology
Session: Designer Proteins Through Genetic Code Expansion

ABS# 264 | Diversifying Simple Synthetic Antibody Chemistries

Mariha Islam[1], Haixing Kehoe[1], Jacob Lissoos[1], Manjie Huang[1], Christopher Ghadban[1], Greg Berumen[1], Hanan Lane[1], James Van Deventer[1,2]
[1]*Chemical and Biological Engineering, Tufts University,* [2] *Biomedical Engineering, Tufts University (Massachusetts, United States)*

Antibodies play key roles in modern therapeutics and diagnostics. However, the limited range of chemical functionality present in canonical amino acids constrains antibodies in their reactivity and covalent antigen binding. In this work, we investigate strategies to expand the range of chemistries accessible in antibodies for enhanced binding and chemical reactivity. Using single chain variable fragments (scFvs) displayed on the yeast surface, we explored the installation of reactive noncanonical amino acids (ncAAs) in antibody complementarity determining regions (CDRs) to enhance binding function. To do this, we identified several synthetic antibodies with nanomolar affinities and high specificities against immunoglobulins from different species. Canonical amino acid residues within the antibody CDRs were site-specifically replaced with different ncAAs and evaluated for binding function, chemical reactivity, and photo-reactivity. Varied effects on binding retention were observed after ncAA incorporation, dependent on the ncAA incorporation site, translation machinery and ncAA side chain. Several substitutions were well tolerated and resulted in retention of mid-nanomolar binding affinities. Additionally, we evaluated the reactivities of antibodies containing azide or alkyne functionalities on the yeast surface through bioorthogonal "click chemistries." Several antibodies supported copper-catalyzed azide-alkyne cycloaddition (CuAAC) and strain-promoted azide-alkyne cycloaddition (SPAAC) reactivity without hindering antibody-antigen binding. Furthermore, we investigated UV-mediated photo-crosslinking with the photoreactive ncAA azidophenylalanine. NcAA site-dependent photo-crosslinking was observed for multiple antibodies. Lastly, evaluations of ncAA-substituted antibodies in solution confirmed key click- and photochemistry observations made on the yeast surface, confirming the utility of yeast display to evaluate antibody properties in high throughput. Overall, our findings showcase critical ways in which antibodies can be readily modified to enhance their chemical diversity while maintaining binding interactions. We expect that further engineering with yeast-displayed antibodies containing reactive ncAAs will form the basis for performing drug discovery on the yeast surface.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 265 | De Novo Design of Ligand-Binding Proteins

Nicholas Polizzi[1], William DeGrado[1]
[1]*UCSF (CA, United States)*

If we understand how proteins work, then we should be able to design proteins that work. While the design of proteins that fold to desired structures is a mature field, the design of proteins that perform desired functions has met with limited success. Most proteins function by first binding a substrate or cofactor, often with exquisite selectivity. Thus, to advance and test our understanding of protein function, we must first understand how proteins recognize small molecules. My approach to understand molecular recognition focuses on the design of small-molecule–binding proteins from scratch. By analyzing thousands of protein structures in the Protein Data Bank, I discovered a structural "code" used by proteins to preferentially bind the chemical groups commonly found in small molecules. I developed a design algorithm called COMBS that uses this code to create ligand-binding proteins from scratch; and I demonstrated its utility by the design of a de novo protein that specifically binds the antithrombotic drug, apixaban. This work sets the stage for the design of custom ligand-binding proteins as antidotes, drug carriers, allosteric switches, and catalysts.

Track: Protein Interactions and Assemblies
Session: Measuring Forces of Biological Systems

ABS# 266 | Calmodulin and S100A1 interactions within the N2A region of titin

Emily Schaffter[1], Sabrina Apel[2], Matthew Gage[3]
[1]Undergraduate Student at University of Massachusetts Lowell, [2]Graduate Student at University of Massachusetts Lowell, [3]University of Massachusetts Lowell (Massachusetts, USA)

Titin, the giant sarcomere protein, consists of multiple domains and regions of repeated structural elements that are essential for sarcomeric integrity of the myofibril. The N2A region is located at the junction between the proximal tandem Ig region and the PEVK region and is comprised of four Ig domains and a unique 117 amino acid insertion sequence (N2A-IS). The insertion sequence resides between the Ig80 and Ig81 domains, and exists in skeletal muscle, as well as some isoforms of cardiac muscle. Our lab has identified a putative binding site for the Ca2+-binding proteins calmodulin and S100A1. Circular dichroism studies have demonstrated that a conformational change occurs when N2A-IS is bound to either calmodulin or S100A1 in the presence of calcium. The individual binding interactions of N2A-IS with the Ca2+-binding proteins has also been quantitated using surface plasmon resonance. S100A1 also has been observed to disrupt the interaction of the N2A region and actin using an actin co-sedimentation assay. These results provide new insights into the complex interactions associated with this region of titin.

Track: Peptides
Session: Allostery & Dynamics in Protein Function

ABS# 269 | Exploring the BH3 activation landscape of pro-apoptotic BAK

Fiona Aguilar[1], Bonnie Su[1], Dia Ghose[1], Robert Grant[1], Molly Carney[2], Sebastian Swanson[1], Amy Keating[1,3]
[1]MIT Department of Biology, [2]UCLA Department of Molecular Cell and Developmental Biology, [3]MIT Department of Biological Engineering (MA, USA)

The Bcl-2 family regulates apoptosis and is comprised of three protein groups: anti-apoptotic proteins, pro-apoptotic proteins, and BH3-only proteins. BAK is one of the two pro-apoptotic proteins, and previous work has shown that binding of certain BH3-only proteins such as truncated Bid (tBid), Bim, and Puma to BAK leads to mitochondrial outer membrane permeabilization (MOMP), release of cytochrome c, and ultimately cell death. This process, referred to as activation, involves the BH3-stimulated conversion of BAK from monomer to dimer, and then to oligomers that promote membrane disruption. Crystal structures of putative intermediates in this pathway, 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. We applied three methods to explore the sequence and structural requirements of activation: 1) yeast surface display, 2) computational structure-based design, and 3) computational screening of the human proteome. We discovered three new human proteins that bind to BAK that have been implicated in apoptosis and identified additional non-native binders. We measured the affinity and activation function of theses peptides. Our results indicate a correlation between peptide affinity and function in which tighter binders inhibit whereas weaker binders activate BAK. We solved crystal structures of BAK-peptide complexes including complexes for two inhibitors and one activator. All three peptides bound in the canonical hydrophobic groove of BAK with a shifted binding mode when superimposed with WT BH3 peptide. Surprisingly, the binding mode of both inhibitors is highly similar to that of the activator, despite their different functions. We frame our results in an energy landscape model of monomer vs. transition-state stabilization.

Track: Single Molecule Studies
Session: Measuring Forces of Biological Systems

ABS# 271 | Revealing DNA Hairpin Conformational Dynamic Using Single-molecule Force-FRET Measurements

Jason Lin[1], Raymond Pauszek[2], Ann Mukhortava[2], Trey Simpson[1]
[1]LUMICKS, Waltham, MA, USA, [2]LUMICKS, Amsterdam, Netherlands (MA, United States)

Complicated yet sophisticated biomolecular networks regulating cellular metabolism are performed by proteins interacting with and processing DNA and RNA amongst other biological molecules. Conformations and conformational dynamics of these biological molecules are the keys to controlling individual biochemical interactions. Therefore, studying how the biomolecular complexes fold is essential to know the complete picture of biological processes. Recent studies have shown that DNA/RNA

hairpins have crucial roles in regulating protein-nucleic acid interactions such as transcription, recombination, or replication. Since DNA/RNA hairpins could be formed with various sequences, understanding the principles of their structure formation is important for elucidating those biological roles. Single-molecule force spectroscopy represents an ideal tool for such studies because of its unique capability to isolate individual biomolecules and observe conformational transitions in real-time while providing control over the population distribution as opposed to approaches in solution. In this work, we report a study on FRET-labelled DNA hairpin folding transitions using high-resolution optical tweezers combined with fluorescence microscopy. We present equilibrium measurements at unprecedented trap distance stability, which allows measuring conformational state distributions over hours without altering the energy landscape. Measuring over longer time periods while keeping the trap distance within nanometer precision is crucial to capture the rare conformational states that might be overlooked in measurements over a timescale that is limited by instrumental drift. These measurements were enabled by integrating trap-distance and temperature stabilization feedback loops to achieve the ultimate stability in distance between the traps. This study shows how correlating force spectroscopy measurements with single-molecule fluorescence detection could complement the investigation of the prevalence and energy landscape of certain conformational states with extra three-dimensional structural information of the biomolecule of interest.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 272 | **Imaging active site chemistry with protons: Modeling the transition states into and out of the tryptophan synthase α-aminoacrylate intermediate with NMR-assisted crystallography**

Leonard Mueller[1]
[1]*University of California - Riverside (CA, USA)*

NMR-assisted crystallography – the synergistic combination of solid-state NMR, X-ray crystallography, and first-principles computational chemistry – holds remarkable promise for mechanistic enzymology: by providing atomic-resolution characterization of stable intermediates in the enzyme active site – including hydrogen atom locations and tautomeric equilibria – it offers insight into structure, dynamics, and function. Here, we make use of this combined approach to characterize the α-aminoacrylate intermediate in tryptophan synthase, a defining species for pyridoxal-5′-phosphate-dependent enzymes on the β-elimination and replacement pathway. By uniquely identifying the protonation states of ionizable sites on the cofactor, substrates, and catalytic side chains, as well as the location and orientation of structural waters in the active site, a remarkably clear picture of structure and reactivity emerges. Most incredibly, this intermediate appears to be mere tenths of angstroms away from the preceding transition state in which the β-hydroxyl of the serine substrate is lost. The position and orientation of the structural water immediately adjacent to the substrate β-carbon suggests not only the fate of the hydroxyl group, but also the pathway back to the transition state and the identity of the active site acid-base catalytic residue. Reaction of this intermediate with benzimidazole (BZI), an isostere of the natural substrate, indole, shows BZI bound in the active site and poised for, but unable to initiate, the subsequent bond formation step. When modeled into the BZI position, indole is positioned with C-3 in contact with the α-aminoacrylate Cβ and aligned for nucleophilic attack.

Track: Chaperones
Session: New Protein Post-Translational Modifications

ABS# 273 | **Protein aggregation and chaperones: nature inspired technology**

Ivan Coluzza[1]
[1]*CIC biomaGUNE (Ivan Coluzza, Spain)*

Protein misfolding and aggregation are critical bottlenecks in the downstream processing of protein recombinant drugs for the pharma industry. Large amounts of proteins are lost, and current methods to restore the folded structure are cumbersome and with limited yield, leading to low throughput and prohibitive costs. Overall, downstream processing has a strong impact on the healthcare system, particularly in crisis times like the current COVID pandemic when we will soon need large amounts of vaccines that are recombinant proteins.Soluble protein aggregates represent a critical problem for protein-based drugs as they can cause severe or even life-threatening side effects. Taking inspiration from natural chaperones, we propose optimally designing soft nanopores through which aggregated proteins can be extruded to refold into their functional structures. The nanopores have a cylindrical geometry and are coated with a dense brush of polymers in good solvent conditions. Protein clusters pushed by a flow against the pore and forced to translocate through the deformable polymer brush in the pore are broken up into isolated

denatured chains. Due to its polymer functionalization, the pore does not get clogged by the protein clusters, as would be the case for hard nanopores. Instead, protein clusters get peeled off by the steric interaction with the brush and leave the cluster with a separation distance that allows the proteins to refold in isolation into their functional structures.

Track: Amyloid and Aggregation
Session: Protein Evolution, Design and Selection

ABS# 274 | Evolution of protein folding and aggregation in mixtures

Ivan Coluzza[1]
[1]*CIC biomaGUNE (San Sebastian, Spain)*

We present a computational study on the folding and aggregation of proteins in an aqueous environment, as a function of its concentration (1). We show how in protein mixtures each component is capable of maintaining its folded state at densities greater than the one at which they would precipitate in single-species solutions. We demonstrate the generality of our observation over many different proteins using computer simulations capable of fully characterizing the cross-aggregation phase diagram of all the mixtures. Dynamic light scattering experiments were performed to evaluate the aggregation of two proteins, bovine serum albumin (BSA) and consensus tetratricopeptide repeat (CTPR), in solutions of one or both proteins. The experiments confirm our hypothesis and simulations. These findings elucidate critical aspects of the cross-regulation of expression and aggregation of proteins exerted by the cell and on the evolutionary selection of folding and non-aggregating protein sequences, paving the way for new experimental tests.(1) Bianco, V. et al. ChemPhysChem 2020, cphc.201900904; Bianco, V.et al. J. Phys. Chem. Lett. 2019, 4800–4804.

Track: Therapeutics and Antibodies
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 275 | Pomegranate-Derived Low-Molecular Weight Water Soluble Protein: Biophysical Characterization and Therapeutic Activity Study.

Shubhi Katiyar[1], Astha Yadav[1], Indranil Banerjee[1]
[1]*Department of Bioscience and Bioengineering, IIT Jodhpur (Rajasthan, India)*

The pomegranate (Punica granatum)-derived formulations have shown a beneficial effect in treating cardiovascular disorders, cancer, diabetes, alzheimer, diarrhea, microbial infection, and immunological complexities. Pomegranate has various bioactive small molecules such as anthocyanins, ellagitannins, gallotannin, sugars, ascorbic acid, hydroxybenzoic acids, flavanols, and amino acids. It also contains different proteins such as globulins, albumins, glutelin, and prolamins. Interestingly, pomegranate-derived proteins are bioactive too but their potential has not been investigated in detail so far. Furthermore, there is a dearth of biophysical characterization of pomegranate-derived protein which limits the present understanding of the properties of these proteins. Keeping this perspective in mind, here we have performed comprehensive biophysical and biochemical profiling of water-soluble proteins from pomegranate arils and evaluated its bioactivity invitro.For this study, the aqueous pomegranate extract was obtained through mechanical grinding of pomegranate arils followed by sequential filtration (0.45 μm and 0.22 μm pore size) of the juicy mixture. The filtrate was subjected to total protein precipitation by the ammonium sulfate method. The protein obtained in this method was lyophilized and quantified. Native and SDS- PAGE analysis revealed that the aqueous extract predominantly contains a single monomeric protein of molecular weight ~28 kDa. The UV-Vis spectrum showed the presence of a peak around 210 nm but not at 280 nm. Fluorimetry confirmed spectral emission in the visible range. Further, FTIR and CD analysis affirmed the presence of characteristic amide peaks and α-helix. Amino acid composition analysis revealed the presence of both hydrophobic and hydrophilic amino acids. The protein exhibited moderate activity against gram-positive and gram-negative bacteria. Cytotoxicity of the protein was studied against cancer cell lines. The studies all together have provided a basic understanding of the biophysical properties and bioactivity of the water-soluble protein derived from pomegranate.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 277 | Designing Proteins for Light-Emitting Diodes

Horst Lechner[1], Tobias Fleiß[1], Gustav Oberdorfer[1]
[1]*Graz University of Technology (Styria, Austria)*

We want to employ artificial fluorescent proteins as down-converting filters for the emitting source of (blue) light-emitting-diodes (LEDs) to create white Bio-LEDs. The proteins are used as hosts for small organic compounds with

fluorescent properties, similar to the ones used in organic LEDs (OLED). This should protect these compounds from photo-deactivation, which represents a central problem with these kinds of fluorescent molecules and often renders them less attractive for the use in white LEDs. Our major aim in this multi-disciplinary project is to computationally identify proteins that are able to accommodate the compounds and redesign their pockets to show specific binding, while retaining high thermal stability after design. Additionally, the fluorescent compounds will be anchored into the binding pocket of the protein by a covalent attachment using a non-canonical amino acid or a cysteine residue. To accomplish this, a suitable anchoring point in the pocket must be installed into the pocket during design. Finally, the derived proteins will be embedded in a polymer matrix to increase their stability against pH changes, temperature and the high photon-flux occurring when the blue LED- emitter is covered with this biological filter. We currently have more than a dozen designs for one of the envisaged dyes expressed and are evaluating their binding, spectroscopic and biophysical properties.

Track: Dynamics and Allostery

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 278 | Solution Conformations of Dimeric Phenylalanine Hydroxylase

Kushol Gupta[1], Emilia Arturo[2], George Merkel[2], Eileen Jaffe[2]
[1]*Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.,* [2]*Molecular Therapeutics, Fox Chase Cancer Center – Temple University Health System, Philadelphia, PA 19111 (PA, United States)*

Phenylalanine hydroxylase (PAH, EC 1.14.16.1) converts L-phenylalanine (Phe) to L-tyrosine (Tyr), maintaining normal Phe blood levels (~50-120 μM). In phenylketonuria (PKU), inherited defects in this enzyme underlie hyperphenylalaninemia, where Phe blood levels can become neurotoxic (up to 2.5. mM). Recent atomic structures of a tetrameric form of this enzyme from rat (rPAH) and human (hPAH), alongside complementary solution scattering analyses have greatly informed our understanding of the dramatic conformational changes that underlie the transition from an auto-inhibited resting state tetramer in the presence of low Phe to an activated tetrameric form that exhibits maximal catalytic activity in the presence of >100 μM Phe. Current structural models for the transition of the resting state enzyme to its activated form suggests a dissociation of the resting-state tetramer into lower-order oligomers that then remobilize into an activated tetramer. However, no data is currently available for the structure of these lower-order intermediates of PAH that exist in the absence of Phe, or those that precede PAH activation. To approach this challenge, we have combined analytical ultracentrifugation (AUC) and size-exclusion chromatography in-line with synchrotron X-ray scattering (SEC-SAXS) to study three unique preparations of rat PAH: 1. the native wild-type rPAH in its resting state, which exists in a dimer-tetramer equilibrium, 2. A unique preparation of rPAH isolated by ion-exchange chromatography that only exists as a dimer in solution, and 3. a series of dimeric oligomers from Phe80 variants recently reported (Arturo et al Biochimie 2021). Modeling of the SEC-SAXS data provides the first insights into the native solution properties of these intermediates in different functional forms and indicates the existence of different dimer assemblies in solution.

Track: Bioinformatics

Session: Protein Structures Through the Lens of Machine Learning

ABS# 279 | Determining the Evolutionary Significance of Palindromic Sequences in Proteins

James Hebda[1], Saif Ahmad[1]
[1]*Austin College (TX, United States)*

The structure and function of a protein is dependent on its amino acid sequence. Many proteins never independently studied have proposed functions based on similarities to known protein sequences. Palindromic sequences in proteins are an overlooked, potentially mechanistically important motif. A nine-residue amino acid palindrome found in human α-B-crystallin has been hypothesized to function as the site of dimerization for the protein, allowing for the protein to have two inverted but nearly identical interactions. We hypothesize that similar palindromic sequences may serve analogous functions in other proteins. To identify and determine if palindromes are overrepresented, and therefore potentially mechanistically important, a program was written in the Python programming language to conduct proteome-wide analysis of palindromes, including determining their sequences, locations, and relationship to each other. This analysis was conducted on E. coli and s. cerevisiae to compare palindrome usage between two prokaryotes and eukaryotes. Both exact and relative palindromes, palindromes that have chemically similar sequences rather than identical amino acids, were identified, grouped, and compared. Sequences were compared to those of randomized proteomes, of which the same analysis

was performed, to determine any evolutionary bias of palindromic sequences in proteins. The palindromic sequences themselves are conserved enough to reestablish the evolutionary relationship between the proteomes analyzed. Although it was found a significant number of palindromes in both prokaryotes and eukaryotes can be accounted for based on random chance and simple repeats, palindromic sequences do more often in nature than expected by chance. This suggests that some protein palindromic sequences are evolutionarily conserved and therefore may have biological significance.

Track: Amyloid and Aggregation
Session: Protein Evolution, Design and Selection

ABS# 280 | Benign or pathogenic? Perplexing variants of the glaucoma-associated myocilin olfactomedin domain

Raquel Lieberman[1]
[1]*Georgia Institute of Technology (GA, USA)*

The effects of nonsynonymous gene mutations can be beneficial, neutral, or detrimental to the stability, structure, and biological function of the encoded protein. In some cases, the effect of any given mutation is not readily predictable, however, particularly where variants can endow a gain of toxic function. Numerous different rare point mutations within the myocilin β-propeller olfactomedin (mOLF) domain are linked to glaucoma; the resulting variants are less stable, aggregation-prone, and sequestered intracellularly, causing cytotoxicity and hastening of glaucoma symptoms. However, destabilization is not a universal consequence of mutation. Some variants identified through population-based genome sequencing are indistinguishable from wild-type mOLF and are likely neutral polymorphisms. Other single point variants we identified exhibit increased stability and do not aggregate, but share key characteristics of glaucoma-causing variants, namely, they adopt partially folded structures. Double mutants combining a stabilizing-yet-partially-folded substitution and a glaucoma-causing mutant can even correct folding, stability, and cellular secretion. Finally, we used the computational method PROSS to identify a 21-residue mOLF variant that is nearly 20 °C more stable than wild-type mOLF while retaining wild-type structure. In summary, although many single point variants are detrimental and likely glaucoma-causing, mOLF has an impressive capacity for additional stabilization. Our study emphasizes the complexities in differentiating between benign and glaucoma-causing variants, with implications to interpreting data from large-scale sequencing projects where clinical and family history data are not available and for future precision medicines for myocilin-associated glaucoma.

Track: Design/engineering
Session: Targeted Protein Degradation

ABS# 281 | Harnessing the APC/C for Targeted Protein Degradation

Cynthia Okoye[1], Laura Itzhaki[1], Catherine Lindon[1]
[1]*Department of Pharmacology, University of Cambridge (Cambridgeshire, United Kingdom)*

PROTACs (PROteolysis Targeting Chimeras) are heterobifunctional molecules that form a ternary complex with a target protein of interest and an E3 ligase, thereby harnessing the ubiquitin proteasome system to eliminate disease-associated target proteins. Most PROTACs that have been developed utilize CRBN or VHL E3 ligases although there are more than 600 E3 ligases in humans. We are designing chimeric proteins that function like PROTACs and that harness the anaphase-promoting complex (APC/C). The APC/C is an important E3 ligase that regulates numerous mitotic proteins including Securin, NEK2A kinase and Aurora kinase A (AURKA). Most natural APC/C substrates contain multiple copies of short linear motifs (SLiMs), termed degrons, that serve as recognition sequences that bind the APC/C and position it for effective polyubiquitin transfer. Consequently, our design of degraders incorporates degrons from AURKA and other natural APC/C substrates, and these were tested in cell-based degradation assays using time-lapse quantitative fluorescence imaging. In the first stage of the design process, we identified novel APC/C degron combinations in binary complex formats that yield significant degradation in cells. We are now advancing to testing the degron combinations in ternary complex formats to develop more potent degraders that harness the APC/C for destruction of disease targets.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 282 | Evidence of a pairwise interaction between the Escherichia coli enzymes EntC and EntB confirms that the enterobactin biosynthetic pathway is fully networked via protein-protein interactions

Sylvie Ouellette[1], Paknoosh Pakarian[1], Navjote S. Kooner[1], Peter D. Pawelek[1]
[1]*Concordia University (QC, Canada)*

Enterobactin is a catecholate-type, triserine lactone siderophore produced by Escherichia coli to scavenge scarce iron from its surroundings. Its biosynthesis involves the coordinated activities of five proteins, EntC, EntB, EntA, EntE and EntF, in a pathway composed of two functional arms: (i) a chorismic acid precursor is transformed through sequential reactions of EntC, EntB and EntA to yield 2,3-dihydroxibenzoic acid (DHB), (ii) DHB is condensed with L-serine through three cycles of condensation involving non-ribosomal peptide synthetase activities (EntE, EntB and EntF).We hypothesize that the Ent biosynthetic enzymes are extensively networked via protein-protein interactions that facilitate substrate channeling in order to enhance efficiency of the enterobactin biosynthesis and secretion. In support of our hypothesis, there is now abundant reported evidence that most of these enzymes interact in a pairwise fashion (EntB-EntA, EntA-EntE, EntE-EntB, EntB-EntF). One possible interaction that has so far eluded detection involves the first two enzymes of the pathway: EntC and EntB. Through a bacterial adenylate cyclase two-hybrid (BACTH) assay we have determined that these two proteins are proximal in vivo. Employing in vivo formaldehyde crosslinking, we demonstrate that EntC and EntB form a specific pairwise interaction in vitro. Using the automated docking server CLUSPRO, we have generated a computational model of the EntC-EntB complex. Finally, we performed an electrostatic surface analysis of the docked complex to identify a possible electrostatic substrate channeling path. Our findings complete the map of demonstrated pairwise interactions for enzymes involved in consecutive steps of the enterobactin biosynthetic pathway.

Track: Intrinsically Disordered Proteins
Session: Protein Evolution, Design and Selection

ABS# 283 | Sequence Determinants of pH-dependent Conformational Change in IDPs

Sophia Manukian[1], Emily Punch[1], Sudarshi Premawardhana[1], Matthew Gage[1]
[1]*University of Massachusetts Lowell (Massachusetts, United States)*

Intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs) have been found to play a role in a variety of cellular processes. Unlike ordered proteins, many intrinsically disordered proteins (IDPs) become more structured under extreme pH conditions. This is thought to occur as a result of the highly charged nature of IDPs. In very basic or acidic conditions, an IDP's similarly charged residues are neutralized allowing for a more compact state, due to decreasing electrostatic repulsion between neighboring amino acids. This hypothesis is supported by the fact that a higher net charge per residue (NCPR) has been shown to be associated with a greater level of pH-dependent conformational change. However, this trend of relating increased NCPR to increased pH sensitivity is inconsistent among IDPs, suggesting that NCPR alone cannot explain the variation in pH sensitivity observed. The objective of this study is to determine how different sequence characteristics affect the pH-sensitivity of a peptide. To do this, the intrinsically disordered poly-E motif from the PEVK segment of the long muscle protein titin was used as a reference sequence. It contains glutamate clusters, which are prevalent among IDPs, and has been shown to exhibit pH-dependent conformational changes. Peptides of varying hydrophobicity, proline content and disorder are being examined over a range of pHs using a combination of Size Exclusion Chromatography and CD. This may have physiological relevance to some microenvironments, such as tumors and active muscle, which have been shown to be more acidic than the regular cytoplasmic environment. A better understanding of how different IDP sequences influence pH-sensitivity could improve the prediction and modeling of IDPs in such environments. In addition, this could lead to more informed design of pH-responsive peptides for drug delivery or other applications.

Track: Intrinsically Disordered Proteins
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 284 | Liquid-like condensates of p53 are modulated by interaction with the papillomavirus E2 master regulator

Silvia Borkosky[1], Karen Campos-León[2], Ramón Peralta Martínez[1], Nicolás Demitroff[1], Kevin Gaston[2], Gonzalo Prat-Gay[1]
[1]*Fundación Instituto Leloir CONICET,* [2]*University of Nottingham (Ciudad Autónoma de Buenos Aires, Argentina)*

Membrane-less organelles are formed by biomolecular condensation of macromolecules driven by the physicochemical process known as liquid-liquid phase separation (LLPS), where further transitions from liquid to solid states impact on a variety of physiological and pathological processes. The formation of ordered aggregates of the tumour-suppressor protein p53, suggests that LLPS plays a key role in its regulation and activities. p53 has been

shown to inhibit replication of human papillomaviruses (HPV), and the HPV E2 proteins can induce p53-dependent apoptosis, both processes involving E2-p53 interactions whose molecular details are largely unknown. By using spectroscopic techniques combined with fluorescence microscopy, here we show that full-length p53 undergoes homotypic LLPS at neutral pH and low ionic strength. In addition, we show that p53 and the HPV16 E2 DNA binding domain (E2C) readily form heterotypic liquid-like droplets. Time-course experiments revealed that the homotypic droplets evolved to amyloid-like aggregates, whereas large spherical droplets containing both proteins remain up to 24 hours. When assessing the effect of specific target DNA duplexes, we found that p53 bound to its specific DNA fail to form droplets or condensates. On the other hand, the complex formed by p53 and its specific DNA recruited E2 to form large solid-like amorphous aggregates. Lastly, cellular transfection experiments showed that p53 and E2 co-localize in the nucleus in a granular chromatin-associated pattern, strongly suggestive of phase-separated condensates. This work demonstrates the capacity of p53 to follow different condensation routes as part of its role in cellular processes and specially highlights the formation of functional condensates and their intertwined connection with gene function control in a cancer-associated virus.

Track: Computational Modeling/Simulation

Session: Protein Structures Through the Lens of Machine Learning

ABS# 285 | Homology Modeling of GPR114/ADGRG5 With Rosetta Elucidates Possible Interactions Between ECL2 And GAIN Domain

Fabian Liessmann[1], Caroline Wilde[2], Anja Wieprecht[2], Maria Sierks[2], Clara Tabea Schoeder[1,3], Sandra Berndt[2], Jens Meiler[1,3], Ines Liebscher[2]

[1]Institute for Drug Discovery, Faculty of Medicine, Leipzig University, Leipzig, Germany, [2]Rudolf Schoenheimer Institute of Biochemistry, Faculty of Medicine, Leipzig University, Leipzig, Germany, [3]Department of Chemistry, Department of Pharmacol (Saxony, Germany)

Adhesion G protein-coupled receptors (aGPCRs) form the second largest family of GPCRs and have strong involvement in important cellular processes, carcinogenesis, immunology, neurological diseases and many more. However, the precise activation mechanism has remained enigmatic because no full-length structure of an intact member has been determined to date. A tethered peptide agonist, the so-called "Stachel", inside the GPCR autoproteolysis-inducing (GAIN) domain activates intracellular G protein-coupled signal after being cleaved. ADGRG5 (former called GPR114) is an aGPCR expressed in leukocytes, colon and thymus with a high constitutive activity but does not need the typical cleavage. The increasing number of resolved GPCRs in recent years as well as the rise of more sophisticated computational modeling studies (e.g., with algorithms like RosettaCM) have greatly improved our understanding of GPCR structure and gave further insight into their activation and signaling.In this work, we constructed models of ADGRG5 using Rosetta homology modeling with a special focus on the unusual long extracellular loop 2 (ECL2) which could allow it to form interactions with the Stachel inside the GAIN domain. The recently first resolved structure of an aGPCR transmembrane area guided the construction of the beta sheet-structured loops. Peptide-peptide docking of both domains provided an interaction matrix. The loops in the GAIN domain normally covering the Stachel were cut and rebuild to retain the flexible aspects of loops. After analyzing the binding interface, site-directed mutagenesis validated the generated models and our hypothesis.Here, leveraging on the available structural information we have investigated the activation mechanism of the first full-length ADGRG5 model and characterized the ECL2 interactions. Moreover, the structural model can be further used in paving the way toward the identification of new ligands.

Track: Structure (X-Ray/NMR/EM)

Session: Allostery & Dynamics in Protein Function

ABS# 286 | Investigating Caspase-6 Mobility via Biomolecular NMR

Nathanael Kuzio[1]

[1]Department of Chemistry, University of Massachusetts Amherst (MA, United States)

Caspases are some of the key protagonists of programmed cell death and homeostasis. However, dysregulation of these proteases can lead to a variety of health concerns including cancer and neurodegeneration. Caspase-6 has been identified as a valuable drug target for neurological conditions such as Alzheimer's disease (AD) due to its cleavage of microtubule-associated proteins tau and tubulin preceding neuronal degeneration. Here, I use biomolecular NMR to investigate unique structural aspects of caspase-6 as well as potential

inhibition mechanisms that can be used to selectively target the protease for therapeutic intervention. More specifically a distinct 130's helix-strand interconversion is associated with a transition between active and inactive conformations. Understanding the rates of exchange and respective dynamics of these interconversions may provide insight into caspase-6's lower activity compared to other caspases. Additionally, harnessing this unique interconversion by locking the protease in its inactive form may provide a mechanism of selective inhibition for caspase-6 among all caspases.

Track: Enzymology

Session: Designer Proteins Through Genetic Code Expansion

ABS# 287 | Site-Specific Incorporation of Non-Canonical Amino Acids to Increase Catalytic Efficieny of a Tannase and a Pyrogallol Dioxygenase

Hanna Parker[1], Thomas Grant[1], Joshua Lister[1], John Pezacki[2], David Prescott[2], Kelly Robinson[2], Antony St-Jacques[2], Matthew Loewen[3], Michele Loewen[1,2]
[1]Department of Chemistry and Biomolecular Sciences, University of Ottawa, [2]National Research Council of Canada, Aquatic and Crop Resources Development Research Centre, [3]Department of Veterinary Biological Sciences, University of Saskatchewan (Ontario, Canada)

Tannins are a common plant metabolite that when found at high levels in animal feed can prevent palatability and nutrient absorption, specifically in monogastric animals. We can use biocatalysts to remove tannin biomolecules and its derivatives to yield a higher value product. In this tannin degradation pathway, tannase hydrolyses the galloyl ester bond within the tannin to produce gallic acid, which is then oxidatively cleaved by pyrogallol dioxygenase (PDase, aka catechol 1,2-dioxygenase) to produce 4-carboxy-2-hydroxy-cis,cis-muconic acid. To optimize tannase and PDase for more efficient catalysis, we are utilizing genetic code expansion (GCE) technology to incorporate the noncanonical amino acid δ-N-methyl-histidine (NmHis) into the catalytic active sites. In addition to reducing the complexity of the hydrogen bonding network in the active site, the extra methyl group of the NmHis will increase the electron donating of the ε-N to the catalytic Fe3+ in PDase, which is hypothesized to increase catalytic activity of the enzyme. The wild-type of both enzymes has been recombinantly expressed and purified, and the kinetic data has been obtained. NmHis will be site-specifically added into our enzymes using an orthogonal pyrrolysyl tRNA synthetase (PylRS)/tRNACUA that integrates NmHis in response to the amber codon UAG. After incorporation of methylated histidines to the catalytic sites of tannase and PDase the enzymes will be assayed and compared to the wild type to verify they display higher activity and a higher turnover. Engineering biocatalysts like these to become more stable and active will produce higher quality pulse meal and canola products, paving the way for future industrially and economically relevant enzymatic engineering. This project is funded by NSERC Alliance Grant #550057-20 to MCL and MEL.

Track: Dynamics and Allostery

Session: Allostery & Dynamics in Protein Function

ABS# 289 | Rap1A molecular dynamics simulations show features distinct from those of Ras

Michael Schwabe[1], Genevieve Holzapfel[2], Carla Mattos[1]
[1]Northeastern University, [2]North Carolina State University (Massachusetts, United States)

GTPases cycle between a GTP-bound state capable of activating effector proteins to propagate cellular signaling pathways and an inactive GDP-bound state. While nearly all members of Ras superfamily contain a catalytic glutamine at position 61, Rap1A, which has the highest sequence conservation compared Ras, contains T61. The proto-oncogenic nature of Ras has resulted in many structures made available in the protein data bank, while Rap1A has fewer structures available for detailed analysis. We have previously proposed a novel mechanism for GTP hydrolysis in the absence of the canonical GTPase activating protein (GAP) that involves a secondary water molecule interacting with the Q61 side chain. It is currently unclear if T61 in Rap1A is involved in a similar intrinsic hydrolysis mechanism. The goal of this project is to explore the Rap1A active site and its mechanism of intrinsic hydrolysis. To answer this question, we have solved a number of Rap1A crystal structures with novel active site conformations and applied 200 ns of accelerated molecular dynamics simulations starting from these structures. Crystal structures show two conformations of T61. Simulation data suggests a predominant conformation that allows T61 to be involved in an intrinsic catalytic mechanism. Beyond the differences seen in the Ras and Rap1A active sites, global differences in allosteric communication pathways are clear.

Track: Protein Interactions and Assemblies
Session: Diffraction Methods are Alive and Well

ABS# 290 | Data-driven model of the KRas4B/calmodulin complex

Monica Ojeda[1], Kendra Marcus[1], Hyunbum Jang[2], Avik Banerjee[3], Ruth Nussinov[2], Vadim Gaponenko[3], Carla Mattos[1]
[1]Northeastern University, [2]Frederick National Laboratory for Cancer Research, [3]University of Illinois, Chicago (Massachusetts, USA)

KRas4B (henceforth called KRas) is a small 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. We present 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 and chemical shift perturbations. The low-resolution envelope of the complex shows that CaM interacts with KRas in an open conformation. The NOEs suggest that the HVR adopts a helical conformation in the region that interacts with CaM. Our low-resolution experimental data, together with published chemical shift perturbations lead to a model of the KRas/CaM complex that fits our SAXS envelop well (X = 1.97). This model will be refined using molecular dynamics simulations.

Track: Chaperones
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 292 | Selective Tuning of Solubility and Structural Accuracy of Newly-Synthesized Proteins by the Hsp70 Chaperone System

Rachel Hutchinson[1], Rayna Addabbo[2], Matthew Dalphin[3], Miranda Mecha[1], Yue Liu[1], Silvia Cavagnero[1]
[1]University of Wisconsin-Madison, [2]University of Minnesota, [3]Catalent Pharma Solutions (WI, United States)

Protein folding within the cellular environment is not well understood, especially the role of the ribosome and molecular chaperones. Translation through the ribosome is sufficient to grant solubility to some proteins even in the absence of molecular chaperones. Yet, many more proteins with widely different sequences are soluble when synthesized in the presence of chaperones. Therefore, chaperones are important for granting solubility to proteins in the earliest stages of their life. Recent findings revealed that, upon release from the ribosome, protein solubility is sensitive to even small variations in amino acid sequence. Once the native state is formed and released from the ribosome, it is kinetically trapped from aggregates. Therefore, all events that accompany co- and immediately post-translational folding are vital for long-term protein solubility and cell function. Interestingly, most mutations do not affect bacterial fitness, suggesting that the cellular machinery, including molecular chaperones, may play a protective role against random mutations. Here, we probe this hypothesis by generating single-point variants of a model globin protein (apomyoglobin, apoMb) and analyzing the role of the Hsp70 chaperone in aggregation prevention. The variants do not differ in their thermodynamic stability and native-state structure compared to wild-type protein but lead to insoluble aggregate formation upon release from the ribosome, in the absence of chaperones. We show that increasing levels of Hsp70 restore 100% solubility. Additionally, we explored another action of the Hsp70 chaperone: maintaining the structural accuracy of proteins. In the absence of chaperones, apoMb forms a mixture of native protein and soluble aggregate. We show that sufficient levels of Hsp70 grant structural accuracy to this protein. These results demonstrate that Hsp70 both enables apoMb to reach its native functional structure and protects it against harmful mutations, and we suggest that these chaperone functions may apply to other proteins with different folds.

Track: Protein Interactions and Assemblies
Session: Protein Evolution, Design and Selection

ABS# 293 | Uncovering the Peptide Binding Specificity of the TRAF6 MATH Domain Using High-throughput Binding Experiments

Jackson Halpin[1], Dustin Whitney[1], Venkat Sivaraman[1], Amy Keating[1]
[1]MIT (MA, US)

Cellular processes depend on the formation of specific protein-protein interactions, many of which are mediated

by modular peptide recognition domains that bind to short linear motifs (SLiMs) in other proteins. SLiM interactions play critical roles in pathways integral to signal transduction and cell motility, but for most SLiM-binding domains, the biochemical features that determine binding affinity and specificity are not understood. This makes it difficult to identify candidate interaction partners or design inhibitors to disrupt SLiM-mediated complexes. In this study we examined protein interactions with TRAF6, a protein involved in a wide array of signaling pathways relevant to disease. TRAF6 binds to SLiMs in partner proteins via its MATH domain. We characterized the peptide binding properties of the TRAF6 MATH domain using a semi-quantitative bacterial cell-surface screening assay called MassTitr, which generated a dataset of ~2000 peptide sequences with apparent binding affinities for TRAF6. Many of the sequences from the screen were found to bind more tightly to TRAF6 than any known ligands. We used the screening data to build position-specific scoring matrices to predict binding and scored sequences in the human proteome to identify candidate TRAF6 interaction partners. Interestingly, no likely sequences in the proteome strongly match the highest affinity sequence space identified in the screen, suggesting that natural TRAF6 binders are not optimized for affinity. Our findings suggest an avenue for designing peptide-based interaction inhibitors that are likely to outcompete most endogenous ligands.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 294 | Structural Characterization of Pulse Allergens from Pea and Lentil

Isabella Bakestani[1], Michele Loewen[2,3]
[1]Undergraduate student, Biochemistry, uOttawa, [2]Senior Research Officer, [3]Corresponding author (Quebec, Canada)

Pulses are one of the most important crops in Canada as they are a sustainable source of plant-based proteins, vitamins, minerals and phytochemicals. Due to recent concerns about food waste and sustainability, modern technologies for the processing and extraction of pulse proteins have been expanded (e.g., High Hydrostatic Processing), yet the data concerning the structural impact and application of these novel technologies is scarce. Even though pulses can be effective for reducing risk factors such as diabetes, processed pulse products commonly cause allergic reactions in susceptible individuals. Additionally, characterization of plant protein allergens is complex as there is a lack of structural data to make predictions about what constitutes an epitope. The purpose of this study is to obtain structural data for wild type pea and lentil allergens of the Cupin superfamily of proteins, in order to compare the structural data of the processed allergens to further understand the allergenicity of the pulse allergens after processing by novel technologies. The allergens were synthesized and cloned into pET28b+ and successfully expressed by IPTG induction. After two-step purification using Histidine-tag affinity chromatography and size-exclusion chromatography, the allergens were shown to be 98% pure. Final protein yields from 100 mL BL21DE3 cell cultures ranging from 7.5 to 24.5 mg were sufficient to establish crystallography trials. Preliminary crystallographic buffer screens on these allergens resulted in the formation of protein crystals and upon further buffer optimization, reproducible protein crystals were obtained for these allergens. Protein crystals will be sent for x-ray diffraction at the Canadian Light Source, to obtain and compile structural data such as 3-dimensional shape and electronic surface characteristics for these wild type proteins. The ultimate goal will be to determine the impacts of novel processing technologies on processed pulse allergens, increasing our knowledge of protein allergenicity and benefitting the Canadian agri-food industry.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 295 | Dynamics of orexin2 receptor of class A GPCRs using molecular dynamics simulations

Shun Yokoi[1], Ayori Mitsutake[1]
[1]Department of Physics, School of Science and Technology, Meiji University (Kanagawa, Japan)

Orexinergic systems which comprise two peptide ligands, orexin-A and -B, and two G protein coupled receptors (GPCRs), orexin1 receptor (OX1R) and orexin2 receptor (OX2R), are involved in regulation of sleep wake rhythm etc. Such kinds of neurological process are caused by the GPCRs activation. It's a process where the ligand binding stabilizes the receptor conformation that allows for the G protein binding and the downstream signaling. However the mechanism of the activation remains unknown.Here, we investigated the dynamics of wild-type and mutants that are in the stable inactive state or the constitutively active by performing long-time all-atom molecular dynamics simulations. We performed over twenty several microsecond MD simulations of the wild-type and the

mutants of the OX2R to extract the characteristics of the structural changes taking place in the active state.In this poster, we first show the results of the MD simulations and investigate the dynamics of the OX2R. Then, we discuss the implications in the activation of the GPCRs.

Track: Proteostasis and quality control
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 296 | Hsp40 Affinity Reveals Proteins Destabilized by Cellular Exposure to Toxicants

Joseph Genereux[1], Guy Quanrud[1]
[1]*University of California, Riverside (CA, USA)*

Oxidative and electrophilic toxicants can damage proteins and induce cellular protein misfolding stress responses. There are limited technologies to identify which proteins misfold in response to specific environmental toxicants. Chaperones, such as Hsp40s, have evolved to recognize the misfolded proteome. We have developed an approach that uses protein association with the human Hsp40 DNAJB8, determined by affinity purification and mass spectrometry, as a proxy for destabilization in the cell. This discovery stage is then followed by targeted limited proteolysis and parallel reaction monitoring as an orthogonal validation assay. We applied this approach to determine the destabilized proteome in response to brief exposure of HEK293T cells to heavy metals or electrophilic chloroacetanilide herbicides. We find that known functional targets of arsenite, such as RNA-binding proteins and components of the pyruvate dehydrogenase complex (PDC), are destabilized in response to brief (15 min.; 500 μm) cellular arsenite exposure. Interestingly, the PDC E1 subunit displays increased proteolytic sensitivity nearly everywhere except at the lipoamide binding site, the canonical target for arsenic. By contrast, cadmium or manganese exposure yields distinct fingerprints of protein destabilization, indicating that different metals have unique targets. The chloroacetanilide herbicides acetachlor, propachlor, and alachlor primarily target proteins with active site cysteines. A majority of their targets are shared, such as thymidylate synthase and acetyl-CoA acetyltransferase, but each chloroacetanilide also targets a unique proteome, suggesting that the haloacetamide warhead does not solely determine the susceptible proteome. Our results demonstrate that Hsp40 affinity profiling is an effective way to determine which proteins are targeted by toxicant exposures.

Track: Therapeutics and Antibodies
Session: Protein Evolution, Design and Selection

ABS# 297 | The high-affinity immunoglobulin receptor FcgRI potentiates HIV-1 neutralization via antibodies against the gp41 N-heptad repeat: implications for vaccine development

Maria Filsinger Interrante[1,2,3], Peter Kim[2,4,5]
[1]*Stanford Medical Scientist Training Program, Stanford University School of Medicine, Stanford, CA 94305,* [2] *Stanford ChEM-H, Stanford University, Stanford, CA 94305,* [3]*Stanford Biophysics Program, Stanford University School of Medicine, Stanford, C (CA, USA)*

The N-heptad repeat (NHR) of the HIV-1 gp41 pre-hairpin intermediate (PHI) is an attractive vaccine target with high sequence conservation across diverse strains. Inhibiting viral fusion by binding to the NHR is the mechanism of action of the FDA-approved drug enfuvirtide, and several neutralizing monoclonal antibodies that bind to this epitope have been identified and characterized. However, to date neither these monoclonals nor NHR-directed antisera have been potently neutralizing, leading to skepticism about the NHR as a vaccine target. Here, I will present our recent discovery that the expression of FcgRI (CD64) on the surface of target cells dramatically increases the neutralization potency of the NHR-directed antibody D5 and its derivative, D5_AR. The neutralizing activity of NHR-directed antisera from immunized animals is also potentiated by FcgRI, enabling neutralization of tier-2 viruses across multiple clades. FcgRI is expressed on cells that are infected during the earliest stages of HIV-1 transmission, including dendritic cells and macrophages, suggesting that these results may have important implications for vaccine development. I will also outline ongoing, unpublished efforts to improve the neutralization potency of NHR-directed antibodies by using high-resolution structural data to design and sort a yeast-display library derived from D5_AR. Taken together, this work motivates renewed interest in the gp41 NHR as a potential vaccine target towards development of a safe and effective HIV-1 vaccine.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 298 | Investigating the Interaction Between Nitrogenase and CowN

Ruchita Kharwa[1], Terrence Lee[1], Emily Wong[1], Max Strul[1], Chloe Garcia[1], Kiersten Chong[1], Michelle Jin[1]
[1]*Chapman University (California, United States)*

Nitrogen is an essential plant nutrient, but the most abundant form of nitrogen, dinitrogen gas (N2), is unreactive. Plants obtain nitrogen from a more reactive form of nitrogen, ammonia (NH3). Industrially, NH3 is produced using the Haber-Bosch process. However, this process is environmentally problematic since it results in greenhouse gas emissions. Certain bacteria are able to produce ammonia under ambient conditions without polluting the environment using an enzyme called nitrogenase. Nitrogenase is inhibited by carbon monoxide (CO). The presence of a small protein called CowN will mitigate the inhibition by CO. CowN is hypothesized to protect nitrogenase from CO through protein-protein interactions. However, interaction between CowN and nitrogenase has not been demonstrated thus far. This project aims to investigate the interaction between nitrogenase and CowN using crosslinking assays and mass spectrometry. We carried out experiments with several crosslinkers, finding that protein-protein crosslinking between CowN and nitrogenase can be detected using a diazirine-based crosslinker. The degree of crosslinking is dependent on CowN concentration and not present in a series of negative controls. We then confirmed the interaction using mass-spectrometry methods that showed that the crosslinking product indeed represents a CowN-nitrogenase crosslink. Together, this data allows us to conclude CowN and nitrogenase, in fact, do interact.

Track: Design/engineering

Session: Protein Structures Through the Lens of Machine Learning

ABS# 299 | The role of histidine near to conserved residues in the spectral shift of biliverdin-binding Slr1393g3

Priyadharshini Kannan[1], Keunwan Park[1]
[1]*Natural Products Informatics Research Center, Korea Institute of Science and Technology, Gangneung, 25451, Republic of Korea (Gangwon-do, South Korea)*

Cyanobacteriochromes (CBCRs) are linear tetrapyrrole bilin-binding photoreceptors that exhibit spectral diversity than canonical phytochromes. CBCRs require only GAF (cGMP-phosphodiesterase/adenylate cyclase/FhlA) domain for the ligation of chromophore and photoconversion. Biliverdin (BV) binding CBCRs absorbs longer wavelength than phycocyanobilin (PCB) and phycoviolobilin (PVB) binding photoreceptors. The single-domain requirement and high spectral response of CBCRs gained attention in optogenetics and bioimaging applications. Slr1393g3, the third GAF domain from Synechocystis sp. PCC 6803 CBCR shows red/green reversible photoconversion upon covalently bound PCB. A recent study used random mutagenesis to generate several far-red sensing BV-binding variants of slr1393g3. We aimed to identify the factors responsible for spectral shifts among these variants. Sequence and structure comparison showed that residue 532 has a high impact on far-red absorbance. Out of H532, I532, K532 and N532 variants, H532 shows low absorbance. Pi-Pi stacking between H532 and W496 (conserved residue among CBCRs) affect the tryptophan interaction with BV. In addition, the distance between H532 and W496 is smaller than I532/K532/N532 and W496. Histidine (H532) is not a suitable mutation because of its interaction with conserved residue. We built a random forest prediction model to classify high and low fluorescence of BV-binding variants. Our model achieved a mean AUC of 0.775 from 10-fold cross-validation. Residue497 had high feature importance for better prediction performance. All variants grouped into high fluorescence class has His, and low fluorescence class has His/Gln at 497. A further structural investigation is required to prove our prediction result of residue 497 importance in maintaining high fluorescence.

Track: Protein Interactions and Assemblies

Session: Protein Structures Through the Lens of Machine Learning

ABS# 300 | Structural Propensity in the C-terminal domain of the Albino3 translocase in thylakoids

Patience Okoto[1], Mercede Furr[1], Dustin Baucom[1], Vivek Kumar[1], Mahmoud Moradi[1], Colin Heyes[1], Ralph Henry[1], Suresh Thallapuranam[1]
[1]*University of Arkansas (AR, United States)*

The c terminus of the Albino3 (cAlb3) translocase in chloroplasts is a region fundamental to the integration of LHCPs into the thylakoid membrane in cooperation with the cpSRP. Alb3 is an integral membrane protein containing five transmembrane helices in an N-in, C-out orientation in the thylakoid membrane. The c terminus of Alb3 is responsible for recruiting the cpSRP43 subunit of cpSRP to the thylakoid membrane for the successful integration of LHCPs. This region of Alb3 has previously been described as intrinsically disordered. In more recent developments, intrinsically disordered proteins have been shown to carry out vital functions within cells. While our findings show that cAlb is predominately disordered, we have discovered a region in this protein that has a high propensity towards an ordered structure. This region was first isolated by secondary

structural sequence analysis using multiple prediction software. Single point mutations which would contribute to the displacement of the structure were made within this region, followed by structural characteristic analysis. Results derived from Far-UV-Circular Dichroism, intrinsic fluorescence, and trypsin digestion reveal that the mutations show a decrease in the overall structure. Inter-residue distance and energetics were acquired from smFRET analysis of this region, in its native state, and upon denaturation. Differences in smFRET histograms show the region is able to be unfolded with an increasing denaturant. Additionally, computational methods show a structural propensity in this region and agree with the smFRET results. These combined biophysical and computational results reveal a region of structure in the N-terminal of cAlb. The local structure found in this study may prove to be important to the binding event with the cpSRP43 subunit of cpSRP.

Track: Protein Interactions and Assemblies
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 302 | Structural and Functional Analysis of Glycyrrhizin Bound to the Scaffold Protein ZO1-PDZ1 of Tight Junctions

Emi Hibino[1], Misaki Hisada[1], Natsuko Goda[1], Takeshi Tenno[1,2], Hidekazu Hiroaki[1,2]
[1]Graduate School of Pharmaceutical Sciences, Nagoya University, [2]BeCerllBar, LLC (Aichi, Japan)

Glycyrrhizin (Grz) is a triterpene glycoside and the principal active ingredient of the medicinal herb licorice, and has been reported to showed various pharmacological activities including anti-inflammation and anti-hepatotoxicity. Grz may act as in an enhancer of epithelial tight junctions (TJs), that are intercellular adhesion complexes, by blocking the inflammatory protein HMGB1. However, other molecular targets for the wide variety of Grz's activities are still unclear. TJs are composed of multiple proteins such as claudins, occludins, and scaffold proteins ZO-1/2/3. We have previously reported that several natural compounds control the open/close of TJs. Recently, we found that Grz directly binds to the first PDZ domain of ZO-1 (ZO1-PDZ1). We measured 1H-15N Heteronuclear Single Quantum Coherence (HSQC) spectra of 15N-labeled ZO1-PDZ1 with and without 1.5 equivalents of Grz and chemical shift perturbation (CSP) was observed. We also performed transferred nuclear Overhauser effect (trNOE) experiments for Grz with ZO1-PDZ1. The distance constraints between the hydrogen atoms of Grz were obtained from the intensity of the trNOE peaks. The complex structure was calculated by the HADDOCK2.4 program using the distance constraints and CSP data. Several key interactions for the molecular recognition of Grz by ZO1-PDZ1 was found in our complex structure. We confirmed the validity of the structure by NMR measurements using structural analogues of Grz as well as ZO1-PDZ1 mutants. To evaluate the physiological effect of Grz on TJs against the epithelial cell monolayer, transepithelial electrical resistance (TEER) of the cultured Caco-2 cells exposed to Grz was assessed.We succeeded in reproduce the results reported by Sakai et al. (1999), in which high-dose Grz could prolong the TJ-opening activity of deoxycholate. Our results suggest that Grz blocks the interaction of TJs through ZO-1, and that the function of Grz depends on the condition since Grz should open the TJs without inflammatory proteins.

Track: Intrinsically Disordered Proteins
Session: Protein Evolution, Design and Selection

ABS# 303 | Membraneless Organelles by Design: The Carboxysome

Abesh Banerjee[1], Richard Booth[2], Christine Keating[2], Ronald Koder[3], Giovanna Ghirlanda[1]
[1]School of Molecular Sciences, Arizona State University, [2]Department of Chemistry, Pennsylvania State University, [3]Department of Physics, City College of New York (AZ, United States)

Membraneless organelles play many vital roles in cell homeostasis and form a discrete cellular subcompartment despite the lack of any physical barrier. This is achieved by complex coacervation whereby molecules segregate into macromolecular dense phase and dilute phase based on their charge. The objective of our study is to develop a bottom-up approach for creating functionally designed organelles using complex coacervation. We hypothesize that proteins with surface supercharging would undergo complex coacervation with a counter-charged polymer. We started with negative supercharging of cytochrome b562 (cytb562), a heme-binding metalloenzyme; we previously showed that cyt b562 catalyzes hydrogen production carbon dioxide reduction when the native heme is replaced with cobalt protoporphyrin IX (CoPPIX). In supercharged cyt b562, the electrostatic repulsion due to close packing of the surface charges leads to the destabilization of the molten globule state resulting in an intrinsically disordered protein (IDP). Our first objective was to recover the folding and function of the artificial enzyme. We show via circular dichroism and UV-visible spectroscopy that the

protein undergoes ligand-induced folding in the presence of CoPPIX and cationic allosteric activation, which is commonly observed in natural IDPs. Using turbidimetric assays and optical microscopy, we demonstrated the formation of coacervates with poly-arginine, a positively charged polymer. Gas chromatographic studies showed the efficient light-activated production of molecular hydrogen by the refolded supercharged CoPPIX-cyt b562. Our results show that the supercharging of a protein creates a viable avenue for complex coacervation with an oppositely charged polymer, without interfering with function. Our ultimate goal is to create a functional membraneless organelle analogous to a carboxysome.

Track: Therapeutics and Antibodies
Session: Protein Evolution, Design and Selection

ABS# 304 | A Universal Chimeric Antigen Receptor (CAR) Adaptor System Based on Engineered Protein GA1 and FAB Scaffolds

Edwin Arauz[1], Ainhoa Arina[2], Zachary P. Schaefer[1], Łukasz Widło[1], Yun-Min Sun[1], Maria Lecka[1], Svitlana Usatyuk[1], Ralph R. Weichselbaum[2], Anthony A. Kossiakoff[1]
[1]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois, USA, [2]Department of Radiation and Cellular Oncology and Ludwig Center for Metastasis Research, The University of Chicago, Chicago, Illinois, USA (Illinois, United States)

Chimeric antigen receptor T (CAR-T) cell therapy has shown extraordinary results for the treatment of hematological cancer. However, many patients fail to respond or relapse after treatment, suggesting additional improvements are urgently needed. Here we present a new fragment antibody binder (Fab)-chimeric-antigen receptor (CAR) pair for cancer immunotherapy applications. This new CAR-T platform is based on an engineered protein G variant (GA1) and a novel Fab scaffold (LRT) that present exquisite specificity and selectivity. We first show that GA1CAR is expressed properly on the surface of immortalized CD4 T cell line Jurkat, as well as on human CD8+T cells isolated from peripheral blood mononuclear cells. As a model system for target cell and Fab, we used engineered HEK cells expressing the bacteria maltose binding protein (MBP) on the cell surface and a conformational specific Fab binding to MBP whose interaction is disrupted in the presence of maltose. Furthermore, we tested GA1CAR-T cells with different Fab-antigen pairs on breast and ovarian cancer cell lines to show the versatility of the system. Finally, using xenograft mouse models harboring two different breast cancer cell lines, we observed that GA1CAR suppressed breast cancer tumor growth in vivo. This highly versatile platform, based on a rigid protein-protein interaction interface, will allow a plug and play strategy to test several synthetically generated Fabs for potential application in CAR-T personalized medicine to prevent escape of antigen loss variants or heterogeneous tumors.

Track: Computational Modeling/Simulation
Session: Protein Structures Through the Lens of Machine Learning

ABS# 305 | In silico analysis of structural and ligands binding properties of alkaline phosphatase from Paenibacillus sp. IPB01

Herman Umbau Lindang[1], Cahyo Budiman[1], Vijay Kumar Subbiah[1], Kenneth Francis Rodrigues[1]
[1]Biotechnology Research Institute, Universiti Malaysia Sabah, Malaysia (Sabah, Malaysia)

Bacterial Alkaline phosphatase (AP) is a metalloenzyme catalyzing the hydrolysis of phosphate bond which structurally contain a crown-like domain (CLD) functioning as the binding site to the metal ions. Nevertheless, the structure and size of crown-like domains are different among the bacterial APs. Earlier, a phosphate solubilizing bacteriaum of Paenibacillus sp. IPB01 was isolated from the tropical rainforest soil and known to harbor alkaline phosphatase (PaeniAP) gene (1,257 bp) in its genome. To date, this is the only PaeniAP reported from the tropical rainforest soil which is predicted to exhibit unique properties in its structure and function. This study aims to determine the structural and ligands binding properties of PaeniAP. Sequence analysis showed that PaeniAP is 44.6 kDa consisting of a putative signal peptide (Met1 – Arg28, 2.6 kDa) followed by the mature region (Gln29 – Lys419, 42.2kDa). Sequence alignment showed that PaeniAP has conserved catalytic sites of Ser94 and Arg158 with some putative metal binding sites at Asp51 and Glu294. Structural homology modelling revealed that this protein assumed a dimeric structure with a unique wing-loop structure that corresponds to the CLD of other bacterial APs. A metal binding site (His381) is found to be located in this loop. Further, molecular docking analysis showed that PaeniAP binds to pNPP and PO4 within its active sites with the binding energy of 5.9 kcal/mol and 2.3 kcal/mol, respectively. Interestingly, Glu294 was found to be the unique residue mediating the interaction between PaeniAP and both ligands. Further, a 100 ns-molecular dynamic simulation for indicated that the dimeric PaeniAP was more stable compared to its monomeric state. During the simulation, the

crown-like domain was found to be highly dynamic which might modulated the mechanism of metal-binding sites on bacterial APs. Structural and functional roles of CLD of this protein was then proposed.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 306 | Single-molecule insights into allosteric regulation of the nuclear receptor protein RXRα

Jakub Jungwirth[1]
[1]*Weizmann Institute of Science (Rehovot, Israel)*

Nuclear receptors are crucial transcription factors that respond to a broad range of external signaling ligands by binding cognate DNA response elements and thereby initiate transcription. As such, these proteins form tightly regulated, multi-component allosteric systems. RXRα is one of the key players in this protein family as it is involved in regulation of many genes by heterodimerization with other nuclear receptors. We employ single-molecule fluorescence techniques to investigate the conformational dynamics of RXRα's ligand binding domain (LBD)—the essential regulatory domain. Ligand binding in known to "activate" the receptor, that is to increase its affinity towards coactivator recruitment. The activation of RXRα's LBD is mediated by a conformational change of helix 12. We characterize the conformational ensemble of this helix and find an unexpected auto-repressed conformation where helix 12 occupies the coactivator binding site. This conformation remains highly populated even after ligand binding. We further investigate this unexpected mechanism of allosteric regulation. In particular, we address the questions: On what time scales do the conformational changes of helix 12 take place? And how are its dynamics affected by modulations in other parts of RXRα and in its binding partners? Our studies will pave the way to better understanding of the complex regulatory machinery embodied in nuclear receptor function.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 307 | An enzymatic approach for external decoration of an artificial protein cage

Szymon Gaweł[1], Yusuke Azuma[1], Jonathan G Heddle[1]
[1]*Małopolska Centre of Biotechnology, Jagiellonian University (Węglówka, Polska)*

Protein cages are hollow nanoparticles composed of protein self-assembly. Such proteinaceous compartments are abundant in nature, e.g. virus-like particles, but recent advance in structural biology and computational design allows us to construct synthetic equivalents using engineered protein building blocks. Such artificial protein cages possess a great potential in use of a number of applications including targeted drug delivery, vaccines, and nanoreactors. In nature, the structure of protein cages is closely related to function and adapted to the optimization of the task performed. Similarly, designed protein cages can be reengineered for specific goals. For example, protein cages surface can be decorated with molecules to target to particular tissues for delivery applications. A potential method to achieve such exterior modification of protein cages is to employ an enzymatic reaction with Sortase A, a bacterial transpeptidase that catalyses amide bond formation. In nature, this sortase can be found in most gram-positive bacteria, where their main role is to attach surface proteins to the cell wall. In this work, we demonstrated that the sortase system can be used to decorate an artificial protein cage composed of an engineered variant of the trp RNA-binding attenuation protein (TRAP) and monovalent gold ions (Malay, A.D., Miyazaki, N., Biela, A. et al. Nature 569, 438–442 (2019).

Track: Membrane Proteins
Session: Protein Evolution, Design and Selection

ABS# 308 | Intra-Helical Salt Bridge Contribution to Membrane Protein Insertion

Gerard Duart[1], John Lamb[2], Arne Elofsson[2], Ismael Mingarro[1]
[1]*Departament de Bioquímica i Biologia Molecular, Insitut Universitari de Biotecnologia i Biomedicina (BioTecMed), Universitat de València. E-46100 Burjassot, Spain.,*
[2]*Science for Life Laboratory and Department of Biochemistry and biophysics, Stockh (Valencia, Espanya)*

Salt bridges between negatively (D, E) and positively charged (K, R, H) amino acids play an important role in protein stabilization. This has a more prevalent effect in membrane proteins where polar amino acids are exposed to a very hydrophobic environment. In transmembrane (TM) helices the presence of charged residues can hinder the insertion of the helices into the membrane. This can sometimes be avoided by TM region rearrangements after insertion, but it is also possible that the formation of salt bridges could decrease the cost of membrane integration. However, the presence of intra-helical salt bridges in TM

domains and their effect on insertion has not been properly studied yet. In this work, we analyze the prevalence of charged pairs of amino acid residues in TM α-helices, which shows that potentially salt-bridge forming pairs are statistically over-represented. We then selected some candidates to experimentally determine the contribution of these electrostatic interactions to the translocon-assisted membrane insertion process. Using both in vitro and in vivo systems, we confirm the presence of intra-helical salt bridges in TM segments during biogenesis and determined that they contribute between 0.5-0.7 kcal/mol to the apparent free energy of membrane insertion (ΔGapp). Our observations suggest that salt bridge interactions can be stabilized during translocon-mediated insertion and thus could be relevant to consider for the future development of membrane protein prediction software.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 311 | Crystallographically resolved preTCR-pMHC complexes reveal the structural basis of β selection during early T cell development

Xiaolong Li[1,2,3], Réka Mizsei[1], Kemin Tan[4], Robert Mallis[1,5,6], Jonathan Duke-Cohan[1,2,3], Aoi Akitsu[1,2,3], Paul Tetteh[1,2], Abhinav Dubey[6,7], Wonmuk Hwang[8,9,10,11], Gerhard Wag
[1]Laboratory of Immunobiology, Dana-Farber Cancer Institute, Boston, MA, USA, [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA;, [3]Department of Medicine, Harvard Medical School, Boston, MA USA, [4]Structural Biology (Massachusetts, United States)

αβ T lymphocytes afford vertebrates protective immunity by recognizing surface-arrayed foreign peptides bound to self-MHC molecules (pMHC) on aberrant cells. Self- vs non-self-discrimination is programmed in the thymus via a sequential refinement process involving myriad pre-T cell receptors (preTCRs) and αβ TCRs, the former being obligate precursors of the latter. PreTCRs, in contrast to αβTCRs, were believed to be ligand autonomous. However, recent studies involving cellular activation and maturation, single-molecule optical tweezers, and solution NMR analyses revealed preTCR-pMHC interactions as crucial for developmental thymocyte expansion and progression linked to bioforce-dependent mechanosensing observed on mature αβ T cells. Here, using X-ray crystallography, we observe an unusual binding mode of preTCRβ to pMHC. While αβTCRs use six canonical CDR loops for pMHC recognition in "vertical" head-to-head binding, preTCRβ uses its twisted concave face of C"C'CFG β-sheet to "embrace" the protruding MHC α2-helix with its CC' and FG loops as a topological fit. This binding ensures that CDR3β will reach the peptide's C-terminus in the MHC groove, establishing a common diagonal docking mode polarity for all αβTCR-pMHC interactions. Nine crystallographically-defined complexes display the same preTCRβ docking mode to the pMHC centered on the α2-helix for MHC I (and α1-helix for MHC II, by analogy) with variation in interface contacts, revealing degenerate ligand recognition specificity that fosters broad pMHC sampling via preTCR CDR3β. "Horizontal" docking diminishes germline CDR1β and CDR2β loop binding. Thus, the preTCR enforces selection of diverse self-pMHC reactivities amongst β chains for retention in the TCRαβ heterodimer repertoire. PreTCR β-chain clonotype expansion at the CD4-CD8- DN thymocyte stage is distinct from positive and negative selection events involving αβTCRs at the more mature CD4+CD8+ DP stage and beyond. Our findings highlight how one subunit successively attunes to mediate recognition processes by multicomponent receptors relevant to T cell biology.

Track: Intrinsically Disordered Proteins
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 312 | Nucleocytoplasmic Transport of Intrinsically Disordered Proteins Studied by High-Speed Super-Resolution Microscopy

Samuel Junod[1], Joseph Kelich[2], Jiong Ma[3], Weidong Yang[1]
[1]Temple University, [2]The Wistar Institute, [3]Fudan University (Pa, United States)

Both natively folded and intrinsically disordered proteins (IDPs) destined for the nucleus need to transport through the nuclear pore complexes (NPCs) in eukaryotic cells. NPCs allow for passive diffusion of small folded proteins while barricading large ones, unless they are facilitated by nuclear transport receptors. However, whether nucleocytoplasmic transport of IDPs would follow these rules remains unknown. By using a high-speed super-resolution fluorescence microscopy, we have measured transport kinetics and 3D spatial locations of transport routes through native NPCs for various IDPs. Our data revealed that the rules executed for folded proteins are not well followed by the IDPs. Instead, both large and small IDPs can passively diffuse through the NPCs.

Furthermore, their diffusion efficiencies and routes are differentiated by their content ratio of charged (Ch) and hydrophobic (Hy) amino acids. A Ch/Hy-ratio mechanism was finally suggested for nucleocytoplasmic transport of IDPs.

Track: Single Molecule Studies
Session: Measuring Forces of Biological Systems

ABS# 313 | An Indestructible Tension Probe for Measuring High-Force Mechanical Events in Cells

Rachel Bender[1], Yuxin Duan[1], Anna Kellner[1,2], Brendan Deal[1], Hiroaki Ogasawara[1], Jennifer Heemstra[1], Yonggang Ke[1,2], Khalid Salaita[1,2]
[1]Emory University, [2]Georgia Institute of Technology (GA, United States)

Cells physically interact with their environment and through mechanotransduction, convert cell, integrin, and matrix interactions in their microenvironment into biochemical signaling pathways that guide downstream signaling outcomes and cell fate. Variations in mechanics caused by traction forces with the extracellular matrix have been associated with the development of skeletal muscle, embryogenesis, and metastasis, highlighting the importance of studying this relationship. DNA-based, surface-immobilized tension probes have enabled measurement of cellular forces in piconewton (pN) ranges. 1 However, the structure of DNA tension probes prevents measurement of forces above ~60 pN, and their susceptibility to nuclease degradation restricts their use beyond a few hours. 2,3 To address these limitations, we have developed peptide nucleic acid (PNA) tension probes. PNA is a nucleic acid analogue that can form conventional Watson-Crick-Franklin base pairings with other nucleic acids. It is a highly attractive as a probe for molecular forces due to its superior thermodynamic stability and resistance to degradation by all known enzymes. We have characterized the thermodynamic properties of a library of PNA probes and quantified their biostability in the presence of nucleases and proteases. We have also demonstrated higher mechanical stability of PNA-based tension probes and have successfully used our probes to image the traction forces of NIH 3T3, Human Airway Smooth Muscle (HASM), and MDA-MB-231 cell lines. References [1] Liu, Y., Galior, K., Ma, V., Salaita, K. Acc. Chem. Res., 50, 12, 2915-2924 (2017) [2] Paluch, E.K., Nelson, C.M., Biais, N. et al. BMC Biol 13, 47 (2015) [3] Chang, Y., Liu, Z., Zhang, Y., et al. J. Am. Chem. Soc., 138, 9, 2901–2904 (2016)

Track: Computational Modeling/Simulation
Session: Protein Structures Through the Lens of Machine Learning

ABS# 315 | Virtual screening of 3.7 billion molecules against SARS-COV-2 targets

Amitava Roy[1], Vishwesh Venkatraman[2], Thomas Colligan[3], Conner Copeland[3], Jeremiah Gaiser[3], Daniel Olson[3], Travis Wheeler[3]
[1]National Institutes of Health, [2]Norwegian University of Science and Technology, [3]University of Montana (MT, USA)

The deadly proliferation of COVID-19 has motivated extensive efforts in the development of therapeutic drugs. Virtual screening of small molecules can expedite the initial phase of drug screening, reducing costs and speeding the discovery process. Here, we report on a project to virtually screen ~3.7 billion small molecules against three SARS-CoV-2 protein targets. To facilitate accurate screening of a massive number of small molecules over a short period, we developed a heuristic search pipeline. The pipeline began with the identification of pockets and possible glycosylation sites in protein targets. Having identified relevant binding pockets, we designed multiple de novo ligands for each pocket and used them as seeds to identify similar molecules from among the full set of candidates ligands. This resulted in a collection of approximately 1.1 million synthetically accessible compounds. These candidates were tested for biological activity using rigid-body docking, and new neural network analysis was performed to predict binding affinity based on predicted docking poses. This machine-learning scoring model was trained and validated using target decoys and actives in the DUD-E as the training set and Lit-PCBA as the test set, resulting in a predicted accuracy that is superior to existing scoring schemes. Finally, we developed an ADMET filter to identify and remove potentially toxic compounds, then ranked the remaining ligands using the machine learning scoring model.

Track: Proteins in Cells
Session: Targeted Protein Degradation

ABS# 316 | Fibrinogen Synthesis by Tumor Cells Predetermines the Type of Tumor

Mariya Konovalova[1], Alexander Generalov[1], Dmitrii Aronov[1], Sergey Akopov[1], Elena Svirshchevskaya[1]
[1]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry (Moscow, Russian Federation)

Mortality from cancer depends on the type of tumor and disease stage. One year survival at early diagnosed pancreatic and colorectal cancers is around 22 and 90% accordingly.The aim of this work was to compare the role of fibrinogen and inflammation cytokines in tumor growth.To this end stable GFP expressing colorectal CT26-eGFP and pancreatic Pan02-eGFP cell lines were generated and s.c. inoculated into genetically compatible BALB/c or C57BL/6 mice accordingly. GFP fluorescence in tumors and parental cell lines was estimated by flow cytometry and confocal microscopy. Fibrinogen a, b, g, IL-6, 10, 25, and 33 gene expression was studied by qPCR.CT26-eGFP induced tumors were characterized by high unlimited growth in vivo quickly producing large soft tumors while Pan02-eGFP tumors were small, fibrotic and grow slowly. Analysis of fluorescent cells in the tumors showed that all Pan02-eGFP cells almost completely lost GFP expression while were able to grow in vitro after tumor homogenates transfer. Contrary to it CT26-eGFP preserved GFP expression on the basic level. Fibrinogen a, b, g expression was 91, 30, and 67 times accordingly higher in Pan02-eGFP in comparison with CT26-eGFP cell lines and 18, 8, and 12 times higher in the corresponding tumors. Besides, gene expression of all cytokines studied were 5-10 times higher both in Pan02-eGFP cells and tumors than in CT26-eGFP ones. This means that high production of fibrinogen results in fibrin accumulation, limitation of the space for tumor cell growth, and, as a result, a higher mutation of tumor cells. Possibly fibrinolytic drugs can be used to ameliorate the aggressiveness of pancreatic tumors by this mean decreasing mortality. This work was supported by RFBR grant 20-04-00521

Track: Enzymology
Session: Protein Evolution, Design and Selection

ABS# 317 | Catalysis in three dimensions: a study of conformational heterogeneity in enzyme active sites

Ioannis Riziotis[1], Antonio Ribeiro[1], Janet Thornton[1]
[1]EMBL-EBI, Wellcome Genome Campus, Cambridge, UK (UK, UK)

The "structure determines function" notion is almost dogmatic in the protein world and especially in the case of enzymes, whose catalytic potential is largely dependent on active site 3D shape. Although active sites are essentially conserved in sequence and structure in similar enzymes, geometrical variation is observable. Evolutionary unrelated enzymes can converge towards similar functions with their active sites adopting similar geometries, while the outer shell of the protein is freer to vary. Our focus is to characterize active site conformational variation within a single enzyme (flexibility) or within homologous enzymes (plasticity), and explore cases of convergent evolution and functional promiscuity. Using enzyme structures from the PDB and catalytic residue annotations for ~900 functionally diverse enzymes extracted from the Mechanism and Catalytic Site Atlas (M-CSA), we perform a systematic structural analysis in evolutionary related enzymes to assess the factors that drive changes in active site geometry. We demonstrate that the correlation between overall sequence similarity and structure similarity tends to be lower in the active site than in the whole protein, since the active site is more disrupted by ligand binding. Furthermore, dissimilar enzymes can share similar active sites. In highly sequence-similar enzymes, substrate and cofactor binding seem to be critical drivers of active site conformational transitions, with the magnitude of these transitions varying among different homologous enzyme families and being highly dependent on the type of ligands binding.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 318 | Icosahedral 60-meric Porous Structure of Designed Protein Nanoparticle TIP60

Ryoichi Arai[1], Norifumi Kawakami[2], Junya Obata[1], Akihisa Tsutsumi[3], Kenji Miyamoto[2], Masahide Kikkawa[3]
[1]Shinshu University, [2]Keio University, [3]University of Tokyo (Nagano, Japan)

Protein nanoparticles have potential in a broad range of applications. Recently, we designed and constructed a uniform hollow protein nanoparticle (molecular mass: ~1 MDa), TIP60 (truncated icosahedral protein composed of 60-mer fusion proteins), self-assembled from a designed fusion protein of a pentameric Sm-like protein and a dimeric MyoX-coil domain based on geometric symmetry (N. Kawakami et al., Angew. Chem. Int. Ed. 57, 12400-12404, 2018). Here, we report the atomic structure of TIP60 solved using single-particle cryo-electron microscopy at 3.3 Å resolution. The TIP60 structure has an icosahedral 60-meric architecture with 20 regular-triangle-like pores. The linker between the two domains is part of a straight and

continuous α-helix from the C-terminal of the Sm-like domain to the first helix of the MyoX-coil domain, suggesting that the straight and rigid structure of the helical linker contributes to the stability and homogeneity of the icosahedral 60-meric structure of the TIP60 nanoparticle. TIP60 and its variants have an array of modifiable target sites on the exterior and interior surfaces. The atomic structure and architecture of TIP60 could be useful for designing biomedical and biochemical nanoparticles for a broad range of future applications, including drug delivery and molecular filtration systems.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 320 | The Role of FlgM in Facilitating Protein Export via the Type III Secretion System

Samuel Fredericksen[1], Matthew Gage[1]
[1]*University of Massachusetts, Lowell (Massachusetts, United States)*

The type III secretion system (T3SS) is a complex molecular apparatus composed of over thirty unique proteins. The main purpose of this structure is to facilitate the export of flagellar proteins from the cytoplasm during the construction of the flagella. Flagellar synthesis is a tightly controlled process, regulated by a "master operon" and a series of class two and three promoters which become activated as the synthesis progresses. Of particular note is the relationship between the flagellar-specific sigma factor (σ28) and its antisigma factor FlgM. Prior to the completion of the hook-basal body (HBB) portion of the flagellar apparatus, σ28 is bound to and inhibited by FlgM. Once the HBB structure is complete, however, the T3SS undergoes a specificity switch and begins to secrete FlgM. We hypothesized that this system might be able to act as a secretion system for expressing recombinant proteins in E. coli. This hypothesis was tested using a FlgM-GFP hybrid to facilitate easy tracking of secreted protein. Our initial results have shown that the FlgM-eGFP hybrid is consistently secreted at amounts equivalent with native FlgM, indicating that the export pathway is available to these recombinant proteins. We have also been able to observe increasing amounts of secreted protein with increasing culture duration, up to 60 hours after induction. This indicates that FlgM is continuously secreted for a prolonged period of time once expression is initiated. This was unexpected, as it has been previously theorized that the rate of FlgM secretion would decrease as a function of time, due to increasing resistance from flagellar proteins forming at the end of the HBB structure. Further testing is ongoing to assess the level of flagellar development of the E. coli in induced cultures to develop additional insights into this process.

Track: Structure (X-Ray/NMR/EM)
Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 323 | Visualizing Molecular Machines in Motion Using Cryo-electron Microscopy and Deep Learning

Joseph Davis[1], Laurel Kinman[1], Ellen Zhong[1], Jingyu Sun[2], Joaquin Ortega[2]
[1]*Massachusetts Institute of Technology,* [2]*McGill University (Massachusetts, United States)*

Macromolecular machines such as the ribosome undergo massive structural changes as they assemble. While we have long appreciated such structural changes exist, experimentally visualizing and analyzing large ensembles of these structures is challenging. Here, I briefly describe cryoDRGN, our newly developed software package to analyze structural heterogeneity in protein complexes visualized by cryo-electron microscopy (cryo-EM). This approach, which uses a purpose-built neural network based on a variational autoencoder, maps individual particle images to a low-dimensional latent space. Direct inspection of this latent space provides insights into the degree of structural heterogeneity in the dataset, provides estimates of particle abundance in each state, and relates the observed states to one another. Users can then visualize structural ensembles of their protein complex by generating three-dimensional structures throughout this latent space using the trained networks. Further, I describe unpublished methods we have recently developed to analyze and interpret the resulting structural ensembles, which include 100s-1,000s of related 3D density maps. Finally, we apply these analysis methods to understand how a bacterial methyltransferase guides assembly of the small ribosomal subunit. In analyzing a series of related cryo-EM datasets, we surprisingly uncovered that this methyltransferase 'proof-reads' the assembling ribosomes by preferentially disassembling ribosomes that have been erroneously constructed. This unexpected insight into the bacterial ribosome biogenesis process was enabled by cryoDRGN and our new

structural ensemble analysis methods, and it highlights the utility of these approaches.

Track: Protein Interactions and Assemblies
Session: Protein Evolution, Design and Selection

ABS# 324 | The Role of Prion-like Domains in Transcriptional Activation by Oncogenic FET-Fusion Transcription Factors

Richoo Davis[1], Priya Banerjee[1]
[1]*University at Buffalo (New York, United States)*

Fusion transcription factors generated by genomic translocations are common drivers of oncogenicity. Oncofusions of the FET (FUS, EWSR1, and TAF15) family involve the prion-like domain (PLD) of FET proteins fused to the DNA-binding domain (DBD) of transcription factors and are the sole cause of cancers in certain sarcomas and leukemias. However, the exact mechanism of aberrant transcriptional reprogramming by FET-fusion proteins is far from understood. We use FUS-DDIT3, a FET oncofusion protein, as a model to study its biophysical properties and the molecular basis for its oncogenicity. We hypothesize that in FET fusion proteins, the association of a PLD with a DBD results in novel functionalities distinct from their parent proteins. The PLDs are multivalent disordered polypeptides with low complexity sequences and can mediate liquid-liquid phase separation of FET and other ribonucleoproteins (RNPs). The DBD, on the other hand, can recruit the fusion transcription factor to specific genomic loci and activate transcription.We test this model utilizing an integrative experimental approach encompassing in vitro biophysical methods, confocal microscopy, and cell culture models. We find that FUS-DDIT3 undergoes liquid phase separation in the nucleus, which is mediated by its PLD. Unlike FUS which shows an RNA-dependent reentrant phase behavior, FUS-DDIT3 condensates are not influenced by nuclear RNA. Nuclear FUS-DDIT3 condensates can enrich essential components of the global transcriptional machinery such as the chromatin remodeler, SWI/SNF. The recruitment of SWI/SNF is driven by heterotypic PLD-PLD interactions between FUS-DDIT3 and core subunits of the SWI/SNF complex. Our results suggest that both partners in the fusion pair (i.e., FUS and DDIT3) contribute to the neomorphic activity of the fusion-protein – the DBDDDIT3 recruit the fusion transcription factor to specific genomic loci, whereas the PLDFUS drives their condensation and enrich chromatin remodelers to activate transcription by ectopic chromatin remodeling.

Track: Intrinsically Disordered Proteins
Session: Protein Evolution, Design and Selection

ABS# 326 | Protein Demixing and the Emergence of Multi-phasic Condensates with Tunable Pattern

Taranpreet Kaur[1], Muralikrishna Raju[2], Ibraheem Alshareedah[1], Richoo B. Davis[1], Davit A. Potoyan[2], Priya R. Banerjee[1]
[1]*Department of Physics, University at Buffalo, Buffalo NY 14260, USA,* [2]*Department of Chemistry, Iowa State University, Ames IA 50011, USA (NY, United States)*

Intracellular biomolecular condensates are complex fluids formed via liquid-liquid phase separation of proteins and nucleic acids. The sequence feature of such scaffold proteins is a critical determinant for the formation and stability of phase-separated condensates. Intriguingly, biomolecular condensates can co-exist without mixing in vivo with variable patterning of co-existing phases in space ranging from phases nested within each other to phases with negligible contact with each other. We hypothesize that such behavior is inherent to multi-component systems with overlapping molecular interactions. To test this hypothesis, we use a minimal three-component system composed of two intrinsically disordered polypeptides (IDPs): prion-like proteins (PLP) and arginine-rich proteins (RRP), and observe that our ternary system can readily form stable biphasic condensates, homotypic PLP condensates, and heterotypic RRP-RNA condensates. Strikingly, PLP and RRP form homogeneous co-condensates in absence of RNA but undergo a spontaneous demixing transition when RNA is introduced to the mixture. Further experiments and molecular dynamics (MD) simulation reveal that the spatial arrangement of the two condensate types can be tuned via RNA-to-protein ratio as well the sequence features of PLP and RRP. Mechanistically, we argue patterning of coexisting condensates is determined by interactions between molecules at the liquid-liquid interface between the condensates. Tuning these microscopic interactions by mixture stoichiometry and protein primary sequences allowed us to predictively control the mesoscopic patterning of the co-existing condensates in our minimal ternary system, giving rise to a broad range of patterns such as totally-engulfed, partially-engulfed, completely-detached, and Janus-like condensates.

Track: Therapeutics and Antibodies
Session: Protein Evolution, Design and Selection

ABS# 328 | The conserved α-helical face of the HIV-1 gp41 C-heptad repeat is a target for neutralizing antibodies

Benjamin Bell[1], Peter Kim[1]
[1]*Stanford University School of Medicine (CA, United States)*

HIV-1 infection requires viral and cell membrane fusion, which relies on the gp41 N-heptad repeat (NHR) and C-heptad repeat (CHR) of the viral glycoprotein Env. When the CHR adopts an α-helical conformation, one face is composed of highly conserved residues and is a validated target for HIV-1 inhibition, suggesting that this conserved α-helical face might be an effective vaccine target. In order to evaluate this potential vaccine target, we used yeast surface display to isolate two novel antibodies A24.3 and A25.2 that target this epitope. Surprisingly, the X-ray crystal structure of A24.3 Fab indicates that CHR binding is concomitant with displacement of the variable light (VL) domain and mediated by germline-conserved framework residues on the variable heavy (VH) domain. This unique binding mode leads to substantial differences in CHR affinity between antibody formats (VH, scFv, Fab, full-length IgG). Nevertheless, these antibodies show viral neutralization activity in an Env-pseudotyped lentiviral assay, with A25.2 VH inhibiting diverse strains across multiple HIV-1 clades. Further, we observed an unusual time-dependent enhancement of neutralization activity for full-length IgG antibody constructs with implications for CHR-targeting vaccine approaches. Taken together, these studies support the utility of the gp41 CHR as a highly conserved target for neutralizing antibodies and potential vaccine approaches.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 329 | Structural Basis for Plazomicin Antibiotic Action and Resistance

Tolou Golkar[1], Angelia Bassenden[1], Albert Berghuis[1], Dev P. Arya[2], T. Martin Schmeing[1]
[1]*McGill University,* [2]*Clemson University (Quebec, Canada)*

The approval of plazomicin broadened the clinical library of aminoglycosides available for use against emerging bacterial pathogens. Contrarily to other aminoglycosides, resistance to plazomicin is limited; still, instances of resistance have been reported in clinical settings. Here, we present structural insights into the mechanism of plazomicin action and the mechanisms of clinical resistance. The structural data reveal that plazomicin exclusively binds to the 16S ribosomal A site, where it likely interferes with the fidelity of mRNA translation. The unique extensions to the core aminoglycoside scaffold incorporated into the structure of plazomicin do not interfere with ribosome binding, which is analogously seen in the binding of this antibiotic to the AAC(2′)-Ia resistance enzyme. The data provides a structural rationale for resistance conferred by drug acetylation and ribosome methylation, i.e., the two mechanisms of resistance observed clinically. Finally, the crystal structures of plazomicin in complex with both its target and the clinically relevant resistance factor provide a roadmap for next-generation drug development that aims to ameliorate the impact of antibiotic resistance.

Track: Computational Modeling/Simulation
Session: Protein Structures Through the Lens of Machine Learning

ABS# 331 | Beyond Fingerprints: Improved Representation of Chemical Compounds

Jeremiah Gaiser[1], Daniel Olson[2], Thomas Colligan[2], Amitava Roy[3], Vishwesh Venkatraman[4], Travis Wheeler[2]
[1]*Department of Biochemistry, University of Montana, Missoula, MT 59801, USA,* [2]*Department of Computer Science, University of Montana, Missoula, MT 59801, USA,* [3]*Bioinformatics and Computational Biosciences Branch, Office of Cyber Infrastructure a (MT, US)*

Computational drug screening requires meaningful representations of proteins and candidate small molecule ligands. One common mechanism for representing small molecules is with molecular "fingerprints", such the ECFP4 bit vector representation. Ideally, such a representation is useful in support of various aspects of drug screening, such as identification of collections of molecules with similar structural and chemical properties; however, current fingerprinting strategies are at best marginally useful in this context. We have developed a mechanism for embedding small molecules in a semantic space that better captures features relevant to drug screening applications, and demonstrate the value of this embedding method in exploring molecular neighborhoods, and also as input to methods for predicting protein-ligand binding affinity.

Track: Chaperones
Session: Targeted Protein Degradation

ABS# 332 | Restoring Chaperone-Client-Chaperone network function is key to Limb-Girdle Muscular Dystrophy Type D1 therapeutic interventions

ANKAN BHADRA[1], Conrad C Weihl[1], Heather True[1]
[1]*Washington University School of Medicine in Saint Louis (MO, United States)*

Limb-girdle muscular dystrophy type D1 (LGMDD1) is a degenerative muscle disease that is caused by the mutation of HSP40 chaperone DNAJB6. DNAJB6 participates in protein folding and disaggregation, however, its role in skeletal muscle protein homeostasis is unknown. DNAJB6 has homology to the yeast DNAJ family member, Sis1, which has known prion protein clients. As DNAJB6 clients have yet to be identified, the yeast system is enabling us to understand the underlying mechanistic basis of disease. Previous studies from our lab suggest that the LGMDD1 mutants in Sis1 not only show substrate but also conformation-specific effects. HSP40s are universal partners for HSP70s, and together they constitute one of the most prolific chaperone systems (HSP70/HSP40 ATPase cycle) across all kingdoms of life. Therefore, we hypothesized that modulating HSP70/HSP40 interactions could rescue the phenotype and potentially provide a therapeutic target for LGMDD1. Our data suggest that the deleterious effect of the G/F domain of DNAJB6 mutants is HSP70-dependent. We found that reducing the binding of HSP70 to HSP40 with the incorporation of a second-site mutation in HSP70 rescued the phenotype of the disease-associated LGMDD1 mutants in yeast. Additionally, inhibiting the interaction of DNAJB6 with HSP70, both genetically or pharmacologically, partially restores HSP70 mobility and rescues muscle phenotypes in mice. Our in vitro aggregation assays (using prion substrate RNQ1) show kinetic differences in the lagtime between the wild type (WT) and mutants. However, we found that all of the mutants are defective in dimerization as well as binding to both substrates and HSP70 (Ssa1). We observed significant delays in the HSP70/40 ATPase cycle with the LGMDD1-associated mutants. Notably, the LGMDD1-associated mutants also poison the WT protein when combined in our assays, which provides some insight into this inherited disease. Together, these data provide crucial insights for future therapeutic options with respect to LGMDD1.

Track: Membrane Proteins
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 333 | Characterization of Folding Stages of TolC, an Outer-Membrane Component of Antibiotic Efflux

Ayotunde Ikujuni[1], Jimmy Budiardjo[2], Emre Firlar[3], Andrés Cordova[1], Jason Kaelber[3], Joanna Slusky[1,2]
[1]*Molecular Biosciences Department, University of Kansas ,* [2]*Centre of Computational Biology, University of Kansas,* [3]*Rutgers New Jersey CryoEM/CryoET Core Facility and Institute for Quantitative Biomedicine,The State University of New Jersey (Kansas, United States)*

Overexpression of tripartite efflux pump systems in gram-negative bacteria has been reported to be linearly correlated with antibiotic resistance in clinical isolates as well as in selected mutants of different bacterial pathogens. The outer membrane subunit of the efflux pump, TolC, is difficult to produce at a sufficient yield for biochemical characterization. We have developed a method of refolding by which TolC remains folded in SDS-PAGE, retains binding to an endogenous ligand, and recapitulates the known crystal structure by single particle cryoEM analysis. Using circular dichroism, thermal denaturation, and proteinase K digestion , we characterized the folding stages of TolC including periplasmic intermediate. We anticipate that our findings will help in developing better efflux pumps inhibitors.

Track: Bioinformatics
Session: Protein Structures Through the Lens of Machine Learning

ABS# 335 | ProTeSSA: A New Secondary Structure Assignment Method

Ford Combs[1], Iosif Vaisman[1]
[1]*George Mason University (Virginia, United States)*

In protein secondary structure assignment (SSA), the three-dimensional structure of a protein is evaluated and each residue is labeled as helix, strand, or coil. Secondary structure regions, which are characterized by a distinct thermodynamic stability and evolutionary conservation, are critical components of a protein's overall structure and function. For this reason, secondary structure plays a significant role in protein classification schemes,

homology modeling, and structure comparison; it is also used to train secondary structure prediction methods, which model secondary structure based on the amino acid sequence alone. The task of SSA is difficult because helices and strands in proteins rarely conform to their theoretical ideals. Most existing SSA methods rely on parameters, such as hydrogen-bond patterns or interatomic distances with arbitrary cutoffs. As a result, various SSA methods generate substantially differing assignments. ProTeSSA (Protein Tessellation-based Secondary Structure Assignment) is a new method that does not require parameters. It is based on the Delaunay tessellation (DT) of a protein's C-alpha coordinates. The DT of a protein is a simplicial complex, where each residue is a member of a set of tetrahedra each forming a group of four natural neighbors. This topological data is then mined to generate a descriptor for each residue, in part using a novel application of persistent homology. Residue-based models were trained on this data and tested on a test set of proteins, that was kept separate from training. The ProTeSSA models achieved greater than 85% accuracy on the residue level, using the protein structure author(s)'s assignments as ground truth, and low misclassification between helices and strands, less than 1 per test protein. The success of ProTeSSA indicates the potential to shift from parameter-based methods to an objective and consistent SSA method that relies solely on protein topology rather than empirical energy and geometry thresholds.

Track: Single Molecule Studies
Session: Protein Evolution, Design and Selection

ABS# 338 | Developing protein tools for single-molecule protein sequencing

Nicholas Callahan[1], Jennifer Tullman[1], Zvi Kelman[1], John Marino[1]
[1]*IBBR (MD, United States)*

One of the central challenges in the development of single-molecule protein sequencing technologies is achieving high-fidelity, sequential recognition and detection of specific amino acids in a peptide. An approach towards achieving this goal is to leverage naturally occurring proteins that function through recognition of amino (N)-terminal amino acids (NAAs). One such protein, the N-end rule pathway adaptor protein ClpS, natively recognizes NAAs on a peptide chain. The native ClpS protein has a high specificity, albeit modest affinity, for the amino acid Phe at the N-terminus but also recognizes the residues Trp, Tyr, and Leu at the N-terminal position. Directed evolution methods were employed to select for ClpS variants with enhanced affinity and selectivity for two NAAs (Phe and Trp). One of the variants was further engineered for attributes necessary for robust application as a biotechnology reagent such as increased thermostability and improved selectivity under different buffer conditions. The stabilized variant was further characterized in terms of selectivity for the N-terminal residue in the context of different peptides with varying residues at position 2 (P2). The implication of the study for the use of ClpS-based reagents in peptide sequencing technologies will be discussed.

Track: Proteostasis and quality control
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 341 | High-throughput screening of sequences that promote proteolysis in bacteria

Patrick Beardslee[1], Priyanka Bheemreddy[1], Karl Schmitz[1]
[1]*University of Delaware (Delaware, United States)*

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 preliminary NGS data from pilot screening experiments. The information gathered from this method we ultimately help us understand the roles that ATP-dependent proteases play in individual pathogenic bacteria.

Track: Membrane Proteins

Session: Protein Evolution, Design and Selection

ABS# 342 | Amino Acid Determinants of Accessory Protein Requirement in Helminth Nicotinic Acetylcholine Receptors

Jennifer Noonan[1], Robin Beech[1]
[1]*McGill University (Quebec, Canada)*

Pentameric ligand-gated ion channels are the targets of many anthelmintic drugs. The expression of these channels is complex and involves accessory proteins at every step of the synthesis pathway. This receptor gene family has expanded in the nematodes, providing us with the opportunity to study specific examples of ion channel functional change while at the same time characterizing potential new targets for anthelmintics. We have found that the nicotinic acetylcholine receptor (nAChR) of D. medinensis ACR-16 does not require the RIC-3 accessory protein for functional expression, whereas the closely related A. suum ACR-16 does. All other characterized ACR-16 receptors also require RIC-3. This means that specific changes occurred in the D. medinensis ACR-16 sequence that regulate its requirement for accessory proteins. It is not known what sequences determine nematode nAChR accessory protein requirement and the objective of this study was to identify this. Using a series of chimeras between A. suum and D. medinensis ACR-16, followed by functional characterization with two-electrode voltage-clamp electrophysiology, we found that three structural regions mediate RIC-3 requirement: the intracellular loop, the transmembrane domain regions and the short C-terminal tail. The majority of the accessory protein requirement is determined by only three amino acids in the transmembrane domain regions and one amino acid in the tail. These residues mapped onto homology models reveals that they are on the outside of the fourth transmembrane helix. These sites may help predict which helminth receptors would need RIC-3 for their expression using heterologous expression systems and help further our understanding of membrane receptor synthesis and functional evolutionary change.

Track: Synthetic Biology

Session: Designer Proteins Through Genetic Code Expansion

ABS# 343 | Identifying Yeast Gene Deletions that Enhance Noncanonical Amino Acid Incorporation

Matthew Zackin[1], Jessica Stieglitz[1], James Van Deventer[1,2]
[1]*Department of Chemical and Biological Engineering, Tufts University,* [2]*Department of Biomedical Engineering, Tufts University (MA, United States)*

Efficient incorporation of noncanonical amino acids (ncAAs) in proteins is a prerequisite for leveraging their chemical diversity in the engineering of proteins with expanded capabilities. Potential applications for ncAAs span from engineering biological therapeutics with chemistries beyond those found in canonical amino acids to expanding our fundamental understanding of protein biosynthesis. Efforts to improve ncAA incorporation have focused on engineering orthogonal translation systems (OTSs): the translation machinery introduced into cells that allows for the encoding of ncAAs in proteins. However, the efficacy of these efforts is limited by complex cellular factors that inhibit genetic encoding of ncAAs at repurposed stop codons. Identification of these cellular factors is necessary to engineer cells that better accommodate expanded genetic codes. In this work, we performed a genome-wide screen of Saccharomyces cerevisiae using a pooled yeast knockout (YKO) collection to identify single-gene deletions that enhance ncAA incorporation efficiency and fidelity. We utilized dual-fluorescent reporter systems that facilitated fluorescence-activated cell sorting (FACS) of the YKO collection to isolate deletion strains with increased ncAA incorporation efficiency. Our early screens identified several unique deletions that appear to exhibit improved stop codon suppression and ncAA incorporation. Identified genes are involved in diverse cellular activities, and genes with no previously characterized function have also been identified. Current efforts focus on further validating these deletions, including verifying that these single-gene deletions enhance ncAA incorporation efficiency in other strains, and quantitatively evaluating ncAA incorporation efficiency and fidelity. Future work will interrogate these single-gene deletion strains to elucidate how they affect ncAA incorporation for applications such as click chemistry. To our knowledge, this work is the first genome-wide investigation of factors that influence ncAA incorporation. Understanding the cellular processes that dictate stop codon suppression and ncAA incorporation are

imperative to augmenting genetic code manipulation strategies for a multitude of applications.

Track: Computational Modeling/Simulation
Session: Protein Structures Through the Lens of Machine Learning

ABS# 345 | Experimental validation of predicted protein structures by Small Angle X-ray Scattering

Susan Tsutakawa[1], Naga Babu Chinnam[2], Aleem Syed[2], John Tainer[2], Greg Hura[1]
[1]*LBNL,* [2]*MD Anderson (CA, US)*

Promising to revolutionize the study of biology and medicine, an artificial intelligence algorithm in 2020 made an astounding breakthrough in solving protein folding problem. The Deepmind AlphaFold algorithm accurately predicted structures for 90% of single chain protein structures, as assessed by Critical Assessment of protein Structure Prediction (CASP) 14. It is likely when these more reliable protein structure predictions become available, scientists will begin to use them. However, there are limitations even for AlphaFold with regard to certain proteins that are difficult or currently impossible to predict with high reliability and accuracy--multimeric, multi-domain, or flexible. SAXS can fill this gap by providing experimental validation and information on conformations occurring in solution. We tested this hypothesis using the AlphaFold model predicted for our protein in the last CASP14 in 2020 and comparing it directly to our SAXS data for the same protein. AlphaFold accurately predicted the Ca backbone, including the relative orientation of the two domains, but flexibility of regions that AlphaFold had predicted structured but scored as low confidence were needed to match the experimental data. This analysis both shows the power of AlphaFold structure prediction, but also its current limitations and the need for efficient, experimental validation.

Track: Protein Interactions and Assemblies
Session: Protein Evolution, Design and Selection

ABS# 346 | Persistent Protein Interactions Reveal the Advantage of Symmetry in Stability

John Bedford[1], Jennifer Poutsma[1], Norou Diawara[1], Lesley Greene[1]
[1]*Old Dominion University (Virginia, United States)*

An investigation into the key determinants of structural stability at the level of individual interactions was performed using two proteins within the β-grasp superfamily, the B1-domain of protein G (GB1) and the small archaeal modifier protein 1 (SAMP1). These proteins are both symmetrical and contain a β-sheet, comprised of two β-hairpins, flanked by a central α-helix. The detailed behavior of each long-range interaction within the proteins was characterized by subjecting each protein to high temperature molecular dynamics simulations. It was found that the most stable region in GB1 was its C-terminal hairpin and in SAMP1 it was the opposite, its N-terminal hairpin. Experimental results for GB1 support this finding. It appears that that the differential stability between GB1 and SAMP1 is dictated by the location and number of hydrophobic long-range interactions, which is accommodated in the β-grasp fold due to structural symmetry. Consequently, the hairpins are interchangeable which lends itself to adaptability and flexibility in nature.

Track: Enzymology
Session: Protein Evolution, Design and Selection

ABS# 347 | How YqeK cleaves Ap4A to regulate cellular response to environmental stresses

Chie Ueda[1], Natalie Chin[1], Maria-Eirini Pandelia[1]
[1]*Brandeis University (Massachusetts, United States)*

YqeK is an HD-domain metalloprotein that acts as a symmetrical diadenosine tetraphosphate (Ap4A) hydrolase. Ap4A is a metabolite involved in cellular stress signaling in both prokaryotes and eukaryotes. Under conditions of stress, including oxidative, thermal and antibiotic stress, Ap4A accumulates in the cell, and regulates mRNA stability by Ap4A incorporation into the 5' cap structure. Ap4A is constitutively synthesized by the non-canonical activities of Aminoacyl-tRNA synthetases and degraded by various hydrolases via asymmetrical or symmetrical cleavage of the phosphates. Symmetrical cleavage of Ap4A in bacteria is performed by ApaH hydrolases or the newly identified YqeK hydrolases. A sequence similarity network analysis of YqeK and ApaH sequences showed their genomic co-occurrence is mutually exclusive; Gram-negative bacteria use ApaH, whereas YqeK is found mainly in Gram-positive bacteria. We also find from a phylogenetic analysis that YqeK sequences segregate into clades of anaerobic and aerobic bacteria. We selected two representative YqeKs from Bacillus halodurans, a facultative anaerobe, and Clostridium acetobutylicum, an obligate anaerobe, for which the crystal structures were solved by the Joint Center of Structural Genomics. Both structures show coordination of a diiron cofactor; however, the role of iron in activity

remains unknown. In the present study we examined a) the relevance of Fe in catalytic activity, b) the redox state of the active diiron cofactor, i.e. diferric (Fe3+-Fe3+), mixed-valent (Fe2+-Fe3+), and diferrous (Fe2+-Fe2+), c) and identify residues near the active site involved in substrate specificity and catalysis. We demonstrate that Fe is a key element in catalysis, and that the diiron cofactors of B. halodurans and C. acetobutylicum possess different redox properties and thus different expected O2-sensitivity, which could serve as a potential mechanism by which the organism determines its level of oxygen tolerance.

Track: Structure (X-Ray/NMR/EM)

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 348 | GTP Allosterically Drives Polymerization of Dynamin-Related Protein 1 Via A Novel Interface

Paul Thomas[1], Loan Doan[1], Arthur Melo[1], Adam Frost[1]
[1]University of California, San Francisco (CA, United States)

Fission dynamin proteins are large GTPases that polymerize helically to constrict and divide membranes. Their association with membranes and their propensity to polymerize have challenged efforts to understand the mechanism of their action. Specifically, the mechano-chemical details of how nucleotide binding, hydrolysis, and release are coupled to dynamin polymerization and membrane fission remain poorly understood. Although dramatic structural differences have been observed for dynamin polymers in different nucleotide states, only one polymerization interface between GTPase domains on separate turns (trans) of a dynamin helix has been shown to be orthosterically nucleotide-dependent and understood structurally. To further probe how nucleotide state affects the polymers conformation we inhibited this polymer interface. This favored the assembly of "1 turn" rings of Dynamin-Related Protein 1 (DRP1), but only in the GTP state. The cryo-EM structure of this assembly reveals that a novel interface forms between the "bundle signalling element" domain and the "stalk" domain of neighboring protomers within a helix turn (cis) that is allosterically dependent on GTP. A subtle increase in affinity of this new interface broke the coupling of nucleotide state and polymeric conformation, revealing details of how the hydrolysis cycle affects the conformation of the DRP1 monomer and subsequently the conformation of the fission dynamin polymer.

Track: Protein Interactions and Assemblies

Session: Allostery & Dynamics in Protein Function

ABS# 349 | Raf promotes Ras dimerization

Morgan Packer[1], Jillian Parker[1], Jean Chung[2], Zhenlu Li[3], Young Lee[2], Trinity Cookis[1], Hugo Guterres[1], Steven Alvarez[2], MD Hossain[1], Daniel Donnelly[1], Jeffrey Agar[1], Lee Makowski[1], Matthias Buck[3], Jay Groves[2], Car
[1]Northeastern University, [2]University of California Berkeley, [3]Case Western Reserve University (MA, United States)

Dimerization is a critical regulatory step throughout the MAPK signaling pathway. While dimerization of the Raf kinase domain is widely accepted and occurs even in the absence of Ras, Ras dimerization has been contested for several decades, but has recently been demonstrated as important for Raf activation. Our work shows that the Ras binding domain of Raf (Raf-RBD) induces robust Ras dimerization on supported lipid bilayers and, to a lesser extent, in solution as confirmed via size-exclusion chromatography coupled to small-angle x-ray scattering. Community network analysis of molecular dynamics simulations of the dimer of the Ras/Raf-RBD complex reveal extensive allosteric connections unifying Raf-RBD into a single allosteric network and bridging the two Raf-RBD D113 residues, located within the predicted galectin scaffold protein binding site and 85 Å apart, at opposite ends of the complex. Our findings suggest that Ras/Raf-RBD binding and dimerization occur simultaneously at the membrane to yield a high-affinity signaling complex poised to form a higher order macromolecular assembly comprising Ras/Raf/galectin complexes for tightly regulated and focused signaling through the MAPK pathway.

Track: Amyloid and Aggregation

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 350 | High Resolution Structural Analysis Of ALS-Associated Mutant SOD1 Inclusion Bodies Reveal Native-Like Aggregation

Michael V. Tarasca[1], Dalia Naser[1], Tyler G.B. Soule[1,2], Harmeen Deol[1], Gyana Gourab Mishra[1], Susan Kelso[1,3], Anna Schaefer[1], Elizabeth M. Meiering[1]
[1]University of Waterloo, [2]University of Calgary, [3]University of Toronto (Ontario, Canada)

Inclusion bodies (IBs) – insoluble structures that can form upon overexpression of proteins- have a diverse array of practical applications, including as functional

nanomaterials, industrial large-scale protein preparation, and potential medical applications. Despite their plentiful applications, there have been very few systematic studies on these aggregates to characterize their structures at high resolution. IBs also provide a tractable model to elucidate the molecular mechanisms of protein aggregation in cells, which also commonly occurs in neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), Parkinson's, and Alzheimer's diseases. The aggregation of Cu, Zn superoxide dismutase (SOD1) variants is associated with ALS, yet the relationships between mutant characteristics and disease properties remain obscure. Here, we report a systematic investigation of SOD1 IBs using a recently optimized powerful method, quenched amide hydrogen-deuterium exchange (HDX), to analyze by NMR the structures of these cellular aggregates and their potential changes upon mutation. Our residue-specific quenched HDX measurements for nine ALS-associated SOD1 point mutants reveal IB structural features and similarities, with their measured protection patterns implying native-like aggregation. Quenched HDX is a robust method that provides a valuable high-resolution view of cellular aggregation, and can be widely applicable to other proteins to define the molecular determinants of their aggregation and solubility.

Track: Membrane Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 351 | Discovery of Potential Positive Allosteric Modulators of Glucagon-Like Peptide 1 Receptor and the Implication for Allosteric Regulation of GPCRs

Zhijun Li[1], Tejashree Redij[1], James McKee[1], Phu Do[1], Jeffrey Campbell[1], Zhiyu Li[1]
[1]*University of the Sciences in Philadelphia (PA, USA)*

The Glucagon-like peptide 1 receptor (GLP-1R) is a member of Class B G-protein coupled receptors (GPCRs) and a well-established target for the treatment of type 2 diabetes. GLP-1R agonist-based therapies are quite successful with several GLP-1 analog drugs available in the market. However, the development of nonpeptidic agonist drugs targeting GLP-1R remains unsuccessful. A promising strategy aims to develop orally bioavailable, small molecule positive allosteric modulators (PAMs) of GLP1-1R. Built upon our previous effort in this direction, we've performed structure-based molecule design. A novel compound was synthesized and confirmed in vitro as a potential GLP-1R PAM. This compound has the molecule weight of 239, the smallest among known GLP-1R agonists and PAMs. When combined with GLP-1, it increased the GLP-1R activity in a dose-dependent manner and it stimulated insulin secretion more than 2.5 fold than when GLP-1 was used alone. When combined with the VIPR peptide agonist, it showed no non-specific activity on VIPR1, another Class B GPCR. The compound reported here has the great potential to be further developed. Further, our approach and data suggest a common allosteric binding site may exist that can be targeted to develop allosteric modulators of other GPCRs.

Track: Computational Modeling/Simulation
Session: Protein Structures Through the Lens of Machine Learning

ABS# 352 | Predicting Binding Affinity using Deep Learning

Daniel Olson[1], Thomas Colligan[1], Amitava Roy[2], Vishwesh Venkatraman[3], Travis J. Wheeler[1]
[1]*University of Montana,* [2]*NIH,* [3]*NTNU (Montana, United States)*

Correctly predicting the binding affinity between protein-ligand pairs is a vital step in computational drug screening, but current methods are computationally expensive or show limited accuracy (or both). We have developed a novel machine learning method to rank potential binding ligands based on techniques from modern Deep Learning. Our method is designed to predict binding affinity for a ligand to a specified protein pocket, and takes as input a set of physical properties from candidate poses produced by docking software such as AutodockVina. Here, we describe a deep neural network architecture that demonstrates binding prediction accuracy that is superior to commonly used measures such as DFIRE, gnina and RF-Score, validated on the DUD-E and LITPCBA benchmarking datasets. Results indicate that deep-learning based approaches to predicting binding affinity are a promising direction for future research.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 354 | Structurally Distributed Surface Sites Tune Allosteric Regulation

James McCormick[1], Marielle Russo[1], Samuel Thompson[2], Aubrie Blevins[1], Kimberly Reynolds[1]
[1]*UT Southwestern,* [2]*Stanford University (Texas, United States)*

Our ability to rationally optimize allosteric regulation is limited by incomplete knowledge of the mutations that tune allosteric function. Are these mutations few or abundant, localized to the allosteric site or structurally distributed? To examine this, we conducted saturation mutagenesis of a synthetic allosteric switch in which Dihydrofolate reductase (DHFR) is regulated by a blue-light sensitive LOV2 domain. Using a high-throughput assay wherein DHFR catalytic activity is coupled to E. coli growth, we assessed the allosteric impact of 1548 viable DHFR single mutations. Fewer than 5% of mutations had a statistically significant influence on regulation with generally modest effect sizes of a few fold. Most allostery disrupting mutations were proximal to the LOV2 insertion site. In contrast, allostery enhancing mutations were structurally distributed and enriched on the protein surface. Combining several allostery enhancing mutations yielded near-additive improvements to dynamic range. Our results indicate a path towards optimizing allosteric function through variation at weakly conserved surface sites.

Track: Structure (X-Ray/NMR/EM)
Session: Protein Structures Through the Lens of Machine Learning

ABS# 355 | Crystal Structure of Rhipicephalus sanguineus Arginine Kinase, a Potential Marker for Tick Exposure

Ana Carolina Gómez Yanes[1], Rogerio Rafael Sotelo Mundo[1], Karina Dalila García Orozco[1]
[1]*Centro de Investigación en Alimentación y Desarrollo (Sonora, México)*

Arginine kinase (AK) is an allergenic invertebrate protein. It may be hypothesized that the AK from the brown tick can induce an immune response and help diagnose exposure to the invertebrate. We present the R. sanguineus arginine kinase crystal structure in the apo form. The recombinant 42 kDa protein was purified by nickel affinity chromatography and gel filtration. The protein product proved to be enzymatically active. Diffraction experiments were carried out at the Stanford Synchrotron Radiation Lightsource. Data were processed with the CCP4 suite to a resolution of 1.53 Å and merged to obtain 95000 unique reflections with an R-meas of 0.37 and completeness of 98.7%. The structure was refined until R-work = 0.1541 and R-free = 0.1792. We are in the process of identifying novel structural epitopes that may provide tools to find an early detection method for tick-borne diseases.

Track: Folding
Session: Allostery & Dynamics in Protein Function

ABS# 356 | The Folding Pathway of 6aJL2

Haven Lopez[1], Sagar Kathuria[2], Daniel Alejandro Fernandez Velasco[1]
[1]*Universidad Nacional Autonoma de Mexico,* [2]*UMASS Medical School (Mexico City, Mexico)*

One-third of the reported cases of light chain amyloidosis are related to the germ line λ6 family; remarkably, healthy individuals express this type of protein in just 2% of the peripheral blood and bone marrow B-cells. The appearance of the disease has been related to the inherent properties of this protein family. A recombinant representative model for λ6 proteins called 6aJL2 containing the amino acid sequence encoded by the 6a and JL2 germ line genes was previously designed and synthesized to study the properties of this family. Previous work on 6aJL2 suggested a simple two-state folding model at 25 °C; no intermediate could be identified either by kinetics or by fluorescence and circular dichroism equilibrium studies, although the presence of an intermediate that is populated at ∼2.4 M urea was suggested by size exclusion chromatography. In this study we employed classic equilibrium and kinetic experiments and analysis to elucidate the detailed folding mechanism of this protein. We identify species that are kinetically accessible and/or are populated at equilibrium. We describe the presence of intermediate and native-like species and propose a five-species folding mechanism at 25 °C at short incubation times, similar to and consistent with those observed in other proteins of this fold. The formation of intermediates in the mechanism of 6aJL2 is faster than that proposed for a Vκ light chain, which could be an important distinction in the amyloidogenic potential of both germ lines.

Track: Folding
Session: Allostery & Dynamics in Protein Function

ABS# 357 | Stability, Coupling, and the Partly-Folded States of Topoisomerase V

Mark Petersen[1], Rebecca Fang[1], Ananya Majumdar[2], Doug Barrick[1]
[1]*Johns Hopkins University,* [2]*Johns Hopkins Biomolecular NMR (Maryland, United States)*

Thermodynamic stability is an important consideration when designing proteins for use as drugs or biochemical tools. Highly stable proteins may have longer shelf lives,

higher tolerance to temperature extremes and denaturants, and better processivity during catalysis. Topoisomerase V (TopoV) from the thermophile Methanopyrus kandleri has gained our attention for its twelve repeated helix-hairpin-helix ((HhH)2) domains. With biological activities including non-specific DNA binding and DNA repair, these TopoV repeats have also been shown to increase the processivity and lifetime of common laboratory polymerases when expressed as either N or C-terminal fusion proteins (Pavlov et al., PNAS, 2002), raising the question of whether the repeated (HhH)2 module gives rise to increased stability, and how repeating (HhH)2 domains affect processivity. To understand how these repeated domains are thermodynamically coupled, we have expressed constructs containing either one or two repeats from TopoV. The individual and tandem repeat constructs have NMR spectra that are characteristic of folded proteins, and their high-helical content makes them particularly amenable to thermodynamic studies by CD. We separate the contributions to folding free energy using a 1D-Ising model, and we find the local stability of domains and interfaces to be highly variable. Surprisingly, the seventh repeat, which lacks electron density in the published crystal structure (PDB 5HM5), is the most stable repeat by far. Using parameters from our small constructs, we build a partition function for the entire array of domains from repeat D to repeat J at the 1D Ising level to determine local stabilities and populations of partly folded states. The resulting partition function accurately describes the global folding transition for the array, suggesting that our method for separating intrinsic and interfacial folding free energies captures the populations of partly folded states in a very complex, multistate system.

Track: Folding

Session: Measuring Forces of Biological Systems

ABS# 358 | **Protein Folding Across Methods**

Colleen Kelly[1]

[1]*University of Massachusetts, Lowell (NY, United States)*

Exploring the mechanical unfolding of proteins provides insight into their roles in a number of biological and physiological events. Methods like atomic force microscopy (AFM), optical tweezers, and magnetic tweezers allow for the mechanical manipulation of a single molecule, whereas thermal and chemical denaturation experiments can observe the average change of millions of billions of molecules in one sample. The existing body of work studying the behavior of proteins in thermal and chemical denaturation experiments is extensive and is often not considered analogous to unfolding data obtained by mechanical methods. This work compares the energetic barriers and folding rates across methods of an immunoglobulin-like domain with high solubility and low stability (I83) from the N2A region of the muscle protein, titin. Tryptophan fluorescence, circular dichroism, and a magnetic tweezer system monitor the structural stability and folding rates of the I83 domain while chemical denaturant, heat, and mechanical force is applied, respectively. The findings suggest that a correlation exists for the folding rates of I83 between chemical and mechanical unfolding as well as for the free energy of unfolding by thermal and chemical means. The similarities and differences observed between methods allow for a comparison of physiologically relevant unfolding behavior and may help to determine parallels that exist across methods for similar globular proteins.

Track: Computational Modeling/Simulation

Session: Allostery & Dynamics in Protein Function

ABS# 359 | **ALS-Causing SOD1 Mutant Simulations Reveal Common Perturbations to Stability and Dynamics during Maturation**

Colin Smith[1], Nicholas Wells[1], Grant Tillinghast[1], Alison O'Neil[1]

[1]*Wesleyan University (CT, USA)*

With over 150 heritable mutations identified as disease-causative, superoxide dismutase 1 (SOD1) has been a main target of amyotrophic lateral sclerosis (ALS) research and therapeutic efforts. However, recent evidence has suggested that neither loss of function nor protein aggregation is responsible for promoting neurotoxicity. Furthermore, there is no clear pattern to the nature or the location of these mutations that could suggest a molecular mechanism behind SOD1-linked ALS. Here, we utilize reliable and accurate computational techniques to predict the perturbations of 10 such mutations on the free energy changes of SOD1 as it matures from apo monomer to metallated dimer. We find that the free energy perturbations caused by these mutations strongly depend on maturational progress, indicating the need for state-specific therapeutic targeting. We also find that many mutations exhibit similar patterns of perturbation to native and non-native maturation, indicating strong thermodynamic coupling between the dynamics at various sites of maturation within SOD1.

These results suggest the presence of an allosteric network in SOD1 which is vulnerable to disruption by these mutations. Analysis of these perturbations may contribute to uncovering a unifying molecular mechanism which explains SOD1-linked ALS and help to guide future therapeutic efforts.

Track: Chaperones

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 361 | Anti-Prion Chaperone Pentosan Polysulfate Interacts Avidly with Unfolded States of the Prion Protein

Shubhadeep Patra[1], Rafayel Petrosyan[1], Uttam Anand[1], Craig R. Garen[1], Michael T. Woodside[1]
[1]*Department of Physics, University of Alberta, Edmonton, AB, T6G 2E1, Canada (Alberta, Canada)*

Prion diseases involve the propagation of misfolded conformers of the prion protein, PrP. Small-molecule pharmacological chaperones can impede conversion of the protein into the diseased form, but how they interact with PrP to prevent misfolding is often unclear. Previous studies of the interactions of two different kinds of pharmacological chaperones—a planar cationic iron tetrapyrrole, Fe-TMPyP, and a linear anionic polymer, pentosan polysulfate (PPS)—with Syrian hamster PrP (SHaPrP) showed that they not only bind to natively folded PrP, stabilizing it thermodynamically and mechanically, but they also bind strongly to partially or fully unfolded PrP. The unexpected interaction of both these chaperones with unfolded states suggests that unfolded PrP plays a crucial role in the prion conversion pathway. Understanding better how such chaperones interact with unfolded states may help improve our understanding of the mechanism of inhibition, but it is challenging to observe the interaction of ligands with unstable and transient unfolded conformers. Here we use single-molecule force spectroscopy to destabilize single molecules of bank vole PrP (BVPrP) and observe the interactions between unfolded BVPrP and PPS. Unlike SHaPrP, BVPrP unfolds and refolds natively via partially folded intermediates in the absence of chaperones. We found that PPS stabilized certain partially unfolded intermediate states of BVPrP, as well as the fully unfolded state, displaying heterogeneous interactions as seen for SHaPrP. By examining the extent of the interactions of PPS with unfolded states at different PPS concentrations, we estimated that PPS bound 10-fold more tightly to unfolded states than to the native state of PrP. These results reinforce the likely importance of unfolded states in the mechanism for propagated misfolding: when natively folded PrP molecules unfold partially or fully during conversion, PPS binds avidly to the unfolded portions, inhibiting conversion.

Track: Chemical Biology

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 362 | Elucidating the Stoichiometry of the Salmonella FraR (Transcriptional Repressor)-DNA Complex using Online Buffer Exchange Coupled to Native-Mass Spectrometry

Angela Di Capua[1], Blake E. Szkoda[2], Sravya Kovvali[3], Joy Shaffer[4], Edward J. Behrman[5], Venkat Gopalan[5], Vicki H Wysocki[1]
[1]*Department of Chemistry & Biochemistry, The Ohio State University, Columbus, OH 43210, USA ; Resource for Native MS Guided Structural Biology, The Ohio State University, Columbus, OH 43210, USA,* [2]*The Ohio State Biochemistry Program, The Ohio State (OH, United States)*

Salmonella enterica serovar Typhimurium (Salmonella) is a foodborne pathogen that causes gastrointestinal illness and can lead to death. The Center for Disease Control and Prevention estimates there are 1.35 million Salmonella-related illnesses in the United States annually. There are no vaccines or antibiotics to specifically combat this bacterium. During inflammation post-infection, Salmonella utilizes fructose-asparagine (F-Asn) as a nutrient. F-Asn is an Amadori product and is converted into glucose-6-phosphate and aspartate through a catabolic pathway that involves different proteins, all encoded by the fra operon. This work was undertaken to characterize gene regulation of the fra operon by FraR, the putative transcriptional factor in this locus.FraR is postulated to be a transcriptional repressor that binds to the fraB promoter (FBP). An inducer metabolite is predicted to govern this repression by binding to the protein and causing a conformational change that releases FraR, permitting transcription. Recombinant FraR was overexpressed in Eschericha coli and purified, and then used in fluorescence-based gel-shift assays and online buffer exchange (OBE)/native mass spectrometry (nMS).With OBE, samples were kept in a non-volatile buffer that favors their native

biological properties and then buffer exchanged into ammonium acetate online for nMS analysis. OBE-nMS was used to confirm the oligomeric state of FraR and FraR-DNA complexes and to investigate 6-phospho-fructose-aspartate (6-P-F-Asp) as a potential inducer. Results from our studies showed that the FraR dimer binds with high affinity to two 26-bp DNA fragments (KD ~1 nM each), and that two 6-P-F-Asp molecules bind to each dimer (KD ~2 μM each). In addition, we show that 6-P-F-Asp acts as the inducer that triggers FraR dissociation from the DNA. These findings provide a first glimpse into the regulation of Amadori metabolism in a clinically significant bacterial pathogen.

Track: Structure (X-Ray/NMR/EM)
Session: Allostery & Dynamics in Protein Function

ABS# 363 | Local disorder of transthyretin modulates its aggregation-prone propensity

Jin Hae Kim[1]
[1]*Daegu Gyeongbuk Institute of Science & Technology (Daegu, South Korea)*

Transthyretin (TTR), in its native tetrameric state, is an essential transporter of thyroxine and holo-retinol binding protein for human. However, its dissociation into the mis-folded monomer facilitates abnormal aggregation of TTR, causing deposition of TTR aggregates typically in the peripheral nervous system or in the heart. Although numerous studies were conducted to elucidate the aggregation mechanism of TTR, it is still elusive which structural features are actually responsible for its aggregation. Here, we determined with nuclear magnetic resonance (NMR) spectroscopy the three-dimensional structures of the two TTR variants: amyloidogenic monomeric TTR and its less-amyloidogenic variant, T119M. Distinctive to the native tetrameric state, the misfolded monomer of TTR presented structural features in which the C-terminal beta-strand is released and the neighboring loops are perturbed. On the other hand, introduction of T119M mutation caused non-native rearrangement of the beta-strand structure; it appears that this mutation damped conformational fluctuations of the C-terminal beta-strand, which is known to be important for TTR aggregation. Finally, we also found that Hsp90 interacts with monomeric TTR more strongly than with tetrameric TTR. Taken together, our results provide the atomistic detail to explain the elevated susceptibility of monomeric TTR for aggregation, as well as the novel insight to develop therapeutic strategies for TTR amyloidosis. References[1] Oroz, J.,* Kim, J.,* Chang, B., Zweckstetter, M. (2017) Mechanistic basis for the recognition of a misfolded protein by the molecular chaperone Hsp90, Nat. Struct. Mol. Biol. 24, 407-413 (*equal contribution).[2] Kim, J., Oroz, J., Zweckstetter, M. (2016) Structure of monomeric transthyretin carrying the clinically important T119M mutation, Angew. Chem. Int. Ed. 55, 16168–16171.

Track: Bioinformatics
Session: Protein Structures Through the Lens of Machine Learning

ABS# 364 | A Novel Method to Identify and Characterize Kinked Alpha-Helices

Shengyuan Wang[1], Iosif Vaisman[1]
[1]*George Mason University (Virginia, United States)*

Virtually all alpha-helices in known protein structures deviate from ideal helices to various degrees. Helices with the largest structural distortions are known as kinked alpha-helices and they have been shown to play important functional roles, particularly in membrane proteins. Kinked alpha-helices can be defined by a number of existing algorithmic and manual approaches, which poorly agree with each other. In this work we describe ValgusHel, a new robust and intrinsically consistent approach for identification and characterization of kinked alpha-helices. ValgusHel is trained on a non-redundant set of 8,826 proteins containing 46,615 alpha-helices. This approach relies on a combination of the angle between axes of the helical subsections consisting of six consecutive residues and topological descriptors of nearest neighbor residues in 3D space as defined by Delaunay tessellation of protein structures represented on a single point per residue level. The thresholds for the angle values (less than 20° for normal helices, 20° to 30° for curved helices, and greater than 30° for kinked helices) were introduced and validated based on parameter-free topological descriptors, helix clustering in structural and sequence spaces, and correlations between sequence similarity, geometrical similarity, and topological similarity measures. Out of 278,010 residues in the dataset, 2,662 (0.96%) residues were annotated as kinked, 13,198 (4.75%) as curved and 262,150 (94.30%) as normal. Comparative sequence analysis of kinked and normal alpha-helices revealed some interesting patterns

which may provide insights into the mechanisms of kink formation and their functional roles in proteins.

Track: Chemical Biology
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 365 | Characterization of the Interactions Between the Leucine Rich Repeat Domain of the Human Commensal Candida. albicans' adenylyl cyclase Cyr1p with Bacterial Peptidoglycan Fragments

Geneva Crump[1], Catherine Leimkuhler Grimes[1]
[1]*University of Delaware (DE, USA)*

The human commensal Candida. albicans recognizes and responds to bacterial peptidoglycan (PG) through the Leucine Rich Repeat domain of its adenylyl cyclase Cyr1p. Upon binding to PG, signaling cascades promote a morphological transition from a budding yeast to a long-extended hyphal format. The Cyr1p receptor thus behaves as a Pathogen Recognition Receptor in the fungi, and is one of the key determinants of the commensal and pathogenic states of this organism in its human host. Our lab has previously quantified the binding between recombinant Cyr1p and the PG fragment Muramyl Tripeptide (MTP) via a Surface Plasmon Resonance assay. Though successful, our previous assay was limited by the ability to obtain soluble, stable and tag free recombinant protein. Given the complexity that exist among bacterial PG, there remains a plethora of unexplored potential ligands for Cyr1p. To attain a compressive scope of ligands that this receptor can bind, we have redesigned the recombinant LRR domain to attain a more stable construct. In doing so, we have learned that this protein largely resides in the cellular membrane as a peripheral membrane protein. With these findings, we biochemically characterize this protein in the presence of the membrane solubilizing lipid Fos-Choline-12. To determine the promiscuity of the Cyr1p-LRR domain, we have conducted Cellular Thermal Shift Assays with various PG fragments and have observed a temperature induced stabilization in comparison to apo-protein. We further characterize these PG fragments for their ability to induce hyphal formation in WT C. albicans cells and observe that the ability to induce hyphal formation does not correlate with the temperature induced stabilization of the individual PG fragment. These findings suggesting that there may be a transcriptional component associated with each ligand binding event that is responsible for hyphal elongation then maintenance.

Track: Amyloid and Aggregation
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 366 | Real-Time Observation of Structure and Dynamics during the Liquid-to-Solid Phase Transition of FUS LC

Raymond Berkeley[1], Maryam Kashefi[1], Galia Debelouchina[1]
[1]*UC San Diego (CA, US)*

Many of the proteins found in pathological protein fibrils and aggregates also exhibit tendencies for liquid-liquid phase separation (LLPS) both in vitro and in cells. Here, we interrogate a model protein for this behavior, FUS LC, using magic-angle spinning NMR in real time as it transitions from a liquid-liquid droplet to a solid. We also apply our methodology to FUS LC G156E, a clinically relevant FUS mutant that exhibits accelerated fibrillization rates. We show that FUS LC and FUS LC G156E form structurally similar fibrils from LLPS droplets via different intermediate gel states and begin to unravel the structural and sequence-specific contributions to this phenomenon with molecular dynamics studies of the phase separated state of FUS LC and FUS LC G156E.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 368 | Directed Evolution of Haloalkane Dehalogenases Benefits from Rationally Designed Libraries with Ancestral and Structural Insights

Benedikt Dolgikh[1], James Van Antwerp[2], Jens Schmidt[3], Daniel Woldring[4]
[1]*First Author,* [2]*Co-Author,* [3]*Collaborator ,* [4]*Corresponding Author (Michigan, United States)*

On a path towards engineered proteins with novel clinical and industrial functionalities, locating proteins at various 'peaks' in the sequence-functional landscape is critical. To this end, a variety of methods guide the prediction of numerous highly functional protein sequences. Protein library design can incorporate tailored amino acid diversity at individual sites based on the composition of other functional homologs, stabilizing motifs, and

natural evolutionary insights. Our objective here is to engineer unique fluorescent ligand binding properties into haloalkane dehalogenases using stability prediction (FoldX and Rosetta), ancestral sequence reconstruction (IQ-Tree), and consensus analysis. We integrate structural and sequence data from a growing body of dehalogenase literature to identify amino acids that are tolerated at individual positions. A large protein library based on these data is then screened on the surface of yeast to identify improved and novel fluorescent ligand binding function. In our analysis, similar positions, reported with improved active site access and catalytic activity, and hydrophobic amino acids which provide stability at the binding pocket, are predicted when comparing to available datasets. Furthermore, the utility of stability prediction and consensus analysis in refining library design choices is further credited as a major driver towards efficient site-wise diversification. By comparing the conserved amino acids among homologues with destabilization prompted by substitutions, it becomes more clear which amino acids and positions should be included in library design. By proposing a narrow examination of amenable positions and substitutable amino acids, structural destabilization and sequence diversity can reveal evolutionary insights. These data provide a deeper understanding of enzymatic characteristics within molecularly-integral regions of haloalkane dehalogenases for the use of cellular imaging and biosensing.

Track: Dynamics and Allostery
Session: Allostery & Dynamics in Protein Function

ABS# 370 | Studies on the Variability of RNA Torsion Angles with Protein Binding

Jarlene Roman[1], Janet Gonzalez[2], Ezekuel Olumuyide[1], Manfred Philipp[3]
[1]Lehman College, City University of New York, [2]LaGuardia Community College, City University of New York, [3]Lehman College and Graduate Center, City University of New York (New York, United States)

The 2MQV pdb file that has been published for the U5-primer binding site of murine leukemia virus RNA provides data on the effects of ligand binding on RNA structure. This pdb file was deposited by D'Souza & Yildiz and published in Nature, 2014 Nov 27;515 (7528):591-5.This study examines the hypothesis that ligand binding can make an RNA structure more flexible than other areas of a double-helical RNA. The 2MQV.pdb structure file was chosen for this purpose because it is the result of in solution NMR experiments. 2MQV.pdb is not a crystal structure, where the crystal lattice can, to a degree, restrict the macromolecular structure in a crystal lattice. The solution experiments that were used to generate the 2MQV.pdb file generated a series of 10 separate pdb structure files. Each of these pdb files was examined by calculating the RNA torsion angles using the x3DNA-DSSR torsion angle server. The results of this server were used to create graphs of various torsion angles as a function of RNA position and pdb model. These graphs were animated to more clearly identify areas of maximum structural and torsion angle variability.This presentation will examine the torsion angles that are most illustrative of torsion angle variability. These results are compared with images and movies of RNA motion derived from visualizations of the multimodel RNA structures.

Track: Chemical Biology
Session: Allostery & Dynamics in Protein Function

ABS# 371 | Modulation of Myb using a Potent and Selective Dual-Site Inhibitor of CBP/p300 KIX

Stephen Joy[1], Matthew Henley[1,2], Matthew Beyersdorf[1,2], Yanira Rodriguez Valdes[1,2], Anna Mapp[1,2]
[1]Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109, [2]Program in Chemical Biology, University of Michigan, Ann Arbor, MI 48109 (MI, United States)

The protein-protein interaction between the transcriptional activator Myb and the KIX domain of the transcriptional coactivator CBP/p300 plays a pivotal role in certain acute myeloid leukemias (AML) and other cancers. However, the CBP/p300 KIX domain is challenging to target potently and selectively due to its multiple binding sites, conformational plasticity, and the existence of multiple structural homologues across the proteome. Here we describe the development of a picomolar inhibitor formed by fusing the KIX-binding Myb and MLL transactivation domains. This dual-site inhibitor (MybLL-tide) has higher affinity for CBP/p300 KIX than any previously reported compounds and possesses 15,000-fold selectivity for the CBP/p300 KIX domain over other similar domains. Modification of the MybLL-tide with a cell penetrating peptide moiety yields a cell active MybLL-tide that potently modulates downstream gene expression and also inhibits AML cell viability. These results show that MybLL-tide can be an effective, modifiable tool to selectively target the CBP/p300 KIX domain to assess transcriptional effects in AML cells and

potentially other cancers that are dependent on aberrant Myb behavior.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 373 | Computational Investigation into Mutational Allosteric Effects on Tau Protein-Antibody Binding

Katherine Lee[1], Eric May[1]
[1]*University of Connecticut (Connecticut, United States)*

Misregulation of post-translational modifications of microtubule-associated protein tau is implicated in several neurodegenerative diseases including Alzheimer's disease. Hyperphosphorylation of tau promotes aggregation of tau monomers into filaments which are common in tau-associated pathologies. Therefore, tau is a promising target for therapeutics and diagnostics. Recently, high-affinity, high-specificity single-chain variable fragment (scFv) antibodies against pThr-231 tau were generated and the most promising variant (scFv 3.24) displayed 20-fold increased binding affinity to pThr-231 tau compared to the wild-type. The scFv 3.24 variant contained five point mutations, and intriguingly none were in the tau binding site. The increased affinity was hypothesized to occur due to allosteric communication between the framework region and binding site. Multi-microsecond all-atom molecular dynamics simulations were conducted for four systems – the wild-type antibody and the mutant with and without tau. Correlation of All Rotameric and Dynamical States (CARDS) software was used to quantify allostery in terms of mutual information (MI), or the dependence between two variables. The total MI of the mutant was significantly higher than that of the wild-type. The MI of the residues was also determined relative to the region that directly binds to the phosphate group on tau. The mutant exhibited much higher MI than the wild-type overall with MI of mutant residues elevated. Diffnets, a supervised autoencoder with a classification task, was used to distinguish the relevant motions separating wildtype from 3.24 mutant. Results showed long range expansion within the mutant stemming from mutation Ile61. Expansion between the loops that comprise the binding site to tau was also observed in the mutant relative to the wildtype in both the unbound and bound states, potentially providing more room for tau to bind and interact. Recent work has been aimed towards quantifying optimal collective variables for discriminating wild-type from mutant ensembles for use in free energy calculations.

Track: Evolution
Session: Protein Evolution, Design and Selection

ABS# 374 | Sequence-Fitness Landscape of a Four-Helix Bundle RNA-Binding Protein using Deep Mutational Scanning

Charu Jain[1], Nishanthi Panneerselvam[1], THOMAS MAGLIERY[1]
[1]*The Ohio State University (OH, United States)*

A protein's sequence structure-function relationship is still poorly understood, and understanding this relationship is crucial for our molecular understanding of disease as well as for the development of biologics. It is generally believed that most mutations of natural proteins are neutral to deleterious, but a few can result in improved stability or function. We are using a model protein, Rop, a small 63 amino acid homodimer four-helix protein that controls the replication of ColE1 plasmids in E coli. Rop performs its function by enhancing the interaction of RNAs that initiate plasmid replication, but how it does that is not understood. We have carried out a comprehensive study to interrogate the sequence-fitness landscape of Rop. We have developed a deep mutational scanning approach for Rop and verified its use with a model experiment. Sixty-two individual point mutant libraries, for each position in Rop, were cloned, and the active mutants were enriched using a growth selection and verified using a fluorescence-based screen. First, three environmentally different position in the core, loop, and surface were enriched separately and together and analyzed using high-throughput sequencing. We found that the loop position was tolerant to mutations and was most abundant in the final round of enrichment, while the core position showed conservative hydrophobic substitutions. The surface position that is believed to be essential for the function was only found with the native Phe and the structurally similar His. We are further analyzing the fitness of these single-point mutants of Rop using a high-throughput thermal scanning assay to measure thermal stabilities. We expect the results of this study will not only better define how Rop functions but also shed light on many similar proteins since RNA binding proteins and four-helix bundle proteins are ubiquitous.

Track: Synthetic Biology
Session: Protein Evolution, Design and Selection

ABS# 375 | New binding partners to cyanobacteriochromes for optogenetic applications

Jaewan Jang[1], Sherin McDonald[2], Maruti Uppalapati[2], G. Andrew Woolley[1]
[1]*University of Toronto,* [2]*University of Saskatchewan (Ontario, Canada)*

Photoactive proteins, which change conformation in response to irradiation, are the core of optogenetic tools. Current optogenetic tools display a limited range of wavelengths, with very few operating at wavelengths in the green or yellow/orange regions of the spectrum. Additionally, for use in whole animals, tools that respond to near IR wavelengths would be ideal. To address these limitations, we have utilized a red/green cyanobacteriochrome (CBCR) domain from Acaryochloris marina that is monomeric in solution. These proteins bind phycocyanobilin and show reversible conversion between red-absorbing (Pr) and green-absorbing (Pg) states. We used phage display of a small protein domain (GA-domain) to find binding partners that bind each photo state of the cyanobacterial GAF domain selectively. In vitro characterization indicates sub-micromolar affinity towards the selected state while showing >10-fold change in affinity versus the other state. We show that the tools developed here can control light-dependent transcription and compartment localization in yeast and living mammalian cells, respectively. This work provides the first cyanobacteriochrome-based optogenetic tools for controlling protein-protein interactions (PPI). Our strategy establishes a general method to develop PPI tools with other CBCRs, which respond to a wide range of wavelengths.

Track: Chemical Biology
Session: Allostery & Dynamics in Protein Function

ABS# 377 | Structure-Based Design of Potent and Selective Inhibitors of Pyruvate Carboxylase

Nicholas Schneider[1], Daniel Burkett[2], Brittney Wyatt[1], Mallory Mews[1], Anson Bautista[2], Ryan Engel[2], Chris Dockendorff[2], William Donaldson[2], Martin St. Maurice[1]
[1]*Department of Biological Sciences, Marquette University,* [2]*Department of Chemistry, Marquette University (WI, United States)*

Pyruvate Carboxylase (PC) is an anaplerotic enzyme that replenishes the TCA cycle intermediate oxaloacetate (OAA), which is a precursor molecule for pathways such as gluconeogenesis, fatty acid synthesis, and amino acid synthesis. PC contributes to glucose-stimulated insulin release in pancreatic beta cells and glucose production in the liver and kidney. Therefore, 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. RNAi studies have further validated PC as a potential target for pharmacological intervention. Currently, there are no known potent and selective inhibitors of PC activity, depriving researchers of a critical tool to assess and manipulate the activity of PC in a variety of diseases and infections. This project is focused on discovering, characterizing, and optimizing small molecule effectors that are capable of potently and selectively altering PC activity. Through structure-based drug design and in collaboration with the additional authors, our lab has developed two compounds (given names 8u and 8v) that are able to effectively inhibit PC at a low micromolar concentrations. Computational docking and competition experiments strongly suggest these compounds bind within the catalytic active site of PC, competing with the substrate pyruvate. 8u and 8v contain an α-keto acid moiety which raises concerns about metal chelation within other metalloenzymes. We have shown that 8u and 8v display reasonable selectivity for PC, displaying limited effects on additional metalloenzyme activity. 8u and 8v also do not alter the activity of enzymes that bind substrates that mimic the substrate of PC. These compounds represent the first major step in the development of potent and selective small molecule effectors of PC. Developing potent and selective molecular probes of PC will represent a vital contribution to the available toolkit for studying the metabolic reprogramming that accompanies type-2 diabetes.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 378 | Enhanced specificity of electrochemical biosensors using small, stable bipartite ligands

Sunanda Dey[1], Daniel Woldring[1], David Hickey[1]
[1]*Michigan State University (Michigan, USA)*

Small changes in appearance or enzymatic modifications to proteins (e.g., glycosylation) can be indicative of the disease progression. Identifying such subtle changes require selective and sensitive diagnostic tools as early detection of aggressive disease can improve patient outcomes. Combining this with continuously monitoring the

level of the relevant biomarkers plays a pivotal role in establishing personalization in the future of medical treatment. Prevalent biosensors use aptamers, antibodies, or enzymes as the bio-recognition element to target relevant biomarkers. These serving as the predominant point-of-care diagnostic tools are often limited by factors such as chemical complexity, stability, and reproducibility. The need to overcome the challenges associated with traditional biosensors motivates our ongoing development of a novel biosensor which incorporates an array binding protein which selectively detect individual biomarkers. We aim to integrate the data obtained from multiple unique, clinically relevant biomarkers to enhance the confidence in predicting the stage and the trajectory of a disease. Our biosensor design is comprised of two small proteins that bind non-competitively to a single target molecule, connected using a long flexible peptide linker. An electrochemically active enzyme is covalently attached to the C-terminus of the tandem binders and the N-terminus of the protein assembly (tandem binders and enzyme) is anchored to the surface of the electrode. When the binding proteins engage in targeting the biomarker simultaneously, the reactive enzyme is pulled closer to the electrode surface which triggers an electrochemical response that can be converted into a readable signal. The goal is to create several such arrays of microelectrodes that can simultaneously bind to an ensemble of biomarkers, thus establishing a non-invasive, real-time, advanced diagnostic tool capable of constant monitoring to inform treatment.

Track: Computational Modeling/Simulation
Session: Protein Structures Through the Lens of Machine Learning

ABS# 379 | Dynamical analysis for protein simulations using relaxation mode analysis

Ayori Mitsutake[1]
[1]*Department of Physics, Meiji University (Kanagawa, Japan)*

Molecular simulation is a powerful method for investigating the structural stability, dynamics, and function of proteins at the atomic level. In recent years, it has become possible to perform simulations on time scales of the order of milliseconds using special-purpose system. Because not only the most stable structure but also meta-stable structures and intermediate structures are included in trajectories in long simulations, it becomes to be necessary to develop dynamical analysis method to automatically extract reaction coordinates that identify these structures. Relaxation mode analysis (RMA) have been developed to investigate dynamics and kinetics of spin [1], polymer [2], and protein [3-8] systems. RMA approximately extracts slow modes and rates from simulations. RMA was applied to folding simulations of a designed mutant of protein G, NuG2, [8] for which simulations were previously performed by Lindorff-Larsen et al.[9]. RMA clarified two main folding pathways. Here, we present RMAs and results of protein simulations.[1] H. Takano and S. Miyashita, J. Phys. Soc. Jpn. 64, 3688 (1995). [2] H. Hirao, S. Koseki, and H. Takano, J. Phys. Soc. Jpn. 66, 3399 (1997).[3] A. Mitsutake, H. Iijima, and H. Takano, J. Chem. Phys.135, 164102 (2011). [4] Y. Maruyama, H. Takano, and A. Mitsutake, Biophysics and Physicobiology, 16, 407-429 (2019).[5] T. Nagai, A. Mitsutake, and H. Takano, J. Phys. Soc. Jpn. 82, 023803 (2013); T. Nagai, A. Mitsutake, H. Takano, Seibutsu Butsuri (Biophysics), 49, Supplement S75, (Abstracts for the 47st annual meeting, The Biophysical Society of Japan) (2009)[6] A. Mitsutake and H. Takano, J. Chem. Phys. 143, 124111 (2015).[7] N. Karasawa, A. Mitsutake, and H. Takano, J. Chem. Phys., 150, 084113 (2019). [8] A. Mitsutake and H. Takano, J. Chem. Phys., 151, 044117 (2019).[9] K. Lindorff-Larsen, S. Piana, R. O. Dror, and D. E. Shaw, Science, 334, 517 (2011).

Track: Chemical Biology
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 380 | Watching S. aureus pump iron: Fluorescent hemoglobin for bacterial heme uptake tracking

Kat Ellis-Guardiola[1], Brendan Mahoney[1], Clarissa Pham[1], Jessica Soule[1], Andrew Goring[1], Robert Clubb[1]
[1]*University of California - Los Angeles (California, USA)*

The Gram-positive pathogen S. aureus utilizes a network of heme-binding receptors, collectively known as Iron-surface determinant (Isd) system, to extract heme from human hemoglobin (Hb) and transport it through the cell wall to nourish the bacterium. These genes are expressed during iron starvation, a condition commonly encountered by pathogens, since host nutritional immunity factors restrict potential sources of soluble iron to thwart infection. The precise mechanism of this heme relay network in vivo has been difficult to study, as bacterial heme utilization is often tracked indirectly through the endpoint measurement of cell growth. In this study we describe the construction of a versatile Hb-fluorophore conjugate (dubbed HbT) that enables the observation of bacterial heme uptake in real-time using a simple fluorescence plate reader assay. We

validate the robustness of this chemical biological tool in vitro with purified Hb receptors IsdB and IsdH, demonstrating that engineering and fluorophore conjugation minimally impact its ability to be recognized by annotated S. aureus Hb receptors. We employ HbT in vivo to determine specific per-cell rates of Hb-sourced heme consumption. Heme uptake rates are measured for S. aureus cultured in the presence of competing iron sources and chelators, allowing the unprecedented comparison of heme uptake rates at different degrees of Isd surface expression. We compare rates of heme utilization with Isd surface abundance measurements, and we present a whole-cell model for S. aureus import of Hb-sourced heme.

Track: Chemical Biology

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 382 | Protect, modify, deprotect (PMD): A strategy for creating vaccines to elicit antibodies targeting a specific epitope

Payton Weidenbacher[1], Peter Kim[1]
[1]*Stanford University (CA, United States)*

The discovery of broadly neutralizing antibodies (bnAbs) has spurred research into techniques aimed to "immunofocus". The epitopes of these bnAbs are highly conserved, while the rest of the protein is highly variable. For example, antibodies which target the stem region on influenza hemagglutinin (HA) are generally broadly neutralizing. The stem epitope is conserved due to its involvement in viral fusion. Meanwhile, the head region of HA is the primary target of antibodies and also frequently mutates from year to year. To this end, we introduce a method termed Protect, Modify, Deprotect (PMD), which is capable of directing immune responses towards epitopes of bnAbs. We pioneered PMD using a stem directed-bnAb against HA as a molecular stencil to focus the immune response to this epitope. We accomplish this by shielding other regions of the native protein through chemical means. The steps in PMD are (i) protection of an epitope on an antigen by binding of a bnAb, (ii) chemical modification of exposed sites to render these exposed sites nonimmunogenic, and (iii) deprotection of the epitope of the bnAb by dissociation of the Ab-antigen complex. This produces an immunogen in which the only unmodified region is the epitope mapped by the bnAb. Using this method, we were able to produce an HA antigen which provides significantly broader protection, against a variety of influenza strains, compared to the unmodified antigen.

Track: Membrane Proteins

Session: Protein Evolution, Design and Selection

ABS# 383 | Uncovering the Novel Targeting Signal Used by TOC159 Receptors to Reach the Chloroplast Outer Membrane

Michael Fish[1,2], Masoud Jelokhani-Niaraki[2], Simon Chuong[3], Matthew Smith[1]
[1]*Wilfrid Laurier University, Department of Biology,* [2]*Wilfrid Laurier University, Department of Chemistry and Biochemistry,* [3]*University of Waterloo, Department of Biology (ON, Canada)*

The dynamic function of plastids relies on the fidelity of protein targeting pathways which shuttle plastid precursor proteins to the organellar surface where they are recognized and imported. The targeting of TOC159 receptors and other key components of the translocation machinery is essential to processes of plastid biogenesis and photomorphogenesis and therefore plant growth and plant development. Building on preliminary fluorescent targeting data, we expand our investigation into the molecular interactions that guide TOC159 receptor targeting specificity, insertion in the chloroplast outer membrane and recruitment to functionally distinct translocation complexes that are responsible for regulating the plastid proteome. Previous studies have revealed that targeting information is found at the C-terminus of TOC159, within the membrane domain. More specifically, the 56-most C-terminal residues were shown to be essential for targeting. It was initially hypothesized that an amphipathic alpha helix downstream of the membrane anchor, similar to those found in typical transit peptides at the N-terminus of plastid precursor proteins, is responsible for targeting. Using fluorescence microscopy, we found that truncated versions of the TOC159 membrane domain lacking the proposed signal sequence were still able to target fluorescent fusion proteins to the plastids of onion epidermal cells after biolistic bombardment. Moreover, the subdomain identified as the membrane anchor alone was necessary and sufficient for targeting to plastids. In light of these results, we propose that the TOC159 targeting signal is comprised of the C-terminal beta-hairpin sequence within the membrane anchor itself. Our most recent fluorescent targeting data will probe the targeting capability of the beta-hairpin sequence alone across Arabidopsis TOC159 receptor homolog sequences to support the evolutionary conservation of the targeting signal.

Track: Bioinformatics
Session: Protein Evolution, Design and Selection

ABS# 384 | Investigation of Age-Related Myosin Heavy Chain Expression in Skeletal Muscle

Smita Chatterjee[1], Matthew Gage[1]
[1]*University of Massachusetts Lowell (Massachusetts, United States)*

Human muscular contraction relies upon the sliding activity of actin and myosin contractile filaments. Previous studies have indicated that the myofibrillar composition of slow and fast-twitch muscles changes as a function age, leading to gradual attenuation of fiber density and strength. In addition, there is a reduction in the fraction of myosin heads capable of reaching the binding state necessary to generate significant contractile force. The myosin heavy chain (MyHC) is regulated by the MYH gene family, with genes Myh1, Myh2, Myh4, and Myh7 encoding for MyHC isoforms Myh-2X, Myh-2A, Myh-2B, and Myh-1 respectively. Differential expression was compared in these 4 genes from infancy to old age using extensor digitorum longus (EDL), psoas and soleus mouse muscle tissues. The transcription levels observed across all 4 genes are congruent with MyHC isoform compositions observed in previous transcriptomic and proteomic data. EDL and psoas, which are fast-twitch muscles predominately of type 2B and 2X, yielded minor expression of Myh2 and Myh7 in comparison to slow twitch soleus (type 1 and 2A). While psoas and EDL exhibit similar transcriptomic profiles with significantly higher transcription levels of Myh1 and Myh4 compared to soleus, the two muscle types demonstrated opposing trends in long-term MyHC isoform expression. We expect the observed MyHC transcriptomic patterns to reflect in the fiber-type composition of fast and slow-twitch muscles across mouse lifespan, with muscles of similar myofibrillar composition differing in long-term expression as a consequence of specific physiological function.

Track: Dynamics and Allostery
Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 385 | CryoEM reveals the stochastic nature of individual ATP binding events in a group II chaperonin.

Yanyan Zhao[1,2], Michael F. Schmid[3,4], Judith Frydman[2,5], Wah Chiu[4,6]
[1]*Biophysics Graduate Program,* [2]*Stanford University,* [3]*Division of CryoEM and Bioimaging, SSRL,* [4]*SLAC National Accelerator Laboratory, Menlo Park, CA 94025,* [5]*Department of Biology, Department of Genetics,* [6]*Department of Bioengineering, Jam (CA, US)*

Chaperonins are homo- or hetero-oligomeric complexes that use ATP binding and hydrolysis to facilitate protein folding. ATP hydrolysis exhibits both positive and negative cooperativity. The mechanism by which chaperonins coordinate ATP utilization in their multiple subunits remains unclear. Here we use cryo-EM to study ATP binding in the homo-oligomeric archaeal chaperonin from Methanococcus maripaludis (mmCpn), consisting of two stacked rings composed of eight identical subunits each. Using a series of image classification steps, we obtained different structural snapshots of individual chaperonins undergoing the nucleotide binding process. We identified nucleotide-bound and free states of individual subunits in each chaperonin, allowing us to determine the ATP occupancy state of each mmCpn particle. We observe distinctive tertiary and quaternary structures reflecting variations in nucleotide occupancy and subunit conformations in each chaperonin complex. Detailed analysis of the nucleotide distribution in each mmCpn complex indicates that individual ATP binding events occur in a statistically random manner for mmCpn, both within and across the rings. The lack of apparent cooperativity between adjacent subunits in the chaperonin molecule suggests the absence of the sequential Koshland–Némethy–Filmer-type (KNF) allosteric mode, specifically in the ATP binding step. In addition, the highly heterogeneous non-concerted conformational change in the chaperonin mmCpn due to random ATP binding in the molecule also suggests the lack of concerted cooperativity by Monod–Wyman–Changeux (MWC) model. Our findings illustrate the power of cryoEM to characterize a biochemical property of multi-subunit ligand binding cooperativity at the individual particle level.

Track: Folding
Session: Protein Evolution, Design and Selection

ABS# 386 | Uncovering Essential Properties of the Truncated Hemoglobin Fold: A Consensus Sequence Study

Jaime Martinez Grundman[1], Eric Johnson[1], Katherine Tripp[1], Soumya Behera[1], Juliette Lecomte[1]
[1]*Johns Hopkins University (MD, USA)*

Artificial proteins derived from the consensus of multiple sequences have gained interest for their increased thermodynamic stability. For b heme proteins, which possess

a dissociable cofactor, several issues merit inspection. We used a set of ~340 sequences from the "truncated" hemoglobin lineage (specifically, group 1 truncated hemoglobins, or TrHb1s, found in prokaryotes and unicellular eukaryotes) to generate a consensus protein (cGlbN). The goals were to determine whether high stability would be apparent in the apoprotein or holoprotein (or both) and what chemical properties would emerge from an average heme environment. By optical and NMR spectroscopy, we show that cGlbN is a highly soluble protein that binds the heme group with two histidine axial ligands without discrimination between the two faces of the porphyrin ring. The stabilities of the apo- and holoproteins are high, but the rate of heme loss from the holoprotein is similar to that measured for extant TrHb1s. Chemical denaturation of the protein with and without heme and monitored by circular dichroism proceeds with a common stable intermediate. NMR analysis of the uniformly 13C and 15N labeled protein identifies secondary structure elements in the apoprotein. We propose that cGlbN can be used as a naïve background within which to encode different functionalities, explore the relation between structure and stability, and assess determinants for heme binding and iron coordination for thousands of TrHb1s. Further work will be aimed at understanding the roles of specific residues (and networks of residues) in determining the inherent pH-linked iron ligation switching behavior of cGlbN, as well as the partial loss of thermodynamic coupling between protein folding and heme binding as a result of consensus design. This work is supported by the NSF through grants MCB-1330488, CHE-2003950 and GRFP-1746891.

Track: Amyloid and Aggregation

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 387 | High Throughput Genetic Dissection of Nucleation Barriers Illuminates the Structure of a Pathogenic Amyloid Nucleus

Tej Kandola[1,2], Randal Halfmann[1], Jiahui Zhang[3], Celeste Sagui[3], Brooklyn Lerbakken[1]
[1]Stowers Institute for Medical Research, [2]The Open University, [3]North Carolina State University (MO, United States)

Protein aggregation into amyloids drives deterministic biological phenomena, including aging, neurodegeneration, and certain forms of programmed cell death. This deterministic aspect of amyloid aggregation derives from the fact that its nucleation -- or initial formation -- is generally far slower than its growth. Elucidating structural features of the rate-limiting nucleus could expedite the development of amyloid-controlling therapeutics, and has, consequently, been a long-standing goal of amyloid research. Nevertheless, the rare and fleeting nature of amyloid nuclei has eluded their characterization by classical biochemistry, structural biology, and computational approaches. We recently developed an assay to circumvent this limitation. DAmFRET uses a photoconvertible fusion tag and high-variance expression systems to quantify protein self-assembly as a function of time and concentration across thousands of femtoliter volume reaction vessels. Using DAmFRET we measured sequence-encoded nucleation barriers across a panel of over one hundred rationally designed variants of a model amyloid-forming protein -- pathologically expanded polyglutamine (Q60). From these data and complementary molecular dynamics and biochemical studies, we deduce that the polyglutamine amyloid nucleus corresponds to the formation of a single steric zipper of three beta strands each containing no less than three glutamine residues. The nine nucleating glutamines are spaced two residues apart in their respective strands. This spacing gives rise to exactly two "reading frames" of polypeptide sequence that encode amyloid nucleation. Remarkably, polypeptides follow alternative nucleating pathways leading to different amyloid structures depending on whether only one, or instead both, reading frames match the pattern. From these constraints we rationally designed and validated a monomeric amyloid nucleus. Finally, we discuss how the characteristic length-dependent pathology and complex phase behavior of polyglutamine emerge directly from the dual reading frames of polypeptide sequence.

Track: Therapeutics and Antibodies

Session: Protein Evolution, Design and Selection

ABS# 389 | Enhancing anti-CoV2 antibody neutralization through peptide fusion

Jonathan Labriola[1], Shane Miersch[1], Gang Chen[1], Francesca Pisanu[2], Arnaldo Caruso[2], Francesca Caccuri[2], Alberto Zani[2], Pier Paolo Pandolfo[3], Jim Rini[4], Gaya Amarasinghe[5], Giuseppe Novelli[2], Sachdev Sidhu[1]
[1]University of Toronto, Department of Molecular Genetics, [2]Tor Vergata, University of Rome, [3]Department of Pathology, Beth Israel Deaconess Cancer Center, Harvard Medical School, [4]University of toronto, [5]Washington University School of Medi (ON, Canada)

The ongoing COVID-19 pandemic is causing major economic strain on most industrialized nations due to

lockdowns and strain on healthcare systems. COVID-19 is caused by a novel severe acute respiratory syndrome coronavirus, termed CoV-2. As with other coronvariuses, it makes use of a Spike protein receptor decorated on its surface to gain entry into its host cell through binding of the angiotensin-converting enzyme 2 (ACE2), found on the surface of alveolar, endothelial, and smooth muscle cells of the lower respiratory tract. Development of therapeutics and vaccines to treat COVID-19 is essential toward restoring normal economic and social life. One therapeutic strategy involves the use of neutralizing monoclonal antibodies (nAbs), which bind the spike protein such that it prevents interaction with the ACE2 receptor. Since the onset of the pandemic, a wide variety of nAbs with therapeutic efficacy against CoV-2 have been developed and given emergency authorization use. However, since CoV-2 is a positive strand RNA virus, variants which which evade nAb neutralization due to mutations in the Spike protein have already emerged. Thus, a longer term solution toward the treatment of COVID-19, is to develop antibody modalities which are both highly potent and broad acting against different CoV-2 variants. Here, we present a strategy for increasing the potency of nAbs, and preventing mutational escape, by fusing a Spike binding peptides to the N-terminus of therapeutic nAbs. These peptide fused nAbs have good drug developability profiles, and neutralize a wide variety of recently emerged CoV2 variants at therapeutically relevant concentrations.

Track: Membrane Proteins

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 390 | Lipid Membrane and Protein Hydration Level Play a Critical Role in GPCR Activation

Nipuna Weerasinghe[1], Steven Fried[1], Andrey Struts[1,2], Suchithranga Perera[1], Michael Brown[1,3]
[1]Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721, USA, [2]Laboratory of Biomolecular NMR, St. Petersburg State University, St. Petersburg 199034, Russia, [3]Department of Physics, University of Arizona, Tucson, AZ 85 (Arizona, United States)

G protein-coupled receptors (GPCRs) are the largest family of cellular transducers in the human genome and influence virtually every aspect of human physiology. Given their importance in health and diseases and their potential for therapeutic intervention through using small molecules as regulators, GPCRs represent the largest family of druggable targets. The standard biochemical view holds that biological lipids and bulk water play negligible roles in the GPCR activation mechanism. Challenging this view, we were able to show that the receptor hydration level and lipid bilayer composition influence the metarhodopsin equilibrium of the archetypical GPCR rhodopsin in native and POPC recombinant membranes. Our results show a flood of ~80 water molecules into the rhodopsin interior during photoactivation, forming a solvent-swollen Meta-II active state. Dehydrating conditions favor Meta-I through the efflux of water from the protein interior, yet favor Meta-II by increasing bilayer thickness and the monolayer spontaneous curvature. The osmotic effect on the protein is more significant than the effect of the lipid bilayer, hence, the overall equilibrium generally shifted to Meta-I. However, small osmolytes favored the Meta-II state, because they can penetrate the protein core, giving a lower excluded volume, decreasing the osmotic effect on the protein. Furthermore, the metarhodopsin equilibrium was shifted towards the Meta-I state in POPC recombinant membranes compared to the native membrane environment. Analysis of transducin C-terminal peptide-binding isotherms revealed that the binding affinity is significantly decreased when the lipid environment is changed from the native lipids to POPC lipids. The POPC lipid membrane has zero-spontaneous curvature that shifts the equilibrium towards the more compact, inactive Meta-I state. By contrast, the native lipid membrane environment has a negative spontaneous curvature that favors the more expanded state of Meta-II. Our results delineate the crucial role of soft matter in regulating the metarhodopsin equilibrium in a membrane environment

Track: Membrane Proteins

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 392 | Effect of voltage on transport kinetics of the sodium dependent sugar transporter vSGLT

Farha Khan[1], Matthias Elgeti[2,3], Samuel Grandfield[1], Aviv Paz[1], Ernest M. Wright[1], Michael Grabe[4], Wayne L. Hubbell[2,3], Jeff Abramson[1], Frank V Marcoline[4]
[1]Department of Physiology, University of California, Los Angeles [2]Jules Stein Eye Institute, David Geffen School of Medicine; [3]Department of Chemistry and Biochemistry; University of California, [4]Cardiovascular Research Institute, University of California, San Francisco (CALIFORNIA, United States)

Sodium-Glucose Transporters (SGLT) are plasma membrane transport proteins critical for maintaining blood glucose levels within a narrow range (4-10 mmol/l). SGLT1 is responsible for absorption of dietary sugars by endothelial cells lining the gut. Both SGLT1 and 2 reside in the proximal tubule of the nephron where they reabsorb glucose from the kidney's filtrate and return it to the bloodstream. Given their unique role in sugar homeostasis, SGLTs are active targets for the treatment of type II diabetes with FDA-approved drugs that specifically inhibit their function: SGLT1 and SGLT2 (sotagliflozin) or solely SGLT2 (dapagliflozin, canagliflozin, and empagliflozin).SGLTs use a transmembrane Na+ electrochemical gradient for the active transport of dietary sugar by transitioning from an outward-open conformation (where they bind Na+ and sugar) to the inward-open conformation (where they release Na+ and sugar) inside the cell. Most of our detailed structural understanding of SGLTs have been derived from the sodium galactose cotransporter of Vibrio parahaemolyticus (vSGLT), a close homolog of human SGLT1 (60% sequence similarity). The previous crystal structures and the double electron-electron resonance (DEER) studies suggest that vSGLT favors an inward-facing conformation. Our present findings indicate that this is mainly due to the use of detergent-solubilized protein samples. This work utilizes a physiologically relevant proteoliposome system with negative membrane potential. We have probed the influence of different membrane potentials on the sugar transport rates and conformation of vSGLT. With this system, we observe that negative membrane potentials increase the rate of sugar transport by shifting vSGLT populations towards outward-open conformations.

Track: Membrane Proteins
Session: Protein Evolution, Design and Selection

ABS# 393 | Transmembrane Domains May Hold the Key to Mitochondrial Localization of Tim17 in Trypanosoma brucei

Chauncey Darden[1], Minu Chaudhuri, PhD[1]
[1]*Meharry Medical College (Tennessee, United States of America)*

Mitochondria are dynamic organelles responsible for many key functions in eukaryotic cells (oxidative phosphorylation, calcium storage, fatty acid oxidation, and etc). These functions are performed by hundreds of mitochondrial proteins of which only ~13-18 are encoded in the mitochondrial genome. The remaining proteins are encoded in the nucleus and imported by mitochondrial protein translocases into the organelle after translation in the cytosol. The translocase of the inner mitochondrial membrane 17 (Tim17) is an integral component of the protein translocase of the inner mitochondrial membrane (TIM complex). In yeast and humans, Tim17 and Tim23 together form one Tim complex and Tim22 forms a separate complex. Tim17, Tim23, and Tim22 are homologous proteins, and each has four transmembrane domains (TMDs). However, in Trypanosoma brucei, which is a protozoan parasite responsible for African Trypanosomiasis, Tim17 is the only homologue that is present in a single TIM complex in a single tubular mitochondrion. TbTim17 is present in a ~1000 kDA protein complex with other TbTim proteins (TbTim62, TbTim54, TbTim42, TbTim50, Rhomboid proteins, and 6 small TbTims). Yet, it is unclear how TbTim17, a nuclear encoded protein, is trafficked to the mitochondria, integrated within the inner membrane, and forms a complex by interacting with other TbTims. Using deletion mutants where we removed the N-termini, C-termini, and each transmembrane domain successively, we found that TbTim17 may possess multiple internal targeting signals (ITS) within TMDs 1 and 4. Among these, ITS located within TMD-4 are capable of translocating a non-mitochondrial protein. Whereas TMD-1 translocates this protein with reduced efficiency. Additionally, using yeast two-hybrid analysis, we were able to show the importance of the C-terminus of TbTim17 in interactions with small TbTims. Further structure-function analyses are on-going to determine the TbTIM17 complex architecture and how this complex engages in translocating different nuclear-encoded mitochondrial proteins in T. brucei.

Track: Single Molecule Studies
Session: Measuring Forces of Biological Systems

ABS# 394 | Enzymatic construction and immoblization of polyprotein for single-molecule force spectroscopy

Peng Zheng[1]
[1]*Nanjing University (Jiangsu, China)*

The recent development of chemical and bioconjugation techniques allows for the engineering of various protein polymers. However, most of the polymerization process is difficult to control. To meet this challenge, we develop an enzymatic procedure to build polyprotein using the combination of a strict protein ligase OaAEP1 (Oldenlandia affinis asparaginyl

endopeptidases 1) and a protease TEV (tobacco etch virus). We firstly demonstrate the use of OaAEP1-alone to build a sequence-uncontrolled ubiquitin polyprotein and covalently immobilize the coupled protein on the surface. Then, we construct a poly-metalloprotein, rubredoxin, from the purified monomer. Lastly, we show the feasibility of synthesizing protein polymers with rationally-controlled sequences by the synergy of the ligase and protease, which are verified by protein unfolding using atomic force microscopy-based single-molecule force spectroscopy (AFM-SMFS). Thus, this study provides a strategy for polyprotein engineering and immobilization.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 399 | Tertiary motifs as building blocks to design protein-binding peptides

Sebastian Swanson[1], Gevorg Grigoryan[2], Amy Keating[1]
[1]*MIT,* [2]*Dartmouth (MA, USA)*

Custom designed protein-binding peptides have the potential to advance research and medicine, but remain difficult to design de novo. Central to the challenge is the large space of candidate binder structures. It is difficult to efficiently sample structures from this space and even harder to determine whether a structure is realizable by amino acids. We propose that protein tertiary motifs (TERMs) can be used to design peptide-protein interactions. TERMs are compact elements that recur in protein structures; they can represent preferred arrangements of residues that are non-local in sequence. We developed a method that uses TERMs to generate segments of backbone structure, or "seeds," around a target protein binding site. Our hypothesis is that seeds can be used as building blocks to generate protein-binding peptides. To test this idea, we evaluated whether seeds could be used to reconstruct peptides in known peptide-protein complex structures. We found that the large majority of peptide interface residues in known structures can be described by a TERM-generated seed from an unrelated protein structure. In contrast, a baseline model that randomly places segments of backbone around the same binding sites describes significantly fewer peptide residues. We developed a protocol to merge overlapping seeds into a complete backbone and used structural comparison metrics and sequence-design algorithms to show that the reconstructed backbones are similar to the original, native peptides. These results suggest that TERMs from the PDB could be applied to designing de novo peptide backbones.

Track: Membrane Proteins

Session: Protein Evolution, Design and Selection

ABS# 400 | Slipknotted and unknotted monovalent cation-proton antiporters evolved from a common ancestor

Joanna Sulkowska[1], Vasilina Zayats[1], Aleksandra Jarmolinska[1], Agata Perlińska[1], Borys Jastrzebski[1], Stanislaw Dunin-Horkawicz[1]
[1]*University of Warsaw (Warsaw, Poland)*

The slipknot topology in proteins has been known for over a decade, however, theirevolutionary origin is still a mystery. We have identified a family of two-domainmembrane transporters as slipknotted, which was overlooked before. Moreover, we foundthat these proteins are distantly related to several families of unknotted membraneproteins, thus we were able to directly investigate the evolution of the slipknot motif.Based on our comprehensive analysis of 17 distantly related proteins families we havefound that slipknotted and unknotted proteins share a common structural motif.Moreover, this region is conserved on the sequence level as well. Our results suggestthat regardless of topology, the proteins we studied evolved from a common unknottedancestor protein containing a single domain. Additionally, our phylogenetic analysissuggests presence of at least seven parallel evolutionary scenarios which led to thecurrent diversity of proteins in question. The tools we have developed in the process cannow be used to investigate the evolution of other repeated-domain proteins.

Track: Structure (X-Ray/NMR/EM)

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 401 | Ligand-induced global conformational changes in TBP-associated factor 1 (TAF1) tandem bromodomains – a novel strategy for targeting the TAF1

Rezaul Md Karim[1], Zachary Schultz[1], Leixiang Yang[1], Justin Lopchuk[1], Jiandong Chen[1], Ernst Schonbrunn[1]
[1]*Moffitt Cancer Center (FL, US)*

Small molecule inhibitors of bromodomains are a viable therapeutic strategy to regulate various cellular functions

of bromodomain-containing proteins, including altered chromatin remodeling and aberrant gene transcription in cancer. Bromodomain-containing protein TBP-associated factor 1 (TAF1) is the largest subunit of general transcription factor TFIID, which triggers transcription initiation by the RNA polymerase II. TAF1 is over-expressed in several cancers and plays a pivotal role in AML1-ETO expressing acute myeloid leukemia (AML). Although two small molecule TAF1 bromodomain inhibitors (BAY299 and GNE-371) have been reported, their mechanism of action in the context of TAF1 tandem bromodomains (TAF1-T) is unknown. In this study, our objectives were to elucidate the binding modes of various TAF1 inhibitors for TAF1-T and discover a novel TAF1-T bromodomain inhibitor. We characterized BAY299 and GNE-371 against TAF1-T and identified AZD6738, an orally bioavailable Ataxia telangiectasia and Rad3-related (ATR) kinase inhibitor, as a TAF1 inhibitor that binds to the second bromodomain (BD2). We used a suite of biochemical and biophysical assays to determine binding affinities of the inhibitors for TAF1 and utilized integrated structural biology techniques to determine high-resolution crystal structures and low-resolution solution models of the TAF1-inhibitor complexes. Our studies with BAY299, GNE-371, AZD6738 and its related analogs show that different inhibitors stabilize different conformational states of TAF1-T through an open-closed transition. We found that BAY299 binds to both the bromodomains (BD1 and BD2) of TAF1 and induces dimerization of TAF1-T. Overall, this study reveals ligand-induced global conformational changes in TAF1 tandem bromodomains, and provides a new structural framework to develop a novel TAF1-ATR dual inhibitor as potential cancer therapeutics.

Track: Chaperones

Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 402 | A very special chaperonin: How does TRiC/CCT achieve tubulin folding?

Daniel Gestaut[1], Yanyan Zhao[1], Junsun Park[2], Boxue Ma[3], Alexander Leitner[4], Miranda Collier[1], Ruedi Aebersold[4], Soung-Hun Roh[2], Wah Chiu[3], Judith Frydman[1]

[1]Department of Biology, Stanford University, Stanford, CA, [2]Department of Biological Sciences, Seoul National University, Seoul, South Korea, [3]Department of Bioengineering, Stanford University, Stanford, CA, [4]Institute of Molecular Systems Bio (CA, United States)

The ring-shaped TRiC/CCT complex is an essential ATP-dependent hetero-oligomer chaperonin responsible for folding ~10% of the proteome, in cooperation with jellyfish-shaped chaperone Prefoldin (PFD). Why TRiC is stringently required for folding of some proteins, such as actin and tubulin, remains unclear. Here we address this question using biochemical and structural approaches on each step of the PFD-TRiC bTubulin folding reaction. PFD-bound bTubulin is highly protease sensitive, indicating a highly dynamic conformation. Of note, substrate binding shifts the conformational dynamics of PFD towards a compact state. Addition of PFD-bTubulin to TRiC in the absence of ATP causes a substantial bTubulin compaction, accompanied by decreased protease sensitivity. cryoEM reconstruction revealed the coiled-coil "tentacle" domains of PFD reach into the TRiC chamber connecting to a substrate-linked but apparently unstructured density at the equatorial bottom of the chaperonin chamber. Addition of nucleotide to the PFD-bTubulin-TRiC complex displaced PFD and leads to formation of fully folded bTubulin within the closed TRiC chamber. Strikingly, CryoEM analysis of bTubulin within the TRiC chamber identified a series of defined and progressively folded bTubulin folding intermediates, in addition to fully folded tubulin. Of note, these folding intermediates engage in specific interactions with specific chaperonin elements inside the closed TRiC chamber wall. As these elements are conserved in evolution, this analysis indicate that TRiC co-evolved with tubulin to meet its folding pathways requirements. Our results, defining how TRiC guides the bTubulin folding pathway and explain its obligate requirement for tubulin folding, have important implications for the coevolution of eukaryotic chaperone-protein networks.

Track: Bioinformatics

Session: Designer Proteins Through Genetic Code Expansion

ABS# 403 | Synthetic Long Peptides as Potential Platform in COVID-19 Vaccine Landscape

Alexandru Tîrziu[1], Florina Bojin[1,2], Oana Gavriliuc[1,2], Virgil Paunescu[1,2]

[1]"Victor Babes" University of Medicine and Pharmacy Timisoara, Romania, [2]Emergency County Hospital "Pius Brinzeu" Timisoara, OncoGen Center, Timisoara, Romania (Timis, România)

INTRODUCTION: Despite the availability of SARS-Cov2 vaccines, there is still an emerging demand of vaccination

platforms. Our candidate vaccine makes use of synthetic long peptides (SLPs), a cancer treatment derived strategy, aiming to induce T cell response mainly from CTLs (cytotoxic T lymphocytes) with cytotoxic effect on infected self-cells.MATERIALS AND METHODS: SLPs comprising a HLA class II-restricted epitope, a cathepsin sensitive linker (LLSVGG) and a HLA class I-specific epitope were designed using an immunoinformatic approach. HLA class I and class II epitopes were predicted using artificial neural networks (ANNs) such as NetMHCPan, IEDB tools and NetMHCIIPan. Their allergenicity (AllerCatPro), toxicity (ToxinPred) and interferon-gamma release (IFNepitope) was tested. Population coverage analysis was performed using IEDB tools. 3D-structure prediction was performed using Rosetta ab initio and structure validation was done using Ramachandran plots and ERRAT score. Molecular docking with Toll-like receptors (TLRs) 2 and 4 was performed using HADDOCK. The structures were refined and their binding energy calculated using PRODIGY web server. Molecular dynamics simulations were performed using GROMACS. Each 10-ns simulation included energy minimization (steepest descent), NVT and NPT equilibration. Temperature, density, RMSD, RMSF and radius of gyration analysis was performed using XMGrace. RESULTS: A pool of 94 SLPs, covering >90% of the most frequent alleles around the globe were tested as being immunogenic, non-allergenic, non-toxic and interferon-gamma-inducing. The ERRAT score of the predicted 3D structures was on average >90%. Molecular docking and refinement provided a mean HADDOCK score of -126 for TLR2 and -119 for TLR4 and a mean binding energy of -12.17 kcal/ mol and -10.4 kcal/mol for TLR4.CONCLUSION: As evidence is supporting the idea that SARS-CoV2 induces a potent cellular response, this approach might be used in designing immune adjuvants or novel vaccine candidates for people who do not have access to the already available vaccines.

Track: Dynamics and Allostery
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 404 | The Role of Protomer Conformation in Multimer Diversity and Dynamics

Eileen Jaffe[1]
[1]*Fox Chase Cancer Center - TUHS (Pennsylvania, United States)*

An unexpected quaternary structure dynamic, first documented twenty years ago, is revealed as a fundamental mechanism for controlling protein function. A homomeric protein assembly dissociated, the protomers underwent a hinge motion disallowed in the multimer, and the new protomer conformation assembled to an architecturally and functionally different multimer. The multimeric interconversion is not in a rapid equilibrium and the hinge motion is the rate determining step. This shape shifting dynamic is a basis for allosteric regulation of enzyme activity, and single amino acid substitutions can dramatically alter the equilibrium among the assemblies, allowing crystal structure determination for both forms. Disturbing this multimeric equilibrium is the molecular basis for an inborn error of metabolism and stabilizing one or another assembly is a mechanism for drug action. This story sets the stage for the general consideration of how different protomer conformations can dictate architecturally different assemblies with different functions. The interconversion of said assemblies can control allostery, allow for alternate moonlighting functions, and/or participate in protein filamentation. Terms used to describe this dynamic include "the morpheein model of allostery", transformers, the "fifth level of protein structure", as well as "breaking the fourth wall". Whatever the name, this quaternary structure shape shifting is a fundamental mechanism for functional protein control. It adds to the myriad ways that Nature defies the one sequence, one structure, one function paradigm that governs the interpretation of biochemical data and bioinformatics analysis.

Track: Therapeutics and Antibodies
Session: Targeted Protein Degradation

ABS# 405 | THE PROTEASOME-ASSOCIATED DEUBIQUITINASE PSMD14 AS A POTENIAL NOVEL ANTI-CANCER THERAPEUTIC TARGET

Clémence MESSMER[1], Saad Menggad[1], Haithem Barbour[1], Salima Daou[1], Nadine Sen[1], Achraf Boutayeb[1], Louis Masclef[1], Oumaima Ahmed[1], Alexandre Rouette[2], Sylvain Meloche[2], Claude Perreault[2], El Bachir Affar[1]
[1]*Centre de Recherche Hôpital Maisonneuve Rosemont (CRHMR),* [2]*Institut de recherche en immunologie et cancérologie (IRIC) (QC, Canada)*

The proteasome, a multi-protein complex, is responsible for degradation of unwanted or improperly folded proteins. This complex has been established as a major target for the treatment of Multiple Myeloma, the second most common blood cancer. Although survival

rates have significantly improved since the introduction of proteasome inhibitors (e.g., bortezomib), ultimately patients develop resistance and relapse. Therefore, this disease remains a major health problem and there is an absolute need for novel therapies. To determine whether deregulation of protein homeostasis can be exploited to treat proteasome inhibitor-resistant cells, we generated bortezomib-resistant cancer cell lines and conducted deubiquitinase (DUB: 100 genes) and ubiquitin-conjugating enzyme (E2: 35 genes) RNAi screens to identify which genes of these families affect protein degradation and subsequently cell survival and cell proliferation. We identified the proteasome-associated DUB PSMD14 as a potential candidate cancer target. Using RNAi or CRISPR/Cas9 approaches applied to sensitive or resistant cancer cells, we found that PSMD14 depletion induces a dramatic decrease of cell proliferation and cell death. Cell death following PSMD14 depletion appears to be caspase-dependent but p53-independent. Moreover, we observed that the extent of histone H2A ubiquitination and HSP70 expression levels are correlated with sensitivity to proteasome inhibition. Thus, these factors could be potential biomarkers for disease prognostic. PSMD14 is required for protein deubiquitination that precedes substrate degradation and its inhibition induces effective killing of bortezomib-resistant cancer cells. Therefore, PMSD14 represents a potential therapeutic target whose inhibition might overcome the acquired resistance to proteasome inhibitors currently used in clinic.

Track: Proteins in Cells

Session: Allostery & Dynamics in Protein Function

ABS# 406 | Protein folding and interactions: from the computer into the test tube and on to the cell

Martin Gruebele[1]
[1]*University of Illinois (Illinois, USA)*

Protein Science has learned much at the molecular level from in vitro experiments, theory, computation, as well as more recently from in-cell and in vivo data. Here I will discuss some of our work on protein folding mechanisms of small, fast-folding globular proteins in vitro, protein folding and binding in the native environment of the cell and in whole organisms, as well as large-scale and long-time molecular dynamics simulations of folding and binding to help us better understand these experiments. I will emphasize the recent in-cell experiments and simulations because the spirit of the Hans Neurath Award is recent advances.

Track: Evolution

Session: Protein Evolution, Design and Selection

ABS# 407 | Computational Enzymology: The structure, function and evolution of enzymes

Janet Thornton[1], Jonathan Tyzack[1], Antonio Ribeiro[1], Ioannis Ribeiro[1], Neera Borkakoti[1], Roman Laskowski[1]
[1]*EMBL-EBI (Cambridgeshire, United Kingdom)*

We seek to understand how enzymes work and how they evolve to perform new enzyme functions using computational biology approaches. In this talk I will focus on three practical topics relevant to understanding enzyme catalysis:- (1) Analysis of basic catalytic machinery in proteins from M-CSA (Mechanism and Catalytic Site Atlas)(2) A new way to estimate substrate transformations and find the most appropriate enzymes or pathways to transform a given substrate into a given product. (3) Towards predicting enzyme mechanisms, given [a reaction+3D enzyme structure+algorithm & rules] Almost all domains that perform catalysis have evolved to include many different family members that work on different substrates. To explore how these have evolved, we have generated family trees, showing the emergence of new specificities, and gaining a broad overview of enzyme evolution as we know it today. But we find that each family is different and careful analysis is required to understand individual mechanisms.These approaches will allow us to analyse complex enzyme families, their mechanisms and their evolution and maybe ultimately help in the design of new enzymes.https://www.ebi.ac.uk/research/thornton/

Track: Therapeutics and Antibodies

Session: Protein Evolution, Design and Selection

ABS# 409 | Identification and Structural Characterization of Anti-Hemagglutinin Antibodies in complex with Hemagglutinin

Nina Moore[1], Hejun Liu[1], Xueyong Zhu[1], Ian Wilson[1]
[1]*Scripps Research (California, United States)*

Influenza viruses are human pathogens that cause respiratory disease and cause seasonal epidemics and occasional pandemics. Influenza remains a persistent human

health threat due to rapid accumulation of mutations over time, which requires annual update of the vaccine strains in the seasonal vaccine. Influenza viruses rapidly evade the immune response by the accumulation of mutations over time (antigenic drift) or through the "mixing" of genes from viruses of different species (antigenic shift). Hemagglutinin (HA) is the glycoprotein on the surface of the virus that is crucial for viral entry into cells and is the main target for antibodies in our immune system. The relentless evolution of influenza virus and accumulation of mutations in the HA highlights the need for generation of a broadly neutralizing antibody response to influenza virus. Here, I present our efforts to address this challenge through characterization of binding affinities of Fabs from newly obtained antibodies to the HA and structural characterization of these Fabs in complex with HA using X-ray crystallography.

Track: Therapeutics and Antibodies
Session: Protein Evolution, Design and Selection

ABS# 410 | The C-terminal domain of P97 protein of Mycoplasma hyopneumoniae activates the Toll-like receptor 5 (TLR5) and increases the immunogenicity of grafted peptides epitopes

Félix Lamontagne[1], Laurie Gauthier[1], Steve Bourgault[1], Denis Archambault[1]
[1]*Université du Québec à Montréal (Quebec, Canada)*

Vaccination remains the most efficient strategy to prevent, or limit, morbidities associated with severe infections. The use of adjuvant in vaccination is often necessary to stimulate a solid immune response and to establish of long-term immune memory. The immunostimulating properties of adjuvants emerge through a myriad of ways; they can facilitate the formation of depot of antigens at the site of injection, enhance delivery to target cells and/or promote the recruitment and activation of immune cells. However, the molecular mechanisms involved are partially understood. Herein, the expression in E. coli of the C-terminal domain of the P97 protein of Mycoplasma hyopneumoniae in fusion with a viral epitope from the influenza A virus (P97c-3M2e) enhanced the amount of antibody directed toward the antigen compared to the epitope alone following mice immunization. It induced a balanced Th1/Th2 with high amount of IgG1 and IgG2a antibodies. Furthermore, we demonstrated that the adjuvant effect of P97c emerges through the activation of the TLR5 and the secretion of the chemokine CXCL8 which is involved in the recruitment of immune cells. Interestingly, P97c shows a degree of conformational similarity with flagellin, the most characterized agonist of TLR5. The present study identifies a novel agonist of the TLR5 and demonstrates its potential as an adjuvant in vaccination.

Track: Membrane Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 412 | Mechanics of an Nramp Family Transition Metal Transporter

Rachelle Gaudet[1]
[1]*Harvard University (MA, United States)*

Transition metal ions (like Mn2+, Fe2+ and Co2+) are nutrients crucial to metabolism in all living cells. The natural resistance associated macrophage protein (Nramp) transporters, found across the tree of life, enable cells to take up a range of essential divalent transition metals. Human Nramps are required for the dietary uptake and endosomal recycling of non-heme iron, and in the innate immune response to intracellular pathogens. Nramps are pH dependent and co-transport protons, and thus have been labeled as proton-coupled symporters. Using crystal structures and biochemistry, we found separate ion pathways for protons and metal ions, and other unusual deviations from the canonical symporter mechanism. I'll present our resulting mechanistic model for Nramp proton-metal co-transport and functional advantages may arise from deviations from the traditional model of symport.

Track: Membrane Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 414 | Elucidating the Binding Pocket of Cell-Free Expressed Neuropeptide Y4 Receptor by Crosslinking Studies

Victoria Behr[1], Annette G. Beck-Sickinger[1]
[1]*Leipzig University (SN, Deutschland)*

The human neuropeptide Y4 receptor (Y4R) belongs to the class A G protein-coupled receptors. Due to its involvement in transducing anorexigenic signals it represents a potential target for the development of drugs against obesity. To specifically target the Y4R, detailed

structural information are necessary. Therefore, we express the receptor using a continuous exchange cell-free system based on the E. coli S30 extract, which is convenient for coupled in vitro transcription/translation. By analyzing pH values, different detergents with varying concentrations and additives, the optimal conditions that allows the successful expression of the Y4R in a soluble form in micelles. For structural investigations, a subsequent photo-crosslinking of the ligand and the identification of the binding pocket by using mass spectrometry is suitable. The required peptides containing the specific photoactive amino acid for photo-crosslinking were successfully synthesized and their activity was confirmed in a cell-based assay. The establishment of this promising system contributes to the understanding of the molecular mechanisms of Y4R-ligand binding.

Track: Chemical Biology
Session: New Protein Post-Translational Modifications

ABS# 415 | Designing Chemoproteomic Probes for Activity-Based Profiling of Tyrosine Phosphatases

Sarah Xi[1], Suk Ho Hong[1], Andrew Johns[1], Neel Shah[1]
[1]*Columbia University (NY, United States)*

Phosphorylation of proteins on tyrosine residues is an important post-translational modification that can modulate protein-protein interactions, protein localization, stability, and enzyme activity. Many phosphorylation events are involved in signaling pathways of high importance, and as such, a wide variety of diseases including cancer may arise from abnormal levels of protein tyrosine phosphorylation. While kinases—enzymes that phosphorylate proteins—have been more widely researched and understood, the regulation and actions of phosphatases—enzymes that dephosphorylate proteins—are still largely unknown. This has raised demand for the development of more tools to study the roles of individual tyrosine phosphatases and their mechanisms of regulation in different signaling events. Here, we describe our efforts to design covalently binding chemical probes that can report on the activity of tyrosine phosphatases across the proteome. We employed a rational design approach from structure-activity relationships of various reported substrates and inhibitors. Candidate compounds were synthesized and characterized in an array of biochemical and cellular assays. Based on these assays, a few candidates that displayed the desired characteristics were augmented with affinity tags and will be explored further in proteomics experiments. We anticipate that our design efforts will yield invaluable new tools to investigate mechanisms of signal transduction by tyrosine phosphatases.

Track: Membrane Proteins
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 416 | Revealing the Structure and Function of Membrane Proteins

Stephen Sligar[1]
[1]*University of Illinois (IL, USA)*

Membrane proteins are involved in numerous vital biological processes, including transport, signal transduction and are the enzymes in a variety of metabolic pathways. Integral membrane proteins account for up to 30% of the human proteome and make up more than half of all currently marketed therapeutic targets. Unfortunately, membrane proteins are inherently recalcitrant to study using the normal toolkit available to scientists, and one is left with the challenge of finding inhibitors, activators and specific antibodies using a denatured or detergent solubilized entity. Often, since membrane proteins are inherently insoluble and prone to aggregation and oligomerization in solution, the active state of interest is obscured. The Nanodisc platform circumvents these challenges by providing a self-assembled system that renders typically insoluble, yet biologically and pharmacologically significant, targets such as receptors, transporters, enzymes, and viral antigens soluble in aqueous media. Because Nanodisc constructs provide a native-like bilayer environment that maintain a target's functional activity, they are a versatile tool in the study of membrane proteins such as ion channels, GPCRs, cytochrome P450s, blood coagulation factors, various toxins and viral entities that form a plethora of pharmaceutical targets. By providing a membrane surface of defined area and composition, Nanodiscs have also been useful in revealing the structure and function of multicomponent complexes that operate at the membrane, such as the Ras cancer signaling cascade. In addition to the opportunities in drug discovery, Nanodiscs provide a nanometer scale vehicle for the in vivo delivery of amphipathic drugs, therapeutic lipids, tethered nucleic acids, imaging agents and active protein complexes. In my presentation I will present our latest results on the use of

Nanodiscs for structural biology, sensing and imaging as well as recent work seeking mechanistic understanding of oncogenic cancer signaling by KRas4b and formation of the focal adhesion complex involved in cell migration.

Track: Enzymology
Session: Allostery & Dynamics in Protein Function

ABS# 417 | Crystal Structure and kinetic studies of UCHL1R178Q reveals the increase in UCHL1 activity in early-onset neurodegeneration

Sebastian Kenny[1], Kwame Brown[1], Aaron Krabill[1], Chad Hewitt[1], Daniel Flaherty[1], Chittaranjan Das[1]
[1]*Purdue University (IN, United States)*

A mutant of Ubiquitin C-terminal Hydrolase L-1 (UCHL1) that was first identified in monozygotic twins of Norwegian origin, UCHL1R178Q, was previously reported to have increased catalytic activity compared to WT UCHL1. This increased activity, interestingly, contributed to early-onset neurodegeneration symptoms. In this report, we investigated some factors that might explain the enhanced activity levels through structure determination of UCHL1R178Q supplemented by fluorescence activity assays and binding studies. UCHL1R178Q was tested for activity by employing a Ubiquitin-AMC (Ub-AMC) hydrolysis assay and showed increased activity in cleaving Ub in vitro. We initially hypothesized that the increase in UCHL1R178Q activity is caused by a better aligned catalytic Cys90-His164 pair, which is misaligned in WT UCHL1. This misaligned catalytic triad is aligned in the wild-type UCHL1 only when ubiquitin chains bind to the the UCHL1, pushing the equilibrium to an active UCHL1 enzyme. The crystal structure of UCHL1R178Q, however, showed that the alignment of its catalytic triad is like those observed in WT UCHL1. Moving forward, we performed dynamic studies to understand the reactivity of the protein in solution. We measured the binding affinity of the different UCHL1 variants with Ub and observed that UCHL1 binding to Ub is three-fold tighter compared to UCHL1R178Q. In addition, we conducted a Michaelis Menten kinetics analysis of this mutant which led us to observe a higher kcat in UCHL1R178Q. We speculate that in solution, the transformation of this catalytic residues to a more aligned solution is more readily achieved, allowing the nucleophilic cysteine to be more reactive, as supported by the higher kcat value.

Track: Membrane Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 418 | Coevolution underlies GPCR-G protein selectivity and functionality

Min Jae Seo[1], Joongyu Heo[1], Kyunghui Kim[2], Ka Young Chung[3], Wookyung Yu[1,4]
[1]*Department of Brain and Cognitive Sciences, DGIST, 333 Techno jungang-daero, Daegu, 42988, Republic of Korea* , [2]*Department of Undergraduate studies, DGIST, 333 Techno jungang-daero, Daegu, 42988, Republic of Korea* , [3]*School of Pharmacy, Sungkyun (Daegu (27), 대한민국)*

G protein-coupled receptors (GPCRs) regulate diverse physiological events, which makes them as the major targets for many approved drugs. G proteins are downstream molecules that receive signals from GPCRs and trigger cell responses. The GPCR-G protein selectivity mechanism on how they properly and timely interact is still unclear. Here, we analyzed model GPCRs (i.e. HTR, DAR) and Gα proteins with a coevolutionary tool, statistical coupling analysis. The results suggested that 5-hydroxytryptamine receptors and dopamine receptors have common conserved and coevolved residues. The Gα protein also has conserved and coevolved residues. These coevolved residues were implicated in the molecular functions of the analyzed proteins. We also found specific coevolving pairs related to the selectivity between GPCR and G protein were identified. We propose that these results would contribute to better understandings of not only the functional residues of GPCRs and Gα proteins but also GPCR-G protein selectivity mechanisms.

Track: Membrane Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 419 | Understanding the role of the CB1 toggle switch in interaction networks using molecular dynamics simulation

Sangho Ji[1], Wonjin Yang[1], Wookyung Yu[1]
[1]*Daegu Gyeongbuk Institute of Science & Technology (Daegu, Republic of Korea)*

The cannabinoid receptor 1(CB1) is a class A G-protein coupled receptor (GPCR) that can exert various effects on the human body through the endocannabinoid system. Understanding CB1 activation has many benefits for the

medical use of cannabinoids. A previous study reported that CB1 has two notable residues referred to as the toggle switch, F3.36 and W6.48, which are important for its activation mechanism. We performed a molecular dynamics simulation with a mutation in the toggle switch to examine its role in active and inactive states. We also examined structural changes, the residue-residue interaction network, and the interaction network among helices and loops of wild-type and mutant CB1 for both activation states. As a result, we found that the aromatic interaction in the toggle switch, similar to an ionic lock, is broken after mutation. We revealed that the energetic changes in the hydrogen-bond network of the Na+ pocket and extracellular ECL2-TM5-ECL3-TM6 interfaces directly linked to the toggle switch contribute to the stability of CB1. Our study explained the role of the toggle switch regarding the energetic interactions related to the Na+ pocket and extracellular loop interfaces, which could contribute to a better understanding of the activation mechanism of CB1.

Track: Protein Interactions and Assemblies
Session: Allostery & Dynamics in Protein Function

ABS# 420 | Colicin-mediated transport of DNA through the iron transporter FepA

Ruth Cohen-Khait[1], Phuong Pham[1], Ameya Harmalkar[2], Melissa Webby[1], Nicholas Housden[1], Emma Elliston[1], Jonathan Hopper[1], Shabaz Mohammed[1], Carol Robinson[1], Jeffrey Gray[2], Colin Kleanthous[1]
[1]Oxford University, [2]Johns Hopkins University (Oxford, United Kingdom)

Colicins are protein antibiotics used by bacteria to eliminate competing Escherichia coli. Colicins frequently exploit outer membrane (OM) nutrient transporters to penetrate through the strictly impermeable bacterial cellular envelope. Here, applying live-cell fluorescence imaging we were able to follow colicin B (ColB) into E. coli and localize it within the periplasm. We further demonstrate that single-stranded DNA coupled to ColB is also transported into the periplasm, emphasizing that the import routes of colicins can be exploited to carry large cargo molecules into bacteria. Moreover, we characterize the molecular mechanism of ColB association with its OM receptor FepA, applying a combination of photo-activated crosslinking, mass spectrometry, and structural modeling. We demonstrate that complex formation is coincident with a large-scale conformational change in the colicin. Finally In vivo crosslinking experiments and supplementary simulations of the translocation process indicate that part of the colicin engages active transport by disguising itself to part of the cellular receptor.

Track: Protein Interactions and Assemblies
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 421 | Unexpected control of protein self-association by translation initiation

Shriram Venkatesan[1], Jeffery Lange[1], Brooklyn Lerbakken[1], Tejbir Kandola[1], Dai Tuschiya[1], Jay Unruh[1], Brian Slaughter[1], Randal Halfmann[1]
[1]Stowers Institute for Medical Research (MO, United States)

Living proteomes are necessarily far from equilibrium. It is paradoxical, then, that reducing protein influx -- which should promote equilibration -- instead, prolongs life. We therefore reasoned that kinetic barriers to equilibration must exist and that these decrease with protein influx. We further hypothesized that the probabilistic nucleation of ordered protein assemblies such as amyloids could underlie those barriers.To investigate the effects of influx on protein self-assembly, we used our quantitative reporter of nucleation-limited protein self-assembly (termed Distributed Amphifluoric FRET or DAmFRET) to probe kinetic barriers to self-assembly of structurally diverse protein assemblies at different rates of translation initiation in living cells. We specifically reduced translation initiation just our protein of interest either by using a uORF or weak Kozak sequences. We achieved comparable total translation of the protein in cells in either case – by reducing plasmid copy number by relaxed selection for fast translating cells. Remarkably, this manipulation dramatically reduced nucleation, even for proteins of diverse structures, and independently of changes in their expression level. Further experimentation revealed that the proteins remained fully competent for polymerization when provided with a pre-existing template, indicating that translation initiation rate specifically influences the kinetic barrier to nucleation. We additionally find that manipulations that enhance polypeptide interaction during translation, enhances nucleation. In addition, our observations are true in both yeast and mammalian cells alike, thereby underscoring the widely conserved nature of this

phenomenon.Microscopy revealed that newly synthesized protein exhibits non-random clustering in the cytosol that decays slower than what simple diffusion would allow for. Given the generality of the effect of translation on the nucleation of a wide range of proteins, the mechanism quite likely involves an increased local concentration of newly translated polypeptides occurring at the polysome. We are exploring the physiological implications of this phenomenon.

Track: Protein Interactions and Assemblies

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 423 | The Structure of Atg11CC3 and its Role in Atg32 Puncta Formation during Mitochondrial Autophagy Initiation

Hannah Margolis[1], Michael Ragusa[2], Kelsie Leary[2], Sarah Katzenell[2]
[1]Massachusetts Institute of Technology, [2]Dartmouth College (Massachusetts, United States)

Selective autophagy is a cellular homeostasis mechanism in which target cargo, including damaged organelles, foreign materials, and protein aggregates, is identified, enveloped in a double membrane sheet, and delivered to the vacuole or lysosome for degradation and subsequent release of basic metabolic building blocks. To ensure the selectivity of the process, selective autophagy receptors (SARs) on the target cargo are activated by damage or other signals and subsequently recruit key autophagic proteins to initiate autophagy. In yeast, recruitment of these key autophagic proteins depends on the scaffolding protein Atg11. Atg11 links damaged cargo to the remaining autophagy machinery via protein-protein interacts at its C and N termini respectively, organizing the autophagy initiation site. Despite its importance for selective autophagy initiation, it is unclear how Atg11 organizes autophagy initiation sites. Here, we provide the first evidence that the middle region of Atg11, coiled coil 3 (CC3), is required to concentrate the mitochondrial autophagy SAR Atg32 into puncta on the mitochondrial surface to induce mitophagy, the selective autophagic capture of mitochondria. We also report the first structure of any part of Atg11, demonstrating that the CC3 forms a parallel homodimer with a dimerization interface provided by a limited number of hydrophobic residues in the center of the domain. Curiously, while we found that CC3 is not required for Atg11 dimerization or interaction with the mitochondrial SAR Atg32, deletion of CC3 or point mutations of this key hydrophobic region led to a loss of both Atg32 puncta formation and delivery of mitochondria to the vacuole. These results suggest that Atg11 self-interactions help to concentrate and organize SARs in a way that is necessary for cargo capture during selective autophagy initiation.

Track: Proteins in Cells

Session: Protein Evolution, Design and Selection

ABS# 424 | Functional Characterization of AlkB homologue protein of Mycobacterium leprae: a possible new drug target.

Pragati Vishwakarma[1], Tapan K. Chaudhuri[1]
[1]Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, New Delhi - 110016, India (Delhi, India)

M. leprae is an obligate intracellular pathogen that has never been cultured axenically due to which several details of its infection, parasitization, and replication are still unknown. However, M. leprae harbors several protein-coding genes that do not have an assigned function yet and are commonly referred to as "hypothetical proteins"1. ML0190 is one of such hypothetical protein involved in DNA repair of bacteria. Recently the researchers reported that the protein contains a Fe (II)/2OG-dependent dioxygenase domain and protects the AlkB-deficient E. coli strain from DNA dealkylation damage2.AlkB is a DNA demethylation enzyme in E. coli. There are nine mammalian homologs of E. coli AlkB that play pivotal role in DNA repair pathway3. These homologs have different substrate preferences and specificity that helps in targeting them for cancer therapeutics (in humans). We are here interested to characterize M. leprae ML0190 based on substrate specificity, further comparing the structure-function of protein with E. coli AlkB and human homologs, which can be used in the future to design the specific target molecule against ML0190. Therefore, we have cloned the ML0190 gene and the expression of the recombinant protein is in progress. The protein will be purified and to be characterized for its physicochemical properties and it's of substrate binding specificities (dsDNA or ssDNA) will be investigated using various spectroscopic tools like fluorescence spectroscopy, CD spectroscopy etc. The outcome of the mentioned studies will throw lights on the protein conformational aspects in finer details, which can be

used to design targeted drugs or inhibitors against M. leprae infection in humans.

Track: Membrane Proteins
Session: Allostery & Dynamics in Protein Function

ABS# 425 | Cell-Free Expression of Photoactivatable Chemerin Variants to Elucidate the CMKLR1 Ligand Binding Site by Crosslinking MS

Kevin Kretschmer[1], Annette Beck-Sickinger[1]
[1]*University Leipzig (SN, Deutschland)*

Obesity is a worldwide problem, which dramatically increases the risk for many diseases like type-2 diabetes, cardiovascular and kidney diseases. Overexpression of the adipokine chemerin occurs primarily in the white fat tissue and correlates with obesity.[1,2] Chemerin conveys its signals through the G protein-coupled receptor chemokine-like receptor 1 (CMKLR1) and leads to differentiation of adipocytes and chemotaxis of leucocytes, which are two driving forces of the disease. To tackle these problems and to modulate the ligand-binding event, it is of major importance to get deeper insights into the chemerin-CMKLR1 binding mode. For a better understanding of protein-receptor interaction, coupled crosslinking and mass spectrometry experiments reveal detailed information of the binding pocket. Photoactivatable protein modifications, which are accessible by continuous cell-free expression, are required for photocrosslinking. By using a S30 extract from E. coli BL21(DE3), photo-methionine was introduced at different positions in chemerin variants. After refolding, biological activity at the receptor CMKLR1 was investigated by inositol phosphate accumulation assays, confirming no loss of activity. Additionally, the CMKLR1 was expressed by cell-free expression and subsequently refolded into DMPC/DHPC bicelles. The correct folding of the synthesized receptor was verified by fluorescence polarization assays. The expression and refolding of the CMKLR1 as well as the photoactivatable chemerin variants are the initial steps for investigating the ligand-receptor interaction in further crosslinking MS analyses. This approach can be used for the development of specific therapeutic agents in the future. References:[1] Bozaoglu K, Bolton K, McMillan J, et al. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology. 2007, 4687-4694.[2] Goralski K. B. et al. "Chemerin, a Novel Adipokine That Regulates Adipogenesis and Adipocyte Metabolism." Journal of Biological Chemistry 2007, 28175–28188.

Track: Peptides
Session: New Protein Post-Translational Modifications

ABS# 426 | Design and synthesis of "on/off-switchable" peptide linkers for the selective immobilization of CyP450 on conductive surfaces

Jan Dirks[1], Annette G. Beck-Sickinger[1]
[1]*University Leipzig (Saxony, Germany)*

J. S. Dirks and A. G. Beck-SickingerLeipzig University, Faculty of Life Sciences, Institute for Biochemistry, Brüderstraße 34, 04103 Leipzig, Germany Electron driven activation of a monooxygenase is of high industrial interest for catalytic processes like synthesis of fatty acids in bioreactors [1]. Recently, Zernia et al. have immobilized the monooxygenase cytochrome P450 (CyP450) from Bacillus megaterium on indium tin oxide (ITO) by strong electrostatic interactions. The C-terminus of the protein has been extended by a peptide linker that consists of mainly basic amino acids. This enabled electron-driven activation of the enzyme without the need of the co-factor NADPH, solely by applying a defined current flow [2]. However, this approach provided an oriented immobilization, but did not distinguish between different orientations of the enzyme on the surface in order to allow an on/off-switchable activity profile. Since the orientation is decisive for the final activity, we used the IMPACTTM [3] system to generate a thioester on the CyP450 C-terminus, which is suitable for peptide linkage. Corresponding α-helical peptides were synthesized on solid support with an N-terminal cysteine for conjugation by native chemical ligation (NCL). These peptide linkers carrying an immobilization sequence and an azobenzene-photoswitch unit, allowed for light-induced enzyme activation. First, a FRET pair was coupled to the peptide linker in order to determine the movement of the peptide structure by toggling the azobenzene unit between an on- and off-state. This novel approach provides insights into the behavior of immobilized protein-peptide constructs on surfaces and generates bioactive surfaces with photo-switchable enzyme activity.[1] C. A. Martinez et al. Curr. Top. Med. Chem. 2013, 13 (12), 1470-1490.[2] S. Zernia et al. ChemCatChem 2018, 10, 525-530.[3] S. Chong et al. Gene 1997, 192, 271-281.

Track: Computational Modeling/Simulation
Session: Protein Structures Through the Lens of Machine Learning

ABS# 427 | Predicting the complex conformations of protein/intrinsically disordered peptide complexes using a method termed a/dPaCS-MD

Duy Tran[1], Akio Kitao[1]
[1]*Tokyo Institute of Technology (Tokyo, Japan)*

Recently, we have developed the method of predicting the complex conformations between protein and intrinsically disordered peptide using so-called a/dPaCS-MD [JCTC 16, 2835 (2020)]. a/dPaCS-MD consists of several cycles of MD simulations followed by the relaxation simulations of selected bound conformations. Each cycle includes multiple independent simulations where the initial structure of each simulation is chosen from the predecessor cycle based on the given criteria. In a/dPaCS-MD, the criteria is switched between association and dissociation mode due to the nature of contacts between the protein and the peptide. Using Markov State Modeling with all obtained trajectories, we show that the method can reliably predict bound conformations as well as kinetic constants.

Track: Peptides
Session: Protein Evolution, Design and Selection

ABS# 428 | Designing Novel Antimicrobial Peptides Against Multi-drug Resistant Bacteria

Shravani Bobde[1], Fahad Alsaab[1,2], Guangshun Wang[3], Monique Van Hoek[1]
[1]*George Mason University,* [2]*King Saud bin Abdulaziz University for Health Sciences,* [3]*University of Nebraska Medical Center (Virginia, United States)*

Antimicrobial peptides (AMPs) are ubiquitous amongst living organisms and are part of the innate immune system with the ability to kill pathogens directly or indirectly by modulating the immune system. AMPs have potential as a novel therapeutic against bacteria due to their quick-acting mechanism of action that prevents bacteria from developing resistance. Additionally, there is a dire need for therapeutics with activity specifically against gram negative bacterial infections that are dangerous and difficult to treat. Development of new antibiotics has slowed in recent years and novel therapeutics (like AMPs) with a focus against gram negative bacteria are needed. We designed eight novel AMPs, termed PHNX peptides, using ab initio computational design (database filtering technology on APD3 dataset of natural AMPs against gram negative bacteria as described by Wang et al.) and assessed their theoretical function using published machine learning algorithms as well as measured their activity in our laboratory. These AMPs were tested by others and demonstrated greater activity against gram-negative MDR Escherichia coli than MRSA (Methicillin Resistant Staphylococcus aureus) bacteria and demonstrated low hemolytic activity against human red-blood cells.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 429 | Self-Assembling Functional Complexes of Protein Nanobuilding Blocks with a Trimeric Lectin

Haruka Kaigawa[1], Hiroaki Tateno[2], Ryoichi Arai[1]
[1]*Shinshu University,* [2]*National Institute of Advanced Industrial Science and Technology (Nagano, Japan)*

Lectins, sugar-binding proteins, are widely distributed in almost all organisms, including animals, plants, bacteria, and viruses. In recent years, applications of lectins become important for screening of potential biomarkers, but lectins have not used broadly because some lectins have a weak affinity for their target glycan. To improve binding affinity, many lectins adopt a strategy of multivalency through oligomerization. Recently, we developed a protein nanobuilding block (PN-Block), WA20-foldon, by fusing an intermolecularly folded dimeric de novo WA20 protein and a trimeric foldon domain of T4 phage fibritin (Kobayashi et al., JACS, 2015). WA20-foldon formed several types of self-assembling nanoarchitectures in multiples of 6-mers, including a barrel-like hexamer and a tetrahedron-like dodecamer. However, development of PN-Block complexes with useful functions have been a future task.In order to create self-assembling functional complexes, we employed a trimeric lectin, Ralstonia solanacearum lectin (RSL), which binds to L-α-Fucose, as a component of PN-Block. Further improvement of avidity may be expected by formation of higher multimeric complexes. We designed and constructed a lectin nanobuilding block (LN-block), WA20-RSL, by fusing the dimeric WA20 and the trimeric RSL. The WA20-RSL fusion

protein was expressed in Escherichia coli and purified by immobilized metal affinity chromatography. Size exclusion chromatography-multi angle light scattering (SEC-MALS) and small angle X-ray scattering (SAXS) analyses indicated that the WA20-RSL forms hexamer, dodecamer, and octadecamer, in multiples of 6-mer. Surface plasmon resonance (SPR) analysis showed that the WA20-RSL complexes strongly bind to L-α-Fucose. These results indicated that functional complexes of the lectin nanobuilding block WA20-RSL was successfully developed.

Track: Enzymology
Session: Designer Proteins Through Genetic Code Expansion

ABS# 430 | Multi point mutagenesis of formate dehydrogenase

Natalia Galanicheva[1,2], Pavel Parshin[1,2,3], Leonid Shaposhnikov[2], Denis Atroshenko[1,2,3], Svyatoslav Savin[1,2,3], Anastasia Pometun[1,2,3], Vladimir Tishkov[1,2,3]
[1]Federal Research Centre "Fundamentals of Biotechnology" of RAS, Moscow, Russia, [2]Lomonosov Moscow State University, Moscow, Russia, [3]Innovations and High Technologies MSU Ltd, Moscow, Russia (Moscow, Russian Federation)

NAD(P)+-dependent formate dehydrogenase (FDH, EC 1.2.1.2.) is a well-known enzyme for fundamental study and practical application. FDH from bacterium Pseudomonas sp. 101 (PseFDH) is one of the most stable enzyme among the known FDHs. Formate dehydrogenase is actively used for coenzyme regeneration of NADH or NADPH in different biocatalytic processes as well as for determination of formate and NAD+ in complex samples.Previously, single point mutant PseFDHs (S131A, E170D, S160A) as well as multi point mutant PseFDH SM4S (major amino acid changes C145S, A198G and C255A) with increased thermal and chemical stability and/or improved Km for both substrates have been obtained in our laboratory. Previously, it was also found that mutation C145A in the wild-type enzyme resulted in improvement Km values followed by decrease of thermal stability. In this work positive amino acid changes S131A, E170D, S160A were sequentially introduced into PseFDH SM4S. In final mutant PseFDH Ser145 was changed with Ala residue.Analysis of thermal inactivation kinetics revealed, that the most thermal stability mutant form is PseFDH SM4S S131A/E170D/S160A. The substitution of glutamic for aspartic acid at position 170, together with the substitution of serine for alanine at positions 131, 160 leads to significant stabilization of PseFDH. The other PseFDH mutants showed stability similar to PseFDH SM4S. The mutant form SM4S/S131A/E170D/S160A/S145A has the highest catalytic efficiency for both substrates.This work was financed by the grant of the President of the Russian Federation to support young Russian scientists (MD-349.2021.1.4).

Track: Chemical Biology
Session: Allostery & Dynamics in Protein Function

ABS# 431 | Developing potential inhibitors for alpha viral proteases

Sparsh Makhaik[1], Kristalle G Cruz[1], Jeanne A Hardy[1]
[1]University of Massachusetts at Amherst (MASSACHUSETTS, United States)

Chikungunya and Mayaro virus are arboviral infections which have infected millions of people across 60 countries in the past 10 years alone. Ineffectiveness in controlling the mosquito vectors that spread these viruses and absence of any antiviral therapy or effective vaccines for their treatment increases the risk of these contagions evolving into explosive epidemics in near future. Pathogens causing these infections are enveloped positive-sense single-stranded RNA viruses which encode a protease that plays a critical role in viral replication and maturation. Since the protease is essential to viral life cycle, it is a lucrative drug target for treating these diseases. Our aim is to develop high quality protease inhibitors for Chikungunya virus protease NSP2 (CHIKVP) and Mayaro virus NSP2 protease (MAYVP) by performing activity-based screens using high throughput screening (HTS). We have synthesized protease substrates with different FRET pairs and varying amino acid lengths to optimize the best conditions for performing HTS. Considering that there are no findings about any sort of inhibitors for these proteases, we aim to develop both active and allosteric site inhibitors to explore which is more effective. To achieve this, we are in a process of screening a library of fragment-based fluorine compounds against the viral protease using biomolecular 1D and 2D NMR to identify suitable hits and ultimately exploit the identified compounds for investigating for structure-activity relationship (SAR). We also aim to thoroughly understand the structural interactions between the inhibitor and protease using NMR and X-ray crystallography techniques and utilize the knowledge thus gained to develop more effective antiviral therapeutics in future.

Track: Folding

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 432 | **Protein-Crowder interactions: Biophysical studies and NMR spectroscopy reveal an interplay of enthalpic and entropic effects on the structure and thermal stability**

Shivkumar Sharma Irukuvajjula[1], Jithender Reddy[2], Ramakrishna Vadrevu*[1]
[1]Department of Biological Sciences, Birla Institute of Technology and Science, Pilani - Hyderabad Campus, Jawahar Nagar, Shamirpet, Hyderabad - 500078, Telangana, India, [2]NMR Division, Department of Analytical & Structural Chemistry, CSIR-Indian Ins (Telangana, India)

Macromolecular crowding is being studied thoroughly on a wide variety of proteins. A plethora of data are available in the literature suggesting the role of entropic excluded volume effect and enthalpic quinary interactions of different crowders on proteins. Yet, a unified theory is absent. The effect of crowding appears to be controlled either enthalpically or entropically and depends on protein, molecular crowder properties and also the crowding conditions. Using a combination of biophysical approaches including far/near UV circular dichroism, tryptophan fluorescence, 8-anilinonapthalene-1-sulfonic acid (ANS) binding, nuclear magnetic resonance (NMR), we probed the effect of molecular crowding using polyethylene glycol (PEG, 20kDa); and Ficoll-70 (70 kDa) on the structure and stability of a bacterial protein involved in signaling pathways. Combined analysis from CD, fluorescence indicates that PEG moderately destabilizes the structure and stability. Noticeable changes in structure and stability are absent in the presence of Ficoll. However, interestingly, NMR chemical shift perturbations (CSPs) and line broadening reveal protein-PEG interactions. Further, evidence for the interaction comes from decreased thermal stability measured in the presence of magnesium and PEG together. By combining the CSPs and decreased thermal stability in the presence of magnesium, it appears that crowding from PEG leads to subtle conformational rearrangement, distorted magnesium binding and loss of magnesium-binding induced enhancement of thermal stability of the protein. Surprisingly, while no CSPs are observed in the presence of Ficoll, still, a decrease of thermal stability in the presence of Ficoll and magnesium together is observed. Thus, based on these data, we propose that enthalpic contributions dominate in the case of PEG, whereas, with Ficoll, there seems to exist a balance between the two effects. However, both crowders appear to be distorting magnesium binding to the protein in a manner that leads to a decrease in the thermal stability of the protein.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 433 | **Hepatitis C Virus Envelope Protein E2 Based Antigen Design and Characterization for Vaccine Development**

Lia Maria Cucos[1], Teodor Asvadur Sulea[1], Ioana Caras[2], Laurentiu Spiridon[1], Crina Stavaru[2], Norica Nichita[1], Costin Ioan Popescu[1]
[1]Institute of Biochemistry of the Romanian Academy, [2] Institute for Medicine-Military Research and Development "Cantacuzino" (Bucharest, Bucharest)

Over 71 million individuals are infected with Hepatitis C Virus (HCV) worldwide. Current antiviral drugs are very efficient but also costly, thus accentuating the need for a prophylactic treatment. The main target in the development of a vaccine is the viral envelope protein E2, which binds the CD81 receptor during viral entry. The HCV E2 412-423 epitope is involved in CD81 binding and it is targeted by broadly neutralizing antibodies (nAbs). This epitope has been found to exhibit multiple conformational states due to protein flexibility. A beta hairpin conformation of the 412-423 epitope was found to co-crystalize with a nAb. The objective of our study is to construct and characterize novel HCV E2 derived antigens that elicit a potent neutralizing humoral response by stabilizing the 412-423 epitope in the beta hairpin conformation. Homology modelling, free energy calculation, corelated motion analysis and molecular dynamics were used to predict mutations which stabilize the beta hairpin conformation of the 412-423 peptide. A subset of HCV E2 mutants were expressed in HEK 293T cells and their stability and intracellular trafficking were determined. An antigen candidate has been expressed in suspension HEK293 cells and purified by affinity and size exclusion chromatography to reach 95% purity. Using ELISA, the purified HCV E2 mutant was shown to have an improved binding to an antibody recognizing the beta hairpin conformation while drastically decreasing the binding to an antibody recognizing the linear form of the 412-423 epitope. The antigen was further used to immunize BALBc mice to determine the immunogenicity. We found that the antigen induced mainly a Th2 response comparable to the wild-type antigen control. The presented data suggest

that by de novo antigen design, the HCV E2 412-423 epitope was stabilized in the desired conformation and the mutant has good immunogenic properties.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 434 | Design, Production and Characterization of Novel HBV Chimeric Antigens

Ana-Maria Madalina Pantazica[1], Olivia-Mihaela Dobrica[1], Cristina Scurtu[1], Catalin Tucureanu[2], Crina Stavaru[2], Adrian Onu[2,3], Norica Nichita[1]

[1]Institute of Biochemistry of the Romanian Academy, [2]Institute for Medicine-Military Research and Development "Cantacuzino", [3]"Titu Maiorescu" University (Bucharest, Romania)

Over 250 million people worldwide are infected with the Hepatitis B virus (HBV) and 800,000 deaths occur yearly due to liver complications. Although treatments exist, preventative vaccination remains the most successful method of controlling HBV infection. While effective, the commercial HBV vaccine, based on the small (S) viral envelope protein, involves high production costs and does not elicit protective antibody titers in 10% of the vaccinated population. Previously, we developed a novel vaccine candidate by incorporating an immunogenic determinant (aminoacids 21-47, genotype D) derived from the preS1 domain of the large envelope protein into the S antigenic loop, which was expressed in both mammalian and plant systems, as a low-cost alternative for antigen production. The antigen had better immunogenic properties than the commercial S vaccine; however, its assembly into subviral particles (SVP) was impaired. Here, we aimed to further optimize the design of chimeric S/preS1 HBV antigens by varying the size and position of the inserted sequence, to improve assembly, secretion, and ability to induce HBV neutralizing antibodies across genotypes. Of the four constructs tested, one had superior properties and was further produced in HEK293T cells and its molecular characteristics were determined by ELISA, western blot, and pulse-chase experiments. The antigen was then purified for immunological studies. Our results showed that the antigen is expressed at high levels in mammalian cells and retains the capacity of assembly into SVPs. Moreover, its purification efficiency was significantly improved when compared to the previously developed antigen. Immunological studies revealed that the novel antigen elicited a strong humoral and cellular immune response, as well as infection neutralizing antibodies, suggesting that it might be a promising candidate for alternative vaccine development. Acknowledgment: The research leading to this work has received funding from the ConVac Grant PCCDI-62 and EEA Grants 2014-2021, SmartVac Project, Contract No 1/2019.

Track: Enzymology

Session: Protein Evolution, Design and Selection

ABS# 435 | Phenylacetone monooxygenase: Coenzyme specificity

Pavel D. Parshin[1,2], Ustina A. Martysuk[1,3], Anna V. Popinako[2], Svyatoslav S. Savin[1,2,4], Vladimir I. Tishkov[1,2,4], Anastasia A. Pometun[1,2,4]

[1]Chemistry Department, M.V.Lomonosov Moscow State University, Leninskie Gory 1-3, 119991, Moscow, Russian Federation., [2]Federal Research Centre "Fundamentals of Biotechnology" of RAS, Moscow, Russian Federation; Leninskiy prospect Str., 33/2, 11907 (Moscow, Russian Federation)

Phenylacetone monooxygenase (PAMO) from Thermobifida fusca is a Bayer-Williger monooxygenase (BWMO) capable of oxidizing aldehydes and ketones to esters. In comparison with most of BWMOs PAMO has high temperature and chemical stability, which makes it promising enzyme for use in real biotechnology processes. In spite of listed advantages, wide usage of PAMO alike with other monooxygenases is obstructed by high cost of required NADPH. There is an efficient way to solve the problem - to create a NADPH regeneration system by another enzyme. For example, formate dehydrogenase (FDH). Although there are several NADPH-dependent FDHs, most of them use NADH. Therefore, it would be much more convenient if PAMO used the non-phosphorylated form (NADH) as a coenzyme.The presence of 3D structure of PAMO in the PDB databank made it possible to use computational modeling methods and select two amino acid residues (T218 and K336) presumably involved in phosphate group binding in active site of PAMO. Moreover, change of similar amino acid residues led to a change in the enzyme specificity towards NADH according to data on related Bayer-Villiger monooxygenases. Here we report results of structure modeling and properties of obtained mutant enzymes. To prepare mutant PAMO variants an

enzyme with increased expression level and stability was used.The reported study was funded by Russian Foundation for Basic Research according to the research project № 20-34-90120

Track: Protein Interactions and Assemblies
Session: Targeted Protein Degradation

ABS# 436 | Endoplasmic Reticulum Signaling Pathways Induced by Hepatitis B Virus Infection

Alina-Veronica Ghionescu[1], Mihaela Uță[1], Cătălin Lazăr[1], Petruța Alexandru[1], Norica Nichita[1]
[1]*Institute of Biochemistry of the Romanian Academy (Bucharest, Romania)*

Hepatitis B virus (HBV) infection is a major global health problem, with approximately 250 million people chronically infected worldwide. HBV is a small DNA virus belonging to the Hepadnaviridae family and is a causative agent of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. HBV consists of an enveloped nucleocapsid containing a partially double-stranded relaxed circular DNA (rcDNA) and viral envelope proteins (S, M, L), which are translocated in the endoplasmic reticulum (ER), where oligomerization, N-glycosylation, and folding occur. Several studies have shown that viruses modulate ER signaling pathways to facilitate their replication via the unfolded protein response and the ER-associated degradation (ERAD). In a previous study, we found that HBV infection activates the ERAD pathway, more specifically by increasing ER degradation-enhancing alpha-mannosidase-like protein 3 (EDEM3) expression, a lectin that recognizes terminally misfolded glycoproteins and delivers them to the ERAD pathway. This work aimed to investigate the mechanism by which ERAD and associated molecular machinery are involved in the HBV life cycle. We observed a significantly enhanced HBV production in EDEM3-overexpressing cells and a reduction in the level of expression of ER stress markers like inositol-requiring enzyme 1 (IRE1), eukaryotic initiation factor 2 subunit α (eIF2α), and phospho-eIF2α. This suggests that by decreasing the ER stress, EDEM3 might promote viral infection. Furthermore, we generated EDEM3 –knockout in the HepaRG cells that are permissive for HBV infection, by using the CRISPR/Cas9 technology. Protein knockdown was confirmed by western blot. Studies are currently conducted to elucidate the role played by the ER stress on the HBV life cycle, which can lead to the development of novel therapies for chronic hepatitis B.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 437 | De Novo Design of Site-specific Protein Binders Using Surface Fingerprints

Sarah Wehrle[1], Alexandra Van Hall-Beauvais[1], Pablo Gainza[1], Zander Harteveld[1], Andreas Scheck[1], Freyr Sverrisson[1], Tan Shuguang[2], Aaron Petruzzella[3,4], Stéphane Rosset[1], Sandrine Georgeon[1], Elisa Oricchio[3,4], Georg
[1]*Institute of Bioengineering, École Polytechnique Fédérale de Lausanne and Swiss Institute of Bioinformatics, Lausanne, Switzerland,* [2]*CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Science (Vaud, Switzerland)*

Protein-protein interactions (PPIs) play a critical role in virtually all cellular functions. While rational protein design approaches are advancing, the computational design of novel PPIs to a defined target site remains a substantial challenge. De novo design of PPIs presents a strict test of our understanding of the underlying principles that drive molecular recognition. Successful design opens new possibilities for the development of protein-based therapeutics and the targeting of yet undruggable sites.Using the MaSIF (molecular surface interaction fingerprinting) software, developed by our lab, we identified complementary molecular surfaces, using learned fingerprints, to two different disease-relevant targets, PD-L1 and the SARS-CoV-2 spike protein. We identified binding fragments from these fingerprints and transferred them to protein scaffolds to confer stability and additional contacts.PD-L1 is an immune checkpoint receptor and an important target in cancer immunotherapy. We designed two binders engaging PD-L1. After experimental optimization both binders showed high affinities to their target. Crystallography of the protein complexes and saturation mutagenesis scanning of the binding interface showed excellent agreement to the computational design.The SARS-CoV-2 virus uses the receptor binding domain (RBD) of the spike protein to interact with ACE2, a receptor on human cells, to enter and infect cells. We designed proteins to bind to RBD, specifically overlapping with the ACE2 binding site. The obtained binder has nM affinity to the target and competes ACE2 binding, further optimization is ongoing.In conclusion, we

computationally designed de novo binders for two highly relevant and structurally diverse targets. The designed binders are specific to the selected sites and disrupt the native binding interaction in vitro. These results highlight that MaSIF represents an opportunity for the development of novel therapeutics and a novel route for the design of novel PPIs.

Track: Amyloid and Aggregation
Session: Protein Evolution, Design and Selection

ABS# 438 | Rational design of self-assembling peptide amyloid material: Study of morphological features and exploring functionality

Aishwarya Natarajan[1], Krishnan Rangan[2], Aruna Jyothi Kora[3,4], Ramakrishna Vadrevu[1]
[1]Department of Biological Sciences, Birla Institute of Technology and Science-Pilani, Hyderabad Campus, Shameerpet, Hyderabad - 500078, Telangana, India, [2]Department of Chemistry, Birla Institute of Technology and Science - Pilani, Hyderabad Campus, (TELANGANA, India)

Self-assembly of peptides is a source of inspiration from nature and evolution for the formation of nano-scale biomaterials. The potential role of peptide based self-assembled amyloid biomaterials with specific applications in various fields, via functionalization is emerging rapidly. Being highly ordered and stable structures, generation of novel biomaterials by harnessing the innumerable potential of such amyloid structures via modulating the amino acid sequence, pH, temperature could result in the formation of self-assembling peptides, with a variety of morphologies such as nanofibers, nanotubes, nano-tapes, hydrogels, etc. Design, structural and morphological characterization of self-assembling structures is of great importance as it can bridge the gap between the understanding of natural biomaterials and adapting lessons from nature for diverse functional roles. Introduction of amino-acid residues to these stable structures for functionality such as metal binding, cell adhesion and/or sensing etc. can be explored. In our attempts to design amyloid forming peptide sequences towards functionalization, a short peptide, KLVFF, that is a part of the longer 42 amino acid peptide that forms amyloid fibrils and implicated in Alzheimer's disease has been chosen as a suitable candidate for generating designable material introducing specificity for heavy metal binding. The specificity for metal binding is explored by judicious placement of amino acid driven by the residues mimicking the metal binding pockets of proteins. The preliminary characterization of the designed peptides, with ThT fluorescence, Scanning Electron Microscopy and Atomic Force Microscopy indicated the formation of self-assembled amyloid like fibrils. The binding of toxic metals to the self-assembling peptides was assessed through spectroscopic techniques and the results of the study suggesting binding of will be presented.

Track: Proteins in Cells
Session: Protein Evolution, Design and Selection

ABS# 439 | The Role of NPC1 Protein in Pigmentation

Adriana-Alina Rus[1], Stefana M. Petrescu[1]
[1]Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy (Bistrita, Romania)

Niemann-Pick type C1 (NPC1) is a late-endosomal/lysosomal transmembrane protein involved in cholesterol and glycolipids transport. The deficiency of NPC1 protein results in the accumulation of cholesterol and glycolipids in the endosomal–lysosomal system of the cells. Our study aims to get insights into the role of NPC1 protein in pigmentation. In the process of pigmentation are involved specific proteins, such as tyrosinase, tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2), are influenced by NPC1 protein.The first step towards the study of the impact of NPC1 protein in pigmentation was the generation of a stable MNT-1 NPC1-knockout cell line. We used the CRISPR-Cas9 system to disrupt the NPC1 gene, which provides the Niemann-Pick type C1 cholesterol storage phenotype in MNT-1 cells. After transfection and cell colony selection, the cell lines were verified by filipin staining for cholesterol subcellular distribution and immunoblotting for NPC1 expression. The phenotype of Niemann-Pick type C1 disease was assessed by several methods including lysosomal measurement by flow cytometry and measurement of glycosphingolipids by HPLC. Further, we focused to identify the cause of hypopigmentation, which was observed in NPC1-KO MNT-1 cell lines. To this aim, we have investigated the expression levels of the specific enzymes involved in pigmentation. The expression of tyrosinase, TRP-1 and TRP-2 was reduced, but the activity of tyrosinase remained unchanged. Also,

the mRNA level of tyrosinase was unaffected by the NPC1 deficiency. Forward we sought to find out if tyrosinase traffic is perturbed or its turnover is affected. Taken together these experiments suggest that the NPC1 protein plays an important role in pigmentation mainly because it influences the tyrosinase pathway.

Track: Proteomics

Session: Protein Evolution, Design and Selection

ABS# 440 | New Potential Melanoma-Associated Prognostic Biomarkers Before and After Vemurafenib Treatment

Ioana V. Militaru[1], Cristian V. A. Munteanu[1], Ștefana M. Petrescu[1]

[1]*Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy (Campina, Romania)*

Currently, new biomarker discovery represents one of the most important concerns, due to rapid drug resistance development and difficulty in accurate diagnosis at the correct time. Finding of a specific set of biomarkers to improve treatment response and patient outcome is in real need. In this research, we aimed to identify and validate biomarkers for early melanoma diagnosis and to propose prognostic biomarkers for advanced melanoma. We achieve these by comparing melanoma cell lines with different metastatic potential. Cells were compared by taking into account their migration strength and were subsequently classified based on this criteria. Several well-known biomarkers were identified following comprehensive LC-MS/MS proteomic analysis. Furthermore, other significantly differentially expressed proteins were correlated to disease severity. Several proteins were associated with high migration capacity. We should mention here the presence in high amounts of integrins, annexins and several other proteins like moesin and CD44, as major players in promoting tumor cell invasiveness. The identified proteins were subclassified according to their subcellular localization and biological process. To obtain insights into cellular pathways involved in drug resistance we also established vemurafenib resistant cell lines and analysed them. Using mass-spectrometry based proteomics, several proteins were found to be upregulated in resistant cell lines and the observed changes might be linked to a more aggressive phenotype. A correct evaluation of cell behavior under long-term vemurafenib treatment can help to understand cellular mechanisms involved in acquiring drug resistance and concerned proteins.

Track: Bioinformatics

Session: Protein Evolution, Design and Selection

ABS# 441 | Searching sequence databases for functional homologs using profile HMMs: how to set bit score thresholds

Jaya Srivastava[1], Ritu Hembrom[1], Ankita Kumawat[1], Petety V Balaji[1]

[1]*Indian Institute of Technology Bombay (Maharashtra, India)*

Profile HMMs can be used to search sequence databases for functional homologs. Choosing an optimal bit-score threshold is a critical step in this approach. This step is not straightforward because sequence homology does not necessarily translate to functional homology and there are not adequate experimental data for many families. The workflow for setting the optimal threshold needs to be customized depending upon the extent of functional divergence and experimental data available. We illustrate ways in which workflow can be customized using two each of single domain and multi-domain protein families as case studies. An ROC curve was used for the UDP-HexNAc 6-dehydrogenase profile. Additional use of a scatter plot of bit scores of hits that were common to UDP-HexNAc 6-dehydrogenase and NDP-hexose 6-dehydrogenase profiles helped in setting / refining thresholds for these two profiles. ROC curve was inadequate for the lysozyme C and lactalbumin profiles; a scatter plot of scores for common hits became essential. SH3 and GT-A domains are typically found as a part of multidomain proteins, and in such proteins, functional annotation necessarily involves demarcation of domain boundaries. The Pfam database has 37 profiles for SH3 domains. Some of the sequences are hits to more than one family, and this issue could not be resolved by merely choosing a higher or lower bit score; instead, specificity was achieved by position weight matrices corresponding to unique motifs. The GT-A domain in GT2 family of glycosyltransferases show high sequence and functional divergence. Using a length threshold became necessary along with bit score threshold to increase the specificity of GT-A profile.This study illustrates the limitations of using profile HMMs for functional annotation and suggests some possible ways to overcome them at least partially.

Track: Structure (X-Ray/NMR/EM)
Session: Allostery & Dynamics in Protein Function

ABS# 442 | The Structural and Dynamic Analysis of the E. coli Clamp Loader

Sam Mahdi[1], Dmitry Khorzhnev[1]
[1]*UConn (Connecticut, United States)*

The bacterial clamp loader is a heteropentamer protein consisting of γ, δ, and δ' in a 3:1:1 ratio, respectively. It is responsible for coordinating leading and lagging strand synthesis, the rate of helicase unwinding, and the displacement of single stranded binding proteins (SSBs) to allow nucleotide addition by the polymerase. In addition, it is also responsible for loading the bacterial processivity factor, the beta-clamp. The bacterial clamp loading activity is poorly understood however, with only static crystal structures available for the apo, ATP, and ADP bound forms of the clamp loader. These structures revealed γ, δ, and δ' bind via their collar domains, and the ATP binds γ in between its mini and lid domains. To probe both the structural and dynamics of the clamp loader and its clamp loading activity, we will be using nuclear magnetic resonance (NMR) to study this system. To mitigate the size limiting factors of NMR, each domain of γ, δ, and δ' has been individually cloned, expressed, and purified. The full-length proteins have also been successfully cloned, expressed, purified, and assembled into the clamp loader. Fusions of the mini and lid domains of γ have also been constructed and NMR titrations have been conducted to study mechanism of binding of the clamp loader to ATP. Information obtained from the individual domains, fused-domains , full length proteins, and the clamp loader will be analyzed together to elucidate its mechanism of action.

Track: Proteomics
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 443 | Understanding Cu Toxicity in E. coli using Protein Folding Stability Measurements

Nancy Wiebelhaus[1], Jacqueline Zaengle-Barone[1], Kevin Hwang[1], Katherine Franz[1], Michael Fitzgerald[1]
[1]*Duke University, Department of Chemistry (North Carolina, United States)*

The characterization of protein-metal interactions, specifically those involving copper, is important for understanding the molecular basis of metal toxicity in both bacteria and humans. The objective of this research was to identify the E. coli protein targets of Cu using a mass spectrometry-based method for the proteome-wide analysis of protein-metal binding, termed one-pot STEPP-PP (semi-tryptic peptide enrichment strategy for proteolysis procedures with pulse proteolysis). As part of this work E. coli cells were treated under conditions that exposed the cells to varying levels of copper stress, the cells were lysed, and the proteins in the cell lysates were analyzed by STEPP-PP analysis to identify E. coli proteins that were differentially stabilized in the presence of increasing amounts of Cu.The STEPP-PP analysis enabled close to 600 E. coli proteins to be assayed across four treatment conditions, which included a control, treatment with Cu alone, and treatment with Cu in combination with two ionophores to facilitate copper delivery. Differentially stabilized proteins across the different conditions were selected by a student two-tailed t-test. A total of 37 protein hits were identified in the low Cu stress condition and between 70-80 protein hits were identified in conditions containing Cu in the presence of ionophores which imparted high Cu stress. A cluster analysis was employed to identify proteins exhibiting stability changes that correlated with cell-associated Cu levels, suggesting they were specific targets of Cu. Of the 31 proteins found to be specific targets of Cu, six were involved in glycolysis or the TCA cycle indicating that unregulated Cu may target central carbon metabolism in E. coli. We also found that two of these hits, GAPDH and IDH, had catalytic activities directly inhibited by Cu. Our work provides evidence that protein folding stability is a powerful probe for interrogating protein-metal interactions on the proteomic scale.

Track: Amyloid and Aggregation
Session: Allostery & Dynamics in Protein Function

ABS# 444 | Probing the Conformational dynamics of Neuroserpin strand 1A via Site-specific Labeling and Implications of helix F in Aggregation Mechanism.

Shoyab Ansari[1], Shahzaib Ahamad[2], Dr. Dinesh Gupta[2], Prof. Aman Jairajpuri[1,3]
[1]*Protein Conformation and Enzymology Lab, Department of Biosciences, Jamia Millia Islamia (A Central University), New Delhi-110025, India,* [2]*Translational Bioinformatics Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), (Delhi, India)*

Background: Neuroserpin (NS) is an inhibitor of tissue plasminogen activator (tPA) and belongs to the serpin

superfamily. Point mutation in NS leads to its aberrant conformational transitions in the brain that is associated with the genetic neurogenerative disease, familial encephalopathy with NS inclusion bodies (FENIB). We have shown the predicted effect of the point mutation in NS helix F and strand 1A (s1A) towards aggregation and inhibition mechanism.Aims: To Prepare cysteine mutants of NS for conformational analysis by using site-specific labeling and evaluate the NS polymerization and inhibition mechanism.Result: By using in-silico analysis, we identified a residue (H138C) of s1A that contributes to NS stability. Molecular dynamic simulations revealed the persistent change around helix F and s1A. MD prediction was further analyzed by preparing the H138C (s1A) and W154C-H138C (helix F-s1A) cysteine mutants. Recombinant strand 1A NS mutants were expressed, purified, and characterized for their stability, secondary structure, tPA-complex formation ability, and inhibitory kinetics. The activity and complex formation analysis against tPA indicated the compromised inhibition in the W154C-H138C variant. Further, we observed an increased rate of aggregation in H138C and W154C-H138C variants by native PAGE and TEM analysis which are largely contributed by helix F as double variant showed the faster appearance of aggregates formation. For site-specific conformational analysis, H138C on s1A was labeled with Alexa fluor C5 maleimide, the result indicated the exposure of s1A in the presence of tPA.Conclusion: We conclude that during the reactive loop centre (RCL) insertion exposure in the s1A of β sheet A required to accommodate the incoming RCL. We also postulate the role of helix F residue towards the correct folding and aggregation process of NS as the mutation in helix F attributed to the exposure of partially folded hydrophobic region and increased aggregation propensity of NS.

Track: Structure (X-Ray/NMR/EM)
Session: Protein Evolution, Design and Selection

ABS# 446 | Interactions of PIP and RIR motifs in TLS Polymerases with PCNA and Rev1-CT

Gianluca Arianna[1], Alex Rizzo[1], Dmitry Korzhnev[1]
[1]UConn Health (CT, US)

Translesion synthesis (TLS) is a mechanism utilized by cells to circumvent accumulated structural damage to DNA which would otherwise impede DNA replication. In TLS, proliferating cell nuclear antigen (PCNA) is monoubiquitinated, which recruits the specialized TLS polymerases (pols): pol η, pol ι, pol κ, pol ζ and Rev1 to DNA. These bind to PCNA via its ubiquitin binding domains and to the interdomain connector loop (IDCL) via their "PCNA interacting Peptide" or PIP motif. The TLS pols also bind to the C-terminal (CT) domain of Rev1, which also serves as a scaffold for the TLS complex, via their "Rev1 Interacting Regions" or RIR motifs. Both PIP and RIR motifs feature two adjacent hydrophobic residues (typically 'FF') which insert into hydrophobic pockets in Rev1-CT or the PCNA IDCL. Certain motifs in yeast have also shown capacity to bind both PCNA and Rev1-CT, contributing to the hypothesis that these are not distinct domains, but in fact, a single family of "PIP" like motifs. To better validate this claim and further elucidate of the mechanism of TLS pol binding in the human system, we sought to compare relative binding affinities of each of the motifs to PCNA or Rev1-CT by performing isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) titrations. We demonstrate that PIP and RIR motifs retained specificity for their canonical partners. However, we have also identified several motifs with overlapping specificities for both PCNA and Rev1-CT. These findings suggest that the protein-protein interactions governing the assembly of the TLS complex may be more complicated than previously assumed and supports the notion of dual acting "PIP-RIR" motifs.

Track: Computational Modeling/Simulation
Session: Allostery & Dynamics in Protein Function

ABS# 447 | Revealing Key Intermediate Dynamics between an Antifungal Drug & Sterol Binding Partners

Kevin Cheng[1], Ashley De Lio[2], Agnieszka Lewandowska[2], Corinne Soutar[2], Martin Burke[2], Chad Rienstra[2], Taras Pogorelov[2]
[1]Center for Biophysics, University of Illinois at Urbana-Champaign, Champaign, IL, USA, [2]Department of Chemistry, University of Illinois at Urbana-Champaign, Champaign, IL (IL, United States)

Amphotericin (AmB) is a potent and effective drug to combat life-threatening fungal infections. However, it is extremely toxic to humans and can lead to fatal complications including kidney damage or heart failure. It was experimentally shown that AmB's anti-fungal activity originates from its binding to ergosterol in fungal cells. Since ergosterol is similar to human cholesterol, the toxicity purported to come from AmB's non-specific binding to both sterols. To develop AmB derivatives that maintain potency while reducing toxicity, structural elucidation of sterol interactions is necessary. The goal of our study is to characterize unique binding modes between sterols and

AmB and reveal an atomistic mechanism behind its fungicidal activity. To this end, we use all-atom molecular dynamics (MD) simulations to capture how an NMR-derived AmB lattice structure interacts with the membrane bilayer. We efficiently explore the structural landscape of AmB-Sterol complexes using extensive ~80 μs of replica exchange MD (REMD). Resulting AmB membrane-embedded structures are then subjected to equilibrium MD with the highly mobile-mimetic (HMMM) model that uses a novel sterol-compatible, in-silico solvent. We connect our results to solid-state NMR experiments by calculating theoretical chemical shifts. Our methodology produced an ensemble of atomistically resolved dynamic AmB-Sterol complexes that are within 8 ppm of experimental chemical shifts. We further support our computational model by showing structures of AmB complexes to satisfy within 3.5 Å the majority of distance restraints derived from NMR-REDOR experiments. This study offers a novel and generalizable workflow that combines distinct enhanced sampling methods, and bridges derived structures with NMR observables. Most importantly, the impact of this work will guide future experiments that focus on the design of AmB derivatives to selectively kill fungal cells and not to harm human cells. Such derivatives have the potential to save many lives.

Track: Peptides

Session: Protein Evolution, Design and Selection

ABS# 448 | Chromatography Optimization for LC-MS/MS Analysis of Host Defense Peptides

Amy Carfagno[1], Paul Russo[2], Barney Bishop[1]
[1]*Department of Chemistry and Biochemistry, George Mason University,* [2]*Center for Applied Proteomics and Molecular Medicine, George Mason University (Virginia, United States)*

Host-defense peptides from diverse organisms have been reported to exhibit activity against human pathogens, prompting interest in their potential therapeutic promise. Host defense peptides are generally cationic and amphipathic, which may complicate analysis via liquid chromatography–mass spectrometry. Tailing and broadening are commonly observed when using silica-based reversed phase columns with formic acid (FA), likely due to binding interactions between peptides and silanol groups, complicating identification and quantification. Trifluoroacetic acid may improve chromatography but can reduce sensitivity. Recent studies indicate that difluoroacetic acid (DFA) may represent an effective compromise, offering improved chromatographic performance and lower signal suppression. Increasing temperature and decreasing gradient time may also reduce broadening. We used a Thermo EASY-nLC 1200 coupled to a Thermo Orbitrap Fusion to compare 0.1% FA, 0.1% FA + 0.02% DFA, and 0.1% DFA in the mobile phase, focusing on the impact on peak shape and peptide identification. Host-defense peptides in water and in a solution containing chymotrypsin-treated bovine serum albumin (BSA) were analyzed using two different temperature and time settings. For the peptide mixture in water, qualitative peak shape in terms of width and symmetry generally improved with the addition of DFA to the mobile phase. An increase in retention time was also observed, which is consistent with the stronger ion pairing effect of DFA. Notably, evaluation of peak height, signal to noise ratio, and peptide feature area suggest that DFA does not compromise sensitivity. The potential advantage of DFA in terms of identification was less clear when considering results for the BSA matrix. However, reducing the gradient time from 60 to 30 min appeared to compromise peptide identification, which was consistent with the expected increase in interference caused by co-eluting species. The above results have informed our ongoing efforts to evaluate strategies for improving peptide identification coverage and reproducibility.

Track: Computational Modeling/Simulation

Session: Protein Structures Through the Lens of Machine Learning

ABS# 449 | Forecasting Avalanches in Branched Actomyosin Networks with Network Science and Machine Learning

Chengxuan Li[1,2], James Liman[2,3], Yossi Eliaz[1,2], Margaret Cheung[1,2,3,4]
[1]*Department of Physics, University of Houston, Houston, Texas 77204, USA,* [2]*Center for Theoretical Biological Physics, Rice University, Houston, Texas 77005, USA,* [3]*Department of Bioengineering, Rice University, Houston, Texas 77030, USA,* [4]*Pacif (Texas, USA)*

We explored the dynamical and structural effects of actin-related proteins 2/3 (Arp2/3) onactomyosin networks using mechanochemical simulations of active matter networks. At ananoscale, the Arp2/3 complex alters the topology of actomyosin by nucleating a daughterfilament at an angle to a mother filament. At a subcellular scale, they orchestrate the formation of branched actomyosin network. Using a coarse-grained approach, we sought to understand how an actomyosin

network temporally and spatially reorganizes itself by varying the concentration of the Arp2/3 complexes. Driven by the motor dynamics, the network stalls at a high concentration of Arp2/3 and contracts at a low Arp2/3 concentration. At an intermediate Arp2/3 concentration, however, the actomyosin network is formed by loosely connected clusters that may collapse suddenly when driven by motors. This physical phenomenon is called an "avalanche" largely due to the marginal instability inherent from the morphology of a branched actomyosin network when the Arp2/3 complex is present. While embracing the data science approaches, we unveiled the higher-order patterns in the branched actomyosin networks and discovered a sudden change in the "social" network topology of the actomyosin. This is a new type of avalanches in addition to the two types of avalanches associated with a sudden change in the size or the shape of the whole actomyosin network as shown in the previous investigation. Our new finding promotes the importance of using network theory and machine learning models to forecast avalanches in actomyosin networks. The mechanisms of the Arp2/3 complexes in shaping the architecture of branched actomyosin networks obtained in this paper will help us better understand the emergent reorganization of the topology in dense actomyosin networks that are difficult to detect in experiments.

Track: Dynamics and Allostery

Session: Allostery & Dynamics in Protein Function

ABS# 450 | Expression, purification and oligomeric state analysis of a bacterial zinc finger protein and a tyrosine mutant

Arkadiusz Szura[1], Lucas Pedraz[2], Claus Smith[1], Carmen Mesas[1], Elena Ruiz[1], Eduard Torrents[2], Maria Sola[1]
[1]Structural Biology Unit, Institute for Molecular Biology of Barcelona, Barcelona, SPAIN, [2]Bacterial Infections: Antimicrobial Therapies, Institute for Bioengineering of Catalonia, Barcelona, SPAIN (Barcelona, Spain)

One potential biomedical target is a DNA-binding Zinc-finger repressor that regulates genes that are essential for Pseudomonas aeruginosa viability, which has a homolog in Escherichia coli. We expressed such a repressor from E. coli in E. coli strain as a fusion protein with an N-terminal SUMO tag to increase the formerly detected low solubility. In this type of construct, at the end of the SUMO protein there is the cleavage site for the SUMO protease. However, in our construct the proteolytic cleavage was inefficient and we suspected a potential interference between the tag and the repressor. As an alternative, we added a cleavage site for the TEV protease between SUMO and repressor genes. However, the fusion protein was not cleavable by TEV under many different conditions (1-2mM DTT, 250-750mM NaCl, pH 6-9). One possible reason was that the TEV cleavage site in the new construct was too close to the Zn-finger protein. However, addition of two aminoacids between the protein and the TEV cleavage site did not allow cleavage. Unexpectedly, high efficient cleavage was achieved for a construct that contained a linker of six aa between the TEV cleavage site and the Zn-finger protein. Optimized purification protocol, includes affinity chromatographies that consist in a first step to isolate the fusion protein and a second to isolate Zn-finger protein upon cleavage. SEC-MALS attempts performed with nucleotides that deactivate the protein, shows the repressor ability to form octamers, while from the other hand, introduction of tyrosine mutation in the active site breaks higher oligomeric state of the protein-nucleotide complex, and deprives the protein ability of nucleotide recognition. Therefore, identification of key residues in the active site is essential to understand protein mechanism of regulation, and afterwards to design new strategies, pivotal to combat P. aeruginosa.

Track: Membrane Proteins

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 451 | Atg23-Dimer Drives Vesicle Tethering and Atg9 Recruitment in Yeast Autophagy

Kelsie Leary[1], Wayne Hawkins[2], Devika Andhare[1], Hana Popelka[2], Daniel Klionsky[2], Michael Ragusa[1]
[1]Dartmouth College, [2]University of Michigan, Ann Arbor (New Hampshire, United States)

Autophagy is a catabolic process necessary for the maintenance of cellular homeostasis. Atg23 is a peripheral membrane protein that is involved in trafficking Atg9-vesicles, which serve as an initial membrane source in yeast autophagy. The function of Atg23, despite being critical to the efficiency of this process, is not well understood due to a lack of biochemical investigations and an absence of any structural information. We used a combination of in vitro and in vivo methods to show that Atg23 exists primarily as a homodimer, facilitated by a putative amphipathic helix. We utilized small angle X-ray scattering to monitor the overall shape of Atg23, revealing that it contains an extended rod-like structure. We also reveal that Atg23 interacts with membranes directly, primarily through

electrostatic interactions, and that these interactions lead to vesicle tethering. Lastly, dimerization of Atg23 is essential for subcellular localization, membrane binding, vesicle tethering, Atg9 binding and autophagic efficiency.

Track: Computational Modeling/Simulation
Session: Protein Evolution, Design and Selection

ABS# 452 | Optimizing the Secretion of Designed Self-Assembling Protein Nanoparticles in Rosetta Software

Chelsea Shu[1,2], John Wang[1,2], Neil King[1,2]
[1]university of washington, [2]Institute of Protein Design (WA, United States)

Viral pathogens, especially those that undergo rapid mutagenesis, pose a significant threat to public health as continuous antigenic drift prevent long-term anamnestic immunity in hosts and make vaccination difficult. To elicit broadly neutralizing responses that are difficult yet necessary to obtain, we propose to encode self-assembling protein nanoparticles alongside the antigen of choice in mRNA vaccines. Our selection of nanoparticles are composed of oligomer subunits that self-assemble through hydrophobic interactions computationally designed in Rosetta Suite. These proteins mimic the effects of adjuvants similar to ferritin and display an organized array of antigens that allow for stronger B-cell crosslinking. Additionally, these designs uniquely offer control over antigen organization, selection, and valency. However, the introduction of hydrophobic residues during the design process to allow for spontaneous interface formation inevitably hinders protein secretion in cells. This is due to secretion or transmembrane insertion of a particular sequence being largely determined by the hydrophobicity of its residues. Previously, we were unable to predict whether or not our computational designs in Rosetta suite would be able traverse the endoplasmic reticulum (ER). However, amino acid-specific contributions to transmembrane insertion had been experimentally determined in a separate model system by the calculation of transmembrane energy. Thus, we proposed to integrate this transmembrane energy calculus into Rosetta suite. The algorithm was then tested using a dataset of 967 transmembrane and 2055 non-transmembrane protein sequences extrapolated from the Protein Data Bank. Our new Rosetta program was not only able to characterize the stability of these proteins but also calculate their transmembrane energies to distinguish their ability to traverse the ER. We will be using this program to generate a new class of designed protein nanoparticles optimized for cellular secretion.

Track: Enzymology
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 453 | Chitinase Activity detection on the surface from Chitinibacter tainanensis Ghost

ZHENG-WEN WANG[1], Jeen-Kuan Chen[2]
[1]Ming Chi University of Technology , [2]Department of Environment and Biotechnology, Refining and Manufacturing (Taiwan, Taiwan)

Chitin is the aminopolysaccharide distributed in microorgamisms, animals and plants. It is the main structuralcompnoents in the exoskeletons of a broad diversity of organisms. A lot of derivatives of chitin analogous have been applied for the broad ranges. Chitin and it derivatives are biodegradable, histo-compatible and nontoxic materials. Therefore, it is a good for circular economy.Now, the monomer of chitin, N-acetylglucosamine (NAG) is applied on treating arthritis, pediatric enteritis and Crohn's disease. In human, NAG also promotes the synthesis of hyaluronic acid to anti-wrinkle. In the markets, the NAG is utilized on pharmaceutics, food and agriculture industires.A decade before, a new species microorganism, Chitinibacter tainanensis, was identified from the soil of southern Taiwan.The NAG can be obteined by the micobes degrading chitin. The yield and the end product reach higher than 90%. The transformation of chitin is subjected on the complex, chitinasome docked on the surface of membrane in C. tainanensis. For the detection of the enzyme complex activity on the production of NAG. A plasmid with autolysis was introduced for ghost production. The enzyme activity is also determined.

Track: Enzymology
Session: Measuring Forces of Biological Systems

ABS# 454 | The Efficiency for the N-acetylglucosamine from Cocoon Degradation by Chitinibacter tainanensis

YI-LUNG CHIEN[1], CHAOLIN LIU[1], Jeen-Kuan Chen[2]
[1]Mingchi University of Technology, [2]Department of Environment and Biotechnology, Refining and Manufacturing Research Institute, CPC Corporation (Taiwan, Taiwan)

Musca domestica is the housefly distributed worldwide. The flies are considered as pests because the adults carry pathogens on their bodies and in their feces, contaminate

food, and contribute to the transfer of food-borne illnesses, while, in numbers, they can be physically annoying. However, there the major components of exoskeleton are chitin. A decade before, an acetylcholinesterase of the housefly head was isolated for the pecticide detection. However, their bodies were left. Chitinibacter tainanensis, was identified from the soil of southern Taiwan.The N-acetylglucosamine (NAG) can be obteined by the micobes degrading chitin. The yield and the end product reach higher than 90%. NAG is applied on treating arthritis, pediatric enteritis and Crohn's disease. In human, NAG also promotes the synthesis of hyaluronic acid to anti-wrinkle. In the markets, the NAG is utilized on pharmaceutics, food and agriculture industires.For the development on the for circular economy, the flies bodies were treated with alkaine, puff, mill, colloidization or two or three of them. Then, the trated bodies were cultured with C. tainanensis. Then the yeild were monitored for the NAG production.

Track: Proteomics

Session: Protein Evolution, Design and Selection

ABS# 455 | PROTEOMIC ASSESMENT OF ARG/PRO AMINO ACID METABOLIC INTERCONVERSION IN SILAC LABELED CANCER CELL LINES

Cristian V.A. Munteanu[1], Gabriela N. Chiritoiu[1], Stefana M. Petrescu[1], Livia E. Sima[1]
[1]*Institute of Biochemistry of the Romanian Academy (Bucharest, Romania)*

Cancer is one of the leading causes of mortality worldwide. Thus, intense cancer research aims to discover novel biomarkers for early disease onset detection or to expand the therapeutic options of cancer patients. Quantitative proteomics has emerged as one the most important methods for proteome characterization in biomarker discovery and currently, stable isotope labeling by amino acids in cell culture (SILAC) is still considered the most refined method ensuring quantitative accuracy. SILAC requires complete labeling of the biological material with heavy amino acids, with an efficiency that is usually cell-line dependent. However, amino acid metabolism can impact the quantitative accuracy, particularly in cancer cells, which tend to have a higher metabolic rate. This was actually observed in SILAC proteomics experiments, which used arginine (Arg) for cell labeling, as Arg can be metabolically interconverted to proline (Pro), resulting in an under-estimation of heavy/light (H/L) ratios of tryptic Pro peptide ratios. Here, we show that the metabolic Arg to Pro interconversion can proceed in cancer cells grown in both Pro deficient and Pro supplemented media. We analyzed two highly used model cell lines: A375 - an amelanotic human melanoma cell line and OVCAR-5 - a cell line established from the ascites of an advanced-stage ovarian patient. For this, we optimized amino acids concentrations for cell culture media supplementation and compared Pro deficient and Pro supplemented cells from each cell line by mass-spectrometry based proteomics. Proteome-wide exploration of labeled and non-labeled cells revealed quantitative proteome transformation during cell culture labeling, mapping possibly disturbed biological pathways. We observed that Arg concentration is important and that titration of Pro concentration into the cell culture medium increases the labeling efficiency, particularly for A375 melanoma cells. These results will further aid identifying proteome modifications in cancer cells. We acknowledge CCCDI-UEFISCDI Grants PN-III-P1-1.1-PD-2019-1242, PN-III-P1-1.1-PD-2019-1278, PN-III-P2-2.1-PED-2019-1543, PNCDI-III-PCCDI-2018-1.

Track: Transcription/translation/post-translational modifications

Session: New Protein Post-Translational Modifications

ABS# 456 | Protein monoaminylation in brain: novel mechanism of neural development, plasticity and disease

Ian Maze[1]
[1]*Icahn School of Medicine at Mount Sinai (NY, USA)*

Human neurological diseases associated with monoaminergic (e.g., serotonin, norepinephrine, dopamine) dysfunction affect millions of individuals worldwide; however, treatments for these neurodevelopmental, neurodegenerative and/or psychiatric disorders remain inadequate. Employing a unique combination of biochemical, molecular and behavioral approaches, we are examining novel, neurotransmission independent roles for monoamines in brain in the direct regulation of neuronal gene expression and synaptic plasticity. Our data indicate that alterations in protein monoaminylation (i.e., the covalent addition of hydrophobic monoamines, such as serotonin, dopamine, etc., to substrate proteins) directly affect the functionalities of modified proteins to modulate neurodevelopmental and synaptic related events. Our work thus aims to integrate expertise from multiple disciplines (e.g., chromatin biochemistry, chemical biology, neuroepigenomics and behavioral neuroscience) to fully characterize these monoaminylation states in the central

nervous system, as well as their potential roles in neurological disease.

Track: Structure (X-Ray/NMR/EM)
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 457 | Structure of the dihydrolipoamide succinyltransferase (E2) component of the human alpha-ketoglutarate dehydrogenase complex (hKGDHc) revealed by cryo-EM and cross-linking mass spectrometry: Implications for the overall hKGDHc structure

Balint Nagy[1], Martin Polak[2], Oliver Ozohanics[1], Zsofia Zambo[1], Eszter Szabo[1], Agnes Hubert[1], Frank Jordan[3], Jiri Novacek[2], Vera Adam-Vizi[1], Attila Ambrus[1]
[1]Department of Biochemistry, Institute of Biochemistry and Molecular Biology, Semmelweis University, Budapest, Hungary, [2]Central European Institute of Technology, Masaryk University, Brno, Czech Republic, [3]Department of Chemistry, Rutgers, The St (Budapest, Hungary)

The alpha-ketoglutarate dehydrogenase complex (KGDHc) plays a key role in the energy balance of cells by catalyzing a rate-limiting step in the Krebs cycle. Its physiological function is to convert KG to succinyl-CoA and generate NADH. Malfunction of and reactive oxygen species (ROS) generation by the human (h) KGDHc have been implicated in numerous pathologies including various neurodegenerative disorders (like Alzheimer's and Parkinson's diseases), ischemia-reperfusion injury and selected cancers. The substantial ROS-generating capacity of the E1–E2 subcomplex of the hKGDHc (hE1k-hE2k) was recently suggested contributing to the clinically very severe disease called E3-deficiency. We performed cryo-EM, cross-linking mass spectrometry (CL-MS) and molecular modeling (MM) analyses to determine the structure of the hE2k component that transfers a succinyl group to CoA and forms the structural core of hKGDHc. We also assessed the overall structure of the hKGDHc by negative-stain EM and MM. Here we report the 2.9 Å resolution cryo-EM structure of the hE2k component. Flexible domains of the hE2k did not lend themselves to examination by the current approach, therefore the final structural model only represents residues 151-386 in the mature enzyme (1-386); the resolved region comprises the entire core domain plus a few additional residues towards the N-terminus. The overall structure of the hE2k component revealed that 24 hE2k chains assembled into a highly symmetrical cubic (8x3) type structure. We also studied the structurally unresolved region in the protein by CL-MS and MM. These analyses demonstrated that each lipoyl domain is oriented towards the core domain of an adjacent chain in the hE2k homotrimer. Based on our results, we also made proposals for the overall chain stoichiometry and subunit symmetry of hKGDHc. hE1k and hE3 are most likely tethered at the edges and faces, respectively, of the cubic hE2k assembly with an overall chain stoichiometry of 24:24:12 [E1:E2:E3].

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 458 | Construction, expression and characterization of a hyperthermophilic tri-functional glycosidase for cellulose hydrolysis to monosaccharides

Fang Huang[1], Trent Yang[1]
[1]National Research Council of Canada (Ontario, Canada)

Construction, expression and characterization of a hyperthermophilic tri-functional glycosidase for cellulose hydrolysis to monosaccharides Fang Hung, Trent C. YangBioprocessing and biocatalysis team, ACRD, National Research council of Canada, Ottawa, Ontario, Canada K1A 0R6ABSTRACTThe β-1,4 endoglucanase (EGPh) from the hyperthermophilic archaeon Pyrococcus horikoshii has strong hydrolyzing activity towards crystalline cellulose. EGPh alone functions as a processive endoglucanase with both endo- and exo-cellulase activities and can hydrolyze cellulose to cellobiose. When EGPh is combined with the hyperthermophilic β-glucosidase from Pyrococcus furiosus (BGPf), cellulose is hydrolyzed to glucose at high temperature suggesting great potential for these enzymes in biomass hydrolysis. To explore the possibility of creating a single, tri-functional glycosidase (endo-, exo-cellulase and glucosidase activities) for cellulose hydrolysis at high temperature, EGPh was linked to BGPf through a proline and threonine rich flexible linker (Lpt) or a α-helix linker (L2). In addition, the effect of a carbohydrate binding motif (CBM) on the enzyme activity was explored. Western blot analysis showed the expression of the full-length molecules with the expected molecular masses. The kinetic analysis with pNP-G2 as the substrate indicated Lpt linker fused recombinant cellulases possess higher affinity toward the substrate and increased activity comparing to their wild type counterparts EGPh and BGPf and L2 linker recombinant cellulases. Both cellulase and glucosidase activities are conserved in the single molecule. Oligomer formation was observed for BGPf and tri-

functional cellulases. Oligomer/monomer transition condition and ratios were analyzed. HPLC analysis of cellulose hydrolysis product profile confirmed that the final product is glucose and hybrid enzymes led to increased glucose/cellobiose ratio suggesting successful construction of a tri-functional glycosidase that functions at high temperature.

Track: Enzymology
Session: Allostery & Dynamics in Protein Function

ABS# 459 | Shake, Rattle, & Roll: Capturing Snapshots of Ribonucleotide Reductase in Action

Catherine Drennan[1,2]
[1]*Massachusetts Institute of Technology,* [2]*Howard Hughes Medical Institute (MA, USA)*

The use of radical-based chemistry allows for amazing transformations in living organisms. To carry out their functions, radical enzymes often need to be flexible and assume different conformational states. In this talk, the conformational gymnastics involved in ribonucleotide reduction are considered. Ribonucleotide reductases (RNRs) are radical enzymes that convert ribonucleotides (the building blocks of RNA) to deoxyribonucleotides (the building blocks of DNA). RNRs are targets for cancer chemotherapies and have been proposed to be candidates for antimicrobial therapies. Here, I will describe how my lab has employed biophysical methods to interrogate how RNRs shake, rattle, and roll to accomplish their critical cellular function.

Track: Computational Modeling/Simulation
Session: Protein Evolution, Design and Selection

ABS# 460 | Design of High-Affinity Peptide Binders to a Major Histocompatibility Complex Class II Allele

Rodrigo Ochoa[1], Alessandro Laio[2], Pilar Cossio[1]
[1]*Max Planck Tandem Group, Biophysics of Tropical Diseases, University of Antioquia, Medellín, Colombia,* [2]*International School for Advanced Studies (SISSA), Trieste, Italy (Medellin, Colombia)*

The Major Histocompatibility Complex (MHC) class II is a key receptor within the human adaptive immune response pathway. The reported mechanism of action involves the interaction with peptides mainly derived from the proteolysis of external pathogen proteins. This has motivated the implementation of computational tools to predict the affinity of MHC peptide binders, using as basis the laws that govern protein-protein and protein-peptide interactions. In addition, knowledge of the dynamic nature of the system aids the rational design of novel peptides, for example, through iterative amino acid modifications. Here, we used a computational peptide-design protocol to predict high-affinity binding peptides to an MHC class II allele, starting from a peptide with known affinity. The protocol mutates and selects optimal amino acids using a stochastic search with molecular dynamics simulations and binding scoring functions. The conformational sampling mimics the natural environment with explicit solvent, thermostat and pressure coupling. We implemented various strategies to perform the modifications on the peptide sequence, and we selected a small set of 17 candidates to be tested experimentally. We found that two peptides have a similar or higher affinity than the initial peptide, and a subset reported significant results when previous biological knowledge is incorporated in the design. Overall, the protocol aims to predict in a computationally efficient manner, bioactive peptides composed of natural amino acids bound to the MHC class II receptor using physico-chemical, structural and dynamical considerations.

Track: Proteostasis and quality control
Session: Targeted Protein Degradation

ABS# 461 | Dietary Dihydroquercetin Mitigates Oxidative Damage via Promoting Protein Quality Control in Heat-Stressed Rainbow Trout

Liudmila Lysenko[1], Irina Sukhovskaya[1], Ekaterina Tushina[1]
[1]*Institute of Biology, KarRC RAS (Petrozavodsk, Russian Federation)*

Rainbow trout is highly vulnerable to environmental stressors, such as temperature rise, under intense rearing. A plant-origin flavonoid, dihydroquercetin, earlier shown to affect fish well-being decreasing lethality and growth depression in heat-stressed fish through the mechanisms yet poorly understood. In order to understand the stress-reducing effects of the antioxidant, we estimated oxidative damage and antioxidant system expression via cellular protein carbonyls, retinol, and glutathione (GSH), as well as catalase, peroxidase (Px), glutathione-S-transferase (GST), proteasome (as a protease core of UPS), cathepsin (autophagy proteases), and calpain activities in the white muscles of trout reared in nature

environmental variables. Our observations show that, under critical summer temperatures, trout fed dihydroquercetin (if compare with supplement-free fish) manifested less oxidative damage due to both lower level of protein carbonyls and pronounced defense reactions such as the antioxidant system (particularly GSH and GST) and protein quality control with a principal activation of the proteasome and lysosomal cathepsins but not calpains. Analyzing the dynamics of macromolecule oxidation and defense response we conclude that dihydroquercetin promotes adaptive responses utmost in environmentally stressed fish with no effects on growth, survival rate, and antioxidant system in fish grown under favorable environment. Our data evidencing the antioxidant capacity of a plant bioflavonoid suggest its benefits in medicine and veterinary practice. The study was funded by the RSF, project no. 17-74-20098.

Track: Membrane Proteins
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 462 | Functional Interactions of GPx4, a Cytoprotective Peripheral Membrane Protein

Courtney Labrecque[1], Brian Fuglestad[1]
[1]*Virginia Commonwealth University (Virginia, United States)*

Glutathione peroxidase 4 (GPx4) is the sole enzyme responsible for protection of membranes from lipid hydroperoxidation. This enzyme engages the membrane and reduces lipid hydroperoxides thereby preventing cellular death via ferroptosis. Due to its central function surrounding ferroptosis, GPx4 has been implicated as a therapeutic target for inflammatory diseases via activation and cancers via inhibition. The dual therapeutic potential of GPx4 makes the enzyme a remarkably interesting drug target that has recently gained much interest.One important step to being able to effectively design activators or inhibitors for GPx4 is a more thorough understanding of its function at high-resolution. While its cellular functions are well characterized, molecular details at high resolution are currently lacking. We report the first NMR assignments and experimental based model of GPx4 engaging with a membrane. We utilize a wide variety of membrane models, including those incorporating native substrates for GPx4, to map the binding interaction of the enzyme with the membrane. Using tryptophan fluorimetry we were also able to determine the affinity of GPx4 with liposomes, as well as experimentally determine that this interaction is electrostatically driven. Mutational analysis of the enzyme confirms the importance of a cationic patch for membrane interaction and lipid substrate engagement. Electrostatic interactions of the cationic patch are also implicated in the moonlighting function of GPx4 as a cross-linker of DNA-bound protamines. The results presented here offer the highest resolution yet of intermolecular GPx4 interactions and illuminate common electrostatic drivers of highly divergent cellular functions.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 463 | Self-Assembling Lectin Nano-Building Blocks Enhance Avidity to Glycans through Polyvalent Effect

Shin Irumagawa[1], Hiroaki Tateno[2], Ryoichi Arai[1]
[1]*Shinshu University,* [2]*National Institute of Advanced Industrial Science and Technology (Nagano, Japan)*

Lectins are carbohydrate-binding proteins that relate to various biological functions, and therefore, they have been attracted as target molecules for biomedical applications. Many lectins have multiple carbohydrate binding domains (CRD) by oligomerization and/or tandem repeats, and bind the specific glycans on surface of target cells.In a previous study, we developed protein nano-building blocks (PN-Blocks) to construct self-assembling supramolecular nanostructures by linking two oligomeric proteins. For example, we constructed the WA20-foldon PN-Block by fusing the WA20, which is the de novo protein forms dimeric 4-helix bundle, to the trimeric foldon domain of T4 phage fibritin. WA20-foldon forms several types of self-assembled nanoarchitectures in multiple of 6 such as a barrel-like hexamer and tetrahedrally shaped dodecamer. For another example, extender protein nanobuilding blocks (ePN-Blocks) were constructed by tandemly joining two copies of WA20 with various linkers. The ePN-Blocks self-assembled into cyclized and extended chain-type nanostructures in multiple of 2.In this study, we developed a new protein nano-building block with a lectin, lectin nano-building block (LN-Block) called WA20-ACG by fusing WA20 to dimeric lectin called Agrocybe cylindracea galectin (ACG). WA20-ACG formed several types of oligomers in multiple of 2 such as dimer, tetramer, hexamer, octamer, and more, and fractions of the oligomers were changed by the linker length between WA20 and ACG. Surface plasmon resonance (SPR) analysis indicated that the avidity of

WA20-ACG for LacNAc were significantly improved compared to original ACG through polyvalent effects of CRD. These results suggest that LN-Blocks are useful for creating polyvalent lectins with high avidity for future biomedical applications.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 464 | Toward Elucidating the mechanism of lytic polysaccharide monooxygenases: Chemical insights from X-ray and neutron crystallography

Gabriela Schröder[1,2], Flora Meilleur[1,2]
[1]*North Carolina State University,* [2]*Oak Ridge National Laboratory (Tennessee, United States)*

Lytic polysaccharide monooxygenases (LPMOs) are copper-center enzymes involved in the oxidative cleavage of the glycosidic bond. LPMOs are responsible for chain disruption of crystalline cellulose, thereby increasing the accessibility of the carbohydrate substrate to cellulases for hydrolytic depolymerization. The enhanced cellulose conversion of biomass makes LPMOs valuable for the generation of biofuels. The LPMO active site is located on the planar enzyme–cellulose binding surface in which a single copper ion is coordinated in a 'histidine-brace' motif. The LPMO reaction is initiated by the addition of a reductant and oxygen to ultimately form an unknown activated copper–oxygen species responsible for polysaccharide substrate hydrogen atom abstraction. Previous work in our group on LPMO9D from Neurospora crassa has provided insight into the binding and activation of oxygen at the LPMO active site as well as the role of the protonation state of a second-shell residue histidine in oxygen-prebinding (O'Dell et al., 2017). Neutron protein crystallography provides a non-destructive technique without beam-induced metallocentre reduction for structural characterization while also allowing the determination of the positions of light atoms such as hydrogen and deuterium which are central to understanding protein chemistry. Neutron cryo-crystallography permits trapping of catalytic intermediates, thereby providing insight into protonation states and chemical nature of otherwise short-lived species in the reaction mechanism. To this end, we collected a cryo-neutron diffraction dataset on an ascorbate-reduced LPMO9D crystal to characterize the activated oxygen intermediate (Schröder et al., 2021). A second neutron diffraction dataset was collected on a LPMO9D crystal exposed to low pH conditions to probe protonation states under acidic conditions. We have characterized the activated oxygen species bound to the copper center directly following copper reduction as well as the protonation state of active site and second-shell residues involved in catalysis, thereby providing insight into the LPMO reaction mechanism.

Track: Protein Interactions and Assemblies
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 465 | Towards solving the hydrogenase maturation mystery

Parag Gajjar[1], Shi Liang[1], Spencer Ward[1], James Moody[1]
[1]*Brigham Young University (Utah, United States)*

We have enough fossil fuels to last for many decades, but they will eventually run out. Burning fossil fuels also releases a lot of CO2 into the atmosphere. We need to identify alternative ways to generate energy. Hydrogen can be one of the main sources of fuel energy in the future. The hydrogenase enzyme is involved in the formation and degradation of hydrogen in various organisms and it can be used as an efficient source to generate hydrogen. The hydrogenase uses a special kind of iron-sulfur cluster (called the H-cluster) to generate hydrogen and this H-cluster is made by three maturase enzymes: HydF, HydE, and HydG. The missing link of the mechanism is the formation of the H-cluster (maturation process). There are two main hypotheses about the maturation process: 1) HydG and HydE bind simultaneously to HydF and 2) HydF binds to HydE and HydG at the same time. Structural data required to differentiate between these hypotheses is lacking. We used chemical cross-linking mass spectrometry (CXMS) and x-ray crystallography to understand the maturation process. We cross-linked all three maturase enzymes with Sulfo-LC-SDA cross-linker and the data suggests a three-way interaction between all three maturase proteins. For x-ray crystallography, we fused a polymer forming crystallization chaperone, human Translocation ETS Leukemia (TEL) protein sterile alpha motif (SAM) domain (TELSAM) with HydE (1TEL-flex-HydE) to increase the rate of crystal formation. The size exclusion chromatography (SEC) data of all three separately purified maturase enzymes mixer also confirmed the involvement of all three maturase enzymes in the complex. Further, we tested different crystallization conditions of the maturation complex. Harvested crystals will be diffracted at Stanford Synchrotron Radiation Light (SSRL) Source to evaluate the structure of the maturation complex toward solving the maturation mystery.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 466 | Fusion to TELSAM accelerate target protein crystallization rate

Sara Soleimani[1], Maria Longhurst[1], Brayden Bezzant[1], Diana Ramirez[2], Maria Pedroza[1], Tobin Smith[1], Tzanko Doukov[3], James Moody[1]
[1]Brigham Young University, [2]California State University Chico, [3]Stanford Synchrotron Radiation Lightsource (Utah, United States)

The development of novel crystallization approaches that require less time, effort, and expense can significantly increase the success rate of target proteins crystallization and accelerate structure determination of many biotechnology and disease-relevant proteins. Specifically, improved crystallization methods will accelerate study of the molecular mechanisms of disease. The long-term goal of this research is to develop crystallization methods that could lead to diffraction-quality crystals from greater than 70% of targeted proteins of interest. Polymer forming crystallization chaperones (PFCCs) are a potentially better type of crystallization chaperone. One potential PFCC is the sterile alpha motif domain (SAM) of the human Translocation ETS Leukemia protein. In this study, the effectiveness of TELSAM protein polymers to reliably form well-diffracting crystals of Capillary Morphogenesis Gene 2 (CMG2) is investigated. CMG2 is involved in cancer, where its overexpression is associated with increased tumor grade and poor patient survival. We generated CMG2 alone, 1TEL-flex-CMG2, 1TEL-flex-CMG2+PGM, 1TEL-flex-DARPin and we crystalized them. TELSAM accelerates the rate of crystal formation by as much as 27-fold versus the target protein alone, likely by using avidity to stabilize weak crystal contacts made by the target protein. In addition, TELSAM-target protein fusions can form well-ordered, diffracting crystals using flexible TELSAM-target linkers. The TELSAM polymers themselves need not directly touch one another in the crystal lattice to form well order crystals. We conclude that TELSAM is a powerful crystallization chaperone warranting future investigation.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 467 | Semi-Explicit Solvation Improves Ligand Binding Site Design in an Allosteric Protein

Zion Perry[1], Anum Glasgow[2], Tanja Kortemme[2]
[1]Yale University, [2]Department of Bioengineering, University of California San Francisco (CT, United States)

A protein's function is closely derived from its structure; the ability to understand and precisely design structures can thus help create proteins with specific functions. An often-overlooked aspect of protein structural design is the placement of individual water molecules, which are normally approximated as bulk solvent. Previous work has shown that specifying key water molecules improves structural predictions. In particular, designing binding sites in proteins could benefit from modeling waters because the binding affinity and resulting response of proteins to their ligands are influenced by water-mediated hydrogen bonds. Here, we applied solvation to the design of the allosteric repressor protein LacI, with the design goal of inverting the inducibility of both an inducing and anti-inducing ligand. We used a statistical semi-explicit solvation model to solvate LacI; this elucidated specific hydrogen bonds we used to inform our computational redesign of the LacI binding site. Although we are in the process of experimentally screening the top designs to measure their response to various ligands, our designs indicate that considering semi-explicit waters yields greater insight into ligand-protein interactions and can improve current protein design protocols.

Track: Enzymology
Session: Targeted Protein Degradation

ABS# 468 | Regulated protein degradation in fish muscles maintains cell osmolarity under drastic salinity changes

Nadezhda Kantserova[1], Nina Nemova[1], Liudmila Lysenko[1]
[1]Institute of Biology, Karelian Research Centre of the Russian Academy of Sciences (Petrozavodsk, Russian Federation)

Anadromous fish like salmonids face drastic salinity changes when migrating from the rivers to the sea (as the smolts) or in opposite direction (for spawning). Protein-degrading activities in smolts and spawners of S. salar and S. trutta were studied to reveal the role of protein degradation in salinity responses. At the pre-migration stage, intense muscle protein accumulation was achieved in two ways including protein synthesis activation and protein catabolism depression. In migrating fish, ion regulation depends on the hormonal interplay of cortisol and the growth hormone (GH)/Igf-I axis resulting in a

decrease in circulating Igf-I that shifts the metabolism to a catabolic state and retards growth. In smoltifying salmonids, increased muscle protein degradation primarily depends on increased activity of cathepsin L (ctsL) but not Igf-I or GH receptor downregulation. Since osmoregulation rearrangement to the changing ambient salinity is an energy-consuming process, it highly depends on the hydrolysis of muscle proteins and resulting amino acids serving both the building blocks for de novo synthesis and the substrates for oxidation and gluconeogenesis. Some salmonids, like brown trout, by the time of migration down to the sea, have neither sufficient protein reserves nor the full-fledged osmoregulatory system and survive in a new environment due to a specific adaptive strategy involving releasing amino acids. Calpain-dependent proteolysis (the other proteases play a minor role if any) of muscle proteins enriches tissues with amino acids, including those with osmolyte properties. Anadromous fish share this strategy of maintaining cell osmolarity with more simple organisms living in unstable environments. In conclusion, amino acids released through various proteolytic pathways, such as calpains or cathepsin L, maintain osmotic stability and the energy-consuming process of osmoregulation. The study was supported by the budget funding by theme 0218-2019-0076.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 469 | Fusion To TELSAM Allows Crystallization In Spite of Minimal Contacts

Supeshala Dilrukshi Sarath Nawarathnage[1], Derick Bunn[1], Cameron Stewart[1], Tzanko Duokov[2], James Moody[1]
[1]Brigham Young University, [2]Macromolecular Crystallography Group, Structural Molecular Biology Resource, SSRL (UT, United States)

X-ray crystallography is required for atomic-level structure determination of proteins too small for cryo-electron microscopy. The Sterile Alpha motif (SAM) domain of human Translocation ETS Leukemia (TEL) protein is a chaperone that is engineered to polymerize at low pH. The goal of this study is to investigate the TELSAM as a viable chaperone. Investigation into covalent protein crystallization chaperones to facilitate the crystallization of novel protein targets is important as it increases the success rate of protein crystallization. Polymerization of the chaperone-target protein fusion lends avidity to strengthen subsequent weak crystal contacts made by the target protein. As the chaperone provides many of the crystal contacts, chaperones should enable protein crystallization with the minimal screening of crystallization conditions, significantly reducing the time and resources required to determine protein structure. The TELSAM monomers were fused to the target protein DARPin to test their ability to form diffraction-quality crystals. The 3TEL-DARPin structure showed that it forms a crystal lattice where polymers do not touch in 1 dimension and stabilize extremely weak inter-DARPin contacts between sheets of polymers. We then crystallized the human TNK1 UBA domain as a fusion to TELSAM. The 1TEL-Flex-UBA crystals appeared in three days whereas the UBA alone crystals only appeared after 183 days. TELSAM thus increased the crystallization by 61-fold. The UBA homology model that was used for the molecular replacement did not fit the electron density data. But the TELSAM polymer allowed the solution of the X-ray phases to build the UBA domain one helix at a time. We conclude that TELSAM merits further investigation.

Track: Proteomics
Session: New Protein Post-Translational Modifications

ABS# 470 | Differential Fatty Acylation Of Influenza A Virus Hemagglutinin Is Sensitive To Mutations In The Cholesterol Consensus Motive

Larisa V. Kordyukova[1], Bodan Hu[2], Marina V. Serebryakova[1], Michael Veit[2]
[1]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia, [2]Institut für Virologie, Freie Universität Berlin, Berlin, Germany (Moscow, Russia)

Hemagglutinin (HA), a major glycoprotein of Influenza A viruses is site-specifically modified with fatty acids bound via thio-ester bonds (S-acylation). Whereas two cysteines in the short cytoplasmic tail of HA contain only palmitate, stearate is exclusively attached to one cysteine located at the cytoplasmic border of the transmembrane region (TMR). A functionally active cholesterol consensus motif (CCM) YKLW was found at the ectodomain border of HA TMR [Hu et al. (2019) J. Virol. 93]. Since both CCMs and S-acylation supposed to fit the HA molecules within membrane nanodomains, lipid rafts, we hypothesized that mutations within the CCMs affect the pattern of S-acylation. The specific mutations were introduced in the

YKLW motif, the virus was generated using the reverse genetics, and the production of infective virus particles was evaluated. No virus was rescued if the whole motif is exchanged (YKLW4A); single (LA) or double (YK2A and LW2A) mutated virus showed decreased titers and a comparative fitness disadvantage. The MALDI-TOF MS analysis of the rescued CCM mutants has revealed that: (1) the stearate amount depends on the host where the virus was grown; (2) the stearate content slightly varies depending on the mutation within the YKLW motif; (3) no underacylated peptides are present. Probably, the HA molecules having mutations in the CCMs are no longer in lipid rafts and therefore they encounter a different DHHC protein S-acylating the Influenza HA. The reported study was funded by RFBR project 20-54-12007 (to L.K.) and DFG project VE 141/14-1 (to M.V.).

Track: Synthetic Biology
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 471 | HDL mimetics facilitate in vivo delivery of self-amplifying mRNA vaccines encoding Chlamydia antigen and induce robust immune response

Wei He[1], Amy Rasley[1], Dina Weilhammer[1], Sean Gilmore[1], Sandra Peters[1], Sukumar Pal[2], Matt Coleman[1], Luis de la Maza[2], Nicholas Fischer[1]
[1]Lawrence LIvermore National Laboratory, [2]University of California, Irvine (United States, US)

The success of synthetic mRNA vaccines against COVID-19 has demonstrated to the world the potential of this technology, which includes protective efficacy, rapid development, and scalable manufacturing. The mRNA vaccines induce robust immune responses against a variety of viral antigens. We developed reconstituted high-density lipoproteins (termed nanolipoprotein particles, NLPs) to package and facilitate in vivo delivery of self-amplifying mRNA (SAM) constructs for vaccine applications. Positively charged NLPs successfully complexed with both traditional mRNAs and SAMs, enhancing their in vivo expression efficiency. Compared to conventional mRNAs, SAMs showed enhanced and prolonged protein expression at lower doses. To assess whether the NLP-SAMs formulation could be used as a vaccine against a bacterial pathogen, we investigated the immunogenicity of NLP-SAMs vaccine encoding the major outer membrane protein (MOMP) of Chlamydia trachomatis. Mice immunized with NLP-SAM vaccines produced significant amounts of serum antibodies. Removal of the bacterial signal sequence resulted in higher antibody levels. Our results suggest the NLP-SAM formulation is a promising candidate for an effective Chlamydia vaccine and that the NLP platform has the potential to formulate mRNA vaccine countermeasure for a wide range of both viral and bacterial pathogens.This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Track: Proteostasis and quality control
Session: New Protein Post-Translational Modifications

ABS# 472 | A quality control mechanism that coordinates the deubiquitinase BAP1

Benjamin Estavoyer[1], Oumaima Ahmed[1], Louis Masclef[1], Salima Daou[2], Haithem Barbour[1], Nadine Sen Nkwe[1], Frank Sicheri[2], El Bachir Affar[1]
[1]University of Montreal, [2]University of Toronto (Quebec, Canada)

The tumor suppressor BAP1 is a ubiquitously expressed deubiquitinase (DUB) that regulates chromatin-associated processes and is mostly involved in the deubiquitination of histone H2A. Interestingly, somatic or germline inactivating mutations of BAP1 have been found in multiple cancers, and this DUB is now established as a major tumor suppressor and the most frequently mutated DUB in human malignancies. Mechanistically, BAP1 assembles DUB complexes with the epigenetic regulators ASXLs in a mutually exclusive manner. These cofactors are necessary to ensure the stability of BAP1 and to stimulate its DUB activity. However, how BAP1 DUB activity is regulated remains largely unknown. Our results suggest that multiple quality control mechanisms regulate BAP1 enzymatic activity and function. Recently, our team has demonstrated that BAP1 interaction with ASXLs promotes the monoubiquitination of the latter. This post-translational modification regulates ASXLs stability, stimulates the DUB activity of BAP1, and is required for mammalian cell proliferation. However, new results from our laboratory suggest that another coordination mechanism of the BAP1/ASXLs complex is at work. These data show that BAP1 is monoubiquitinated in a mutually exclusive manner with the ubiquitination of ASXLs proteins, possibly ensuring a quality control

regulation that fine-tunes this epigenetic complex. Consequently, BAP1-mediated monoubiquitination could be tightly orchestrated, thus coordinating the expression of certain genes involved in cell proliferation, genome integrity, and tumor suppression.

Track: Proteomics
Session: New Protein Post-Translational Modifications

ABS# 474 | Developing a Method for Detecting Protein Adducts of Benzo[a]pyrene-7,8-diol-9,10-epoxide Using High Resolution Mass Spectrometry

Kavi Vaidya[1], Stanislau Stanisheuski[1,2], Monica Maier[1,2], Claudia Maier[1,2]
[1]Oregon State University, [2]Linus Pauling Institute (OR, United States)

Protein adducts of polycyclic aromatic hydrocarbons (PAHs) are of interest as emerging biomarker candidates of exposure to PAHs. In our work we focus on Benzo[a]pyrene (BaP), which is produced during incomplete combustion of organic compounds and to which humans can be exposed when cooking, smoking, working at foundries and bakeries, or during forest fires. The main metabolite of BaP is Benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE), a carcinogenic compound that is able to modify DNA and proteins by forming chemically stable covalent adducts. While the DNA adducts have been studied extensively over the last years, there is less known about the efficacy of protein adducts of BPDE as a potential biomarker of exposure. A major bottleneck that needs to be overcome is that there are not yet \ reliable and robust methods available that detect, identify and characterize the potential protein adducts. Very few works describe how high resolution mass spectrometry can be used to detect protein-BPDE adducts. In our opinion, two major impediments make this task challenging: 1) the exceptional lability of BPDE modification which makes the determination of precise adduct site location nearly impossible when collision-based methods of fragmentation are used and 2) very low abundance of protein-BPDE adducts in biological fluids. In our work, we address the first challenge by using Electron Transfer Dissociation to preserve the information about the adduct site and to achieve gentle dissociation of the peptide backbone. The second challenge is addressed by multistep sample enrichment using semi-preparative offline high pH reverse phase chromatography. Our ultimate goal is to enable assessment of exposure on an individual level using protein adducts as retro perspective exposure biomarkers when personal monitoring is inadequately available, for instance, as for firefighters during large wildfires.

Track: Computational Modeling/Simulation
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 475 | Combating COVID-19 Using High Resolution Computational Protein Docking with Natural Drug Products

Zirui Wang[1], Theodore Belecciu[1], Daniel Woldring[1], Michael Bachmann[1]
[1]Michigan State University (Michigan, United States)

The COVID-19 pandemic has resulted in a staggering number of deaths worldwide. Although multiple vaccines have been developed, the problem is still unsolved for people in areas that lack vaccines and an adequate medical infrastructure. A possible approach to solve this problem lies in using phytochemicals (plant chemicals) to inhibit the function of essential SARS-CoV-2 proteins. Generally, these phytochemicals are safe, cheap to extract, and many of them have documented antiviral properties. To identify potential therapeutic phytochemicals, we selected approximately 300 phytochemicals from the USDA Phytochemical and Ethnobotanical Databases to computationally simulate their interactions with SARS-CoV-2 proteins. In this study, we used Rosetta to implement in-silico protein-ligand docking and obtain the estimated binding affinities of the phytochemicals. For improved accuracy and performance, we modified the standard Rosetta ligand docking protocol to contain the following features among others: a protonation step that ensures the ligands have a protonation state that corresponds to a specified pH, modified ligand-related parameters in Rosetta Docking Script, and an algorithm that reads potential binding pocket information and gives docking starting positions. Within this new workflow, we conducted a comparison between several leading Rosetta score functions to identify the one best-suited for docking ligands onto our collection of proteins. Based on correlation coefficients between REU (Rosetta Energy Units) and RMSD obtained from docking simulations, the Talaris2014 score function was determined to yield the most consistent results. Using our improved docking workflow, we identified

18 potentially effective phytochemical binders for various SARS-CoV-2 proteins. Among these lead molecules, we also discovered a collection of binders like Bilobetin that have a high binding affinity towards multiple SARS-CoV-2 proteins. Overall, this study will provide valuable insight for plant-based drug development in the context of COVID-19 treatment. Our newly established protocol is well-suited for a broad range of protein-ligand docking scenarios.

Track: Therapeutics and Antibodies

Session: New Protein Post-Translational Modifications

ABS# 476 | Specific modification of IgG1 antibodies using the glutamine-walk strategy

Adem Hadjabdelhafid-Parisien[1], Lukas Deweid[2], Sebastian Bitsch[2], Arturo Maccaron[2], Kiana Lafontaine[1], Harald Kolmar[2], Joelle N. Pelletier[1]
[1]*Université de Montréal,* [2]*Technical University Darmstadt (Quebec, Canada)*

Antibody-drug conjugates are composed of a monoclonal antibody bound to a cytotoxic drug, combining the efficacy of chemotherapy with the high specificity of the antibody for the treatment of cancer. Microbial transglutaminase (mTG) allows the conjugation of amino molecules on a glutamine (Q295) of the human crystallizable fragment (hFc) of deglycosylated antibodies1. However, the deglycosylation process reduces antibody stability and solubility, limiting its use for industrial-scale preparation. We propose to investigate 80 positions of IgG1 hFc for conjugation by mTG on a glycosylated antibody. Glutamines are introduced individually into the hFc that is then expressed in prokaryotic cells. The reactivity of mTG towards the new variants is determined by a conjugation reaction using amino fluorophores2. The I253Q variant was identified as allowing conjugation by mTG and was validated in the context of a complete antibody, trastuzumab, used for the treatment of Her2+ breast cancer. Characterization of mutated trastuzumab conjugated with monomethylauristatin E, a cytotoxic drug, shows that antigen binding and compound cytotoxicity are maintained. These results are promising for the use of mTG in the synthesis of antibody-drug conjugates.1. Jeger, S.; Zimmermann, K.; Blanc, A.; Grunberg, J.; Honer, M.; Hunziker, P.; Struthers, H.; Schibli, R. Angew Chem Int Ed Engl 2010, 49 (51), 9995-7.2. Rachel, N. M.; Quaglia, D.; Lévesque, É.; Charette, A. B.; Pelletier, J. N. Protein Science 2017, 26 (11), 2268-2279.

Track: Folding

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 477 | Characterizing the Folding and Stability of the Ultrafast-Folding Protein λ-Repressor in Living Cells

Charnice Hoegnifioh[1], Edward Knab[1], Caitlin Davis[1]
[1]*Yale University, Department of Chemistry (VA, United States)*

Over the past three decades, ultrafast-folding proteins have served as models for comparison between experiments and computer simulations. While these studies have shown good agreement between protein folding mechanisms, timescales, size, conformations, and thermostability in vitro, they have yet to consider steric crowding and non-steric chemical interactions arising from the native cellular environments. To experimentally test in-cell protein folding that can be directly compared to simulation, I propose designing a fluorescent resonance energy transfer (FRET) labeled ultrafast-folding construct. I will determine the melting temperature of the all-α protein λ-repressor using temperature-dependent FRET microscopy, circular dichroism, and tryptophan fluorescence. After quantifying the differences between in vitro and in cells, the thermostability and compaction of λ-repressor within the cellular environment will be reproduced in vitro using artificial crowding and sticking agents. I hypothesize that steric interactions arising from macromolecular interactions inside cells will enhance the thermostability and compact λ-repressor tertiary structure by destabilizing extended protein conformations. These experiments will provide experimental data that can be used to validate computational models of protein folding in cells.

Track: Transcription/translation/post-translational modifications

Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 478 | Contribution of nascent polypeptides of increasing length to the apparent stability of the bacterial ribosome

Meranda Masse[1], Angela Varela[1], Aniruddha Srivastava[1], Wanting Wei[1], Valeria Guzman-Luna[1], Silvia Cavagnero[1]
[1]*University of Wisconsin-Madison (Wisconsin, United States)*

The ribosomes' role in the cell extends beyond catalysis of peptide-bond formation. For instance, the ribosome is emerging as a key player in cotranslational protein folding. Conversely, whether and how the ribosome assists nascent-protein folding, and how the nascent protein may in turn affect the ribosome, is poorly understood. Recent results suggest that the ribosome facilitates protein folding by directly interacting with nascent chains. Yet, whether these interactions have any influence on the ribosome and or promote proper protein folding is currently unknown. Via sucrose-gradient assays, we found that empty ribosomes are more sensitive to urea denaturation than ribosomal complexes carrying either aminoacyl-tRNA or nascent-chains. Further, by taking advantage of tryptophan fluorescence emission, we probed the effect of nascent chains with variable sequence and length on the apparent-stability of ribosomal proteins (rProteins). Our results show no statistically significant difference between the contribution of different nascent chains, even though crosslinking-urea titrations show differences in the strength of rProtein and nascent-chain interactions taking place outside of the ribosomal exit tunnel. These results suggest that nascent-chain-to-rProtein interactions are either weak or thermodynamically compensated.Hence, there is no difference between the sensitivity to urea of ribosomal initiation complexes carrying aminoacyl tRNA and ribosomal complexes harboring peptidyl tRNA. In all, the above findings lead us to conclude that aminoacyl tRNA, likely in conjunction with mRNA, increases the apparent stability of the ribosome, while the nascent polypeptide does not. Finally, our data led us to propose a multi-step model for the disassembly of ribosome-nascent-chain complexes.

Track: Proteostasis and quality control

Session: New Protein Post-Translational Modifications

ABS# 479 | Parkinson's disease-related PINK1 mutations impact PINK1-TOM complex assembly and Parkin mitochondrial recruitment in mammalian cells.

Mohamed Eldeeb[1], Armaan Fallahi[1], Emma MacDougall[1], Edward Fon[1]
[1]*Department of Neurology, Montreal Neurological Institute, McGill University (QC, Canada)*

PTEN- induced putative kinase 1 (PINK1) is a mitochondrial Ser/Thr kinase that was originally characterized as an autosomal recessive gene for familial recessive early-onset Parkinson disease (PD). Notably, previous work revealed that PINK1 is involved in a mitochondrial quality control pathway with the E3 ubiquitin ligase Parkin, another autosomal recessive gene product associated with PD, where Parkin operates downstream of PINK1. Upon mitochondrial damage elicited by genetic or chemical depolarization of mitochondria, Parkin is recruited to mitochondria and mediates the autophagic clearance of this damaged organelle. Importantly, recruitment of Parkin to damaged mitochondria requires the accumulation of PINK1 on the outer mitochondrial membrane (OMM) and the formation of high molecular weight PINK1-TOM complexes. Thus, fine-tuning the levels of PINK1 and Parkin is crucial for maintain a healthy mitochondrial network and for regulating autophagic clearance of deregulated mitochondria through mitophagy. Recent findings point to the regulation of PINK1 levels as an essential factor in regulating mitochondrial quality control. Although it is known that the loss of mitochondrial membrane potential elicits mitochondrial PINK1 accumulation, the biochemical elements within PINK1 sequence that link biochemical stresses to PINK1 mitochondrial accumulation remain poorly understood. Herein, we have examined the role of some Parkinson's disease (PD)-associated mutations on the mitochondrial accumulation of the mitochondrial kinase PINK1, on its biochemical association with the TOM mitochondrial complex, and on Parkin mitochondrial recruitment, a crucial step involved in mitophagy or the targeted clearance of damaged mitochondria. Our work on the functional ramifications of some PD-related mutations of PINK1 underscores the role of PINK1 PD-mutations in altering the molecular function of wild-type PINK1 and highlights some features of the molecular basis of PD. In ongoing work, we are investigating the role of additional PD-related mutations on PINK1 degradation and their functional impact on mitophagy.

Track: Therapeutics and Antibodies

Session: Protein Evolution, Design and Selection

ABS# 480 | Transfer Learning as a Superior Platform in Binding Affinity Prediction for Non-Immunoglobulin Scaffolds

Mehrsa Mardikoraem[1], Daniel Woldring[1]
[1]*Michigan State University (MI, United States)*

Protein Engineering is among the focal points for improving the current trends in medical applications from diagnostics to therapeutics. However, the field is in need of more advanced techniques to improve the efficiency and throughput of novel drug discovery. Accordingly, with

the rapidly increasing availability of protein sequence and structure data available and development on computational tools, especially high-performance GPU, machine learning is gaining more attention in this platform for prediction tasks that improve our understanding of the protein sequence-structure-function dynamics. However, a myriad of issues prevent the undemanding use of machine learning algorithms and precluding them from acquiring accurate predictions. Astronomical mutational space and complex epistatic relationships are among the inherent challenges in proteins. Additionally, biased datasets (high diversity in experimental conditions, high sequence similarity between datasets, etc) containing insufficiently characterized amino acid sequences (i.e. unlabeled) introduce further challenges for achieving high-performance predictions in ML algorithms. Yet, hidden within the protein sequence-structure-function landscape there exists a language (considering amino acids as words) from which protein features and functionality can be extracted. Here we aim to use transfer learning to take advantage of unlabeled data from deep sequencing datasets and apply the acquired embedding for our downstream task of linking amino acid sequence to protein binding affinity. This platform is used to predict the fitness of a non-immunoglobulin scaffolds against biomarkers pertinent to either treating or diagnosing human disorders. This approach offers a promising route for increased therapeutics and diagnostics applications in protein engineering.

Track: Structure (X-Ray/NMR/EM)
Session: Diffraction Methods are Alive and Well

ABS# 481 | Structural Characterization of an Insect Methyltransferase Involved in Juvenile Hormone Biosynthesis

Marie-Ève Picard[1], Rong Shi[1], Michel Cusson[1,2]
[1]Université Laval, [2]Natural Resources Canada (Québec, Canada)

Juvenile hormone (JH) is almost exclusively found in insects and plays a major role in their development and reproduction. Inhibition of JH production in insects leads to physiological consequences threatening the survival of these organisms. The development of bio-rational insecticides targeting the JH biosynthesis is an interesting direction for controlling insect pests.Insects of the order Lepidoptera (butterflies and moths) produce forms of JH with a particular feature, namely the presence of bulkier ethyl branches, that suggest enzymes of the JH biosynthetic pathway could be targeted to develop enzyme inhibitors specific to this order of insects which includes several forest and agricultural pests.Here, we report the structure of a Lepidopteran methyltransferase involved in the JH biosynthesis pathway in complex with its S-adenosyl-L-methionine (SAM) cofactor. The structure was obtained by X-ray crystallography. Our results allow for a better understanding of the active site structure, and will help guide future attempts to develop specific inhibitors for this order of insects.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 482 | Harnessing the PDB to Build TRAF6 Inhibitors

Avinoam Singer[1], Dustin Whitney[1], Amy Keating[1], Craig Mackenzie[2]
[1]MIT, [2]Dartmouth College (MA, United States)

It has recently been shown that the PDB can be decomposed into a set of reoccurring backbone tertiary motifs, or "TERMs", which can be combined to produce the backbone structure of any given protein (MacKenzie et al., 2016). The same TERMs that constitute protein folds can be used to describe – and construct –protein interfaces. dTERMen is a computational protein design method that mines sequence and structural data from TERMs, with the purpose of redesigning the sequences of structures with known backbone geometries and generating structures de novo. We have used dTERMen to design inhibitors of the C-terminal domain of TNF-associated factor 6 (TRAF6), a signaling protein involved in inflammatory responses. The TRAF6-C domain recognizes linear motifs with the sequence PxExxθ (where "θ" is an aromatic or acidic residue and "x" is unrestricted).A high-affinity peptide sourced from a screen for TRAF6-C binders was modeled for binding to TRAF6. The backbone of this peptide served as an anchor, to which auxiliary regions, generated de novo using TERMs with the goal of introducing stabilizing distal contacts, were tethered by a short linker. This methodology was used to generate four classes of designs, grouped by the structure of their auxiliary region, but with variable amino acid sequences. Seven of fifteen constructs bound to TRAF6, and four were selected for further analysis of the auxiliary region's contribution to binding.Truncation of the auxiliary region had a modest effect on the affinity of three designs for TRAF6. The fourth construct, 5_1, exhibited a 25-fold decrease in binding relative to the full-length. Mutational analysis of 5_1 suggests that this construct binds as designed. Notably, 5_1 binds to TRAF6

with a 4-fold higher affinity than the tightest screen-generated binder, and with a Kd of 6.4±0.4 uM, represents one of the highest affinity TRAF6-C binders discovered to date.

Track: Membrane Proteins
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 483 | Overcoming aggregation of a mitochondrial membrane protein

Ramiro Illanes Vicioso[1], Elena Ruiz López[1], Maria Solà[1]
[1]*IBMB-CSIC (Cataluña, España)*

We are interested in a transmembrane protein located at the mitochondrial inner membrane. The protein contains three domains of unknown function. The N- terminal domain is placed at the intermembrane space, a transmembrane helix crosses the inner membrane, and it is followed by the C-terminal domain at the matrix. About thirty constructs were tested in order to produce the most stable protein fragment, suitable for expression and crystallization. Nevertheless, very few genetic constructs, all corresponding to the N-terminal domain, were soluble. Only one construct, N4, showed good expression levels in Escherichia coli and could be isolated. However, N4 showed a high aggregation tendency, suggesting its stability was not enough. We will present the different purification strategies and the re-thinking of the construct to overcome these problems and find the key to solve protein stability and production in high yields.

Track: Amyloid and Aggregation
Session: Allostery & Dynamics in Protein Function

ABS# 484 | Salmonella Typhimurium CsgC Delays the Aggregation of Human Islet Amyloid Polypeptide

Karen Guerrero[1], George Orwa[1], Sajith Jayasinghe[1]
[1]*California State University San Marcos (CA, United States)*

The Curli specific gene (Csg) C product is a bacterial periplasmic protein involved in the assembly of bacterial Curli. Curli is a type of proteinaceous cell surface filament found on gram-negative bacteria such as E. Coli and Salmonella that serve to play an essential role in cell-cell interactions and host cell colonization. Curli, composed primarily of the protein CsgA and to a lesser extent the protein CsgB, is classified as a functional amyloid. The assembly of Curli involves four other proteins, CsgC, CsgE, CsgF, and CsgG. CsgC, CagE, and CsgF are thought to act as chaperones to help prevent the premature aggregation of CsgA and/or CsgB in the periplasm and transport of these proteins through the periplasm to the outer membrane protein CsgG. CsgA, CsgB, and CsgF are secreted through CsgG to the cell surface where they assemble to form Curli. CsgC has been shown to influence the in-vitro aggregation of CsgA as well as human a-synuclein which is thought to play a role in Parkinson's disease. We investigated the ability of the Salmonella Typhimurium CsgC to influence the aggregation of the amyloidogenic human islet amyloid polypeptide (hIAPP). We find that CsgC is able to delay the in-vitro aggregation of hIAPP at stoichiometric and sub-stoichiometric concentrations. Coupled with data from a bioinformatics analysis we are carrying out in-vitro experiments to further our understanding the mechanistic details of CsgC's ability to influence the aggregation of hIAPP.

Track: Design/engineering
Session: Protein Evolution, Design and Selection

ABS# 486 | Engineering of the polyproline type II helical layer fold in antifreeze proteins

Connor Scholl[1], Laurie Graham[1], Peter Davies[1]
[1]*Queen's University, Kingston, Ontario, Canada (Ontario, Canada)*

Antifreeze proteins (AFP) allow organisms living at subzero temperatures to avoid freezing damage by binding to, and preventing further growth of, ice crystals. Many different AFP structures, including alpha helices, beta solenoids, and globular folds, have been characterized to date. The polyproline type II (PPII) helical layer is a newly discovered AFP fold found exclusively in Collembola, a subclass of arthropods commonly called springtails. The PPII helix contains exactly three residues G-X1-X2 per turn, where X1 is often Gly. The Gly residues in positions 1 and 2 of the repeat form the core of the protein, with the lack of sidechains allowing a hydrogen bonding network to form directly between the peptide backbones of neighbouring helices. The outfacing surface of one PPII layer, which is the ice-binding site (IBS), is flat and somewhat hydrophobic, while the surface of the other is uneven and contains polar and charged residues. The IBS organizes surface waters into an ice-like arrangement that facilitates binding to the ice lattice. Isoforms of other AFPs have shown a correlation between the surface area of the IBS and antifreeze

activity. Due to the regularity of the PPII fold, the area of the IBS can potentially be altered by changing the length of each helix and/or by changing the number of helices. Larger and smaller versions of a natural 9-helix PPII AFP were modelled and evaluated in silico before being expressed in E. coli Arctic Express™. Modifications to the area of the IBS had large effects on antifreeze activity, which nearly doubled with the addition of one helix to the IBS and decreased 20-fold with the removal of a helix. Protein engineering studies of this type will allow a better understanding of the structure-function relationship of these proteins and help explore the upper limits of antifreeze activity.

Track: Membrane Proteins
Session: Protein Evolution, Design and Selection

ABS# 487 | Deletion and mutation analyses of an ice nucleation protein reveal its similarity to antifreeze proteins

Jordan Forbes[1], Akalabya Bissoyi[2], Lukas Eickhoff[3], Naama Reicher[4], Chris Bon[1], Virginia Walker[5], Thomas Koop[3], Yinon Rudich[4], Ido Braslavsky[2], Peter Davies[1]
[1]Department of Biomedical and Molecular Sciences, Queen's University, [2]The Robert H. Smith Faculty of Agriculture, Food and Environment, Institute of Biochemistry, Food Science, and Nutrition, The Hebrew University of Jerusalem, [3]Faculty of Chemi (Ontario, Canada)

Outer membrane-associated ice nucleation proteins (INPs) in certain Gram-negative bacteria can cause frost damage to plants by nucleating ice in supercooled water. Pseudomonas borealis INP (PbINP) has a large central domain comprised of 65 tandem sixteen-residue repeats that are flanked by short N- and C-terminal domains of uncertain function. A model for the structure of the central repeat region suggests that INPs resemble a much larger version of some beta-solenoid antifreeze proteins. Whereas antifreeze proteins may organize ice-like water molecules to facilitate ice adsorption, the larger INPs likely order sufficient water molecules to initiate ice nucleation. When PbINP was expressed in Escherichia coli, it triggered ice nucleation at high sub-zero temperatures within a degree or two of native P. borealis activity. Successive deletions of the central 65-repeat region incrementally lowered the nucleation temperature in droplet freezing assays, with a precipitous loss of activity mediated by INPs with fewer than 15 repeats. Mutation of a subset of putative water-organizing residues caused a decrease in nucleation activity comparable to that observed when the corresponding segment was deleted. Nano-droplets of serially diluted cultures containing zero, one, or a few bacteria showed an all-or-nothing response, suggesting that recombinant bacteria had a uniform, reproducible phenotype that reflected the extent of INP truncation. Deletion of the 42-residue C-terminal domain resulted in the complete loss of ice nucleation activity, as did deletion of the last few sixteen-residue repeats. These data show the correlation between central domain length and INP activity and support the hypothesis that repetitive water-ordering motifs are necessary for ice nucleation.

Track: Amyloid and Aggregation
Session: Allostery & Dynamics in Protein Function

ABS# 488 | Probing structural hotspots responsible for aggregation of ALS-associated SOD1 mutations

Harmeen Deol[1], Elizabeth Meiering[1]
[1]University of Waterloo (Ontario, Canada)

Toxic misfolded and aggregated protein species are commonly present inneurodegenerative diseases and are implicated as major drivers for disease. Highresolutionfeatures of aggregate precursors and their mechanisms of formationremain obscure. Additionally, for neurodegenerative diseases like amyotrophiclateral sclerosis (ALS) that exhibit tremendous disease heterogeneity, multiplemechanisms may be involved. Mutations in Cu,Zn superoxide dismutase (SOD1) areassociated with familial ALS. This metalloenzyme undergoes several posttranslationalmodifications in vivo to reach a stable homodimer. Recent studiessupport more immature forms of the protein play a critical role in the diseasepathology via their toxic aggregation. Aggregation studies traditionally focus on thefinal aggregate structure and discount protein conformations responsible forinitiating aggregation. Here we investigate the aggregation mechanism of immatureALS-mutant SOD1s. Using nuclear magnetic resonance (NMR) and dynamic lightscattering (DLS), we identify structural hotspots involved in initiating aggregation. Interestingly, residues in the metal-binding loop and the dimer interface regionsshow significant perturbations under varying solution conditions, suggesting theircritical role in initiating SOD1 aggregation. Our results are compared to diseaserelevant characteristics like disease duration to understand the link between keystructural hotspots responsible for initiating aggregation and disease heterogeneityin ALS.

Track: Chaperones
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 489 | Translation-coupled protein folding assay by using a reconstituted translation system

Tatsuya Niwa[1,2], Eri Uemura[1], Yuki Matsuno[2], Hideki Taguchi[1,2]
[1]*Cell Biology Center, Institute of Innovative Research, Tokyo Institute of Technology,* [2]*School of Life Science and Technology, Tokyo Institute of Technology (Kanagawa Pref., Japan)*

Protein folding is one of the most important issues in protein science. However, it is a quite complicated physicochemical phenomenon and still difficult to fully understand. To uncover a part of this, we have been conducting comprehensive analyses by using a reconstituted cell-free translation system, named the PURE system. Our previous analysis revealed more than one thousand proteins in Escherichia coli fail in spontaneous folding and form amorphous aggregates in the absence of any molecular chaperone. Further analysis showed two major chaperone systems, GroEL/ES and DnaK/DnaJ/GrpE, can prevent these proteins from forming aggregates. However, since these methods are based on a solubility evaluated by a simple centrifugation, the method to directly monitor folding status should be needed for a precise evaluation. To accomplish this, we focused on Lon protease, which is known as one of the major E. coli cytosolic proteases and can distinguish folding status of proteins. By combining Lon protease and the PURE system, we have established a direct monitoring method for protein folding. With this method, we investigated randomly chosen ~90 E. coli proteins in the absence of chaperones. The results suggested that most of highly soluble proteins evaluated by the previous analysis fold spontaneously. In addition, the analysis with the combination of the two chaperone systems and Lon protease revealed that GroEL/ES has a stronger effect on completing the folding of aggregation-prone proteins than DnaK/DnaJ/GrpE.

Track: Chemical Biology
Session: Targeted Protein Degradation

ABS# 490 | Haven't Got a Glue: Translating Molecular Insights on Chromatin and Ubiquitin Ligases into Novel Therapeutics

Nico Thomä[1]
[1]*Friedrich Miescher Institute for Biomedical Research (BS, Switzerland)*

Molecular glue compounds induce protein-protein interactions that, in the context of a ubiquitin ligase, lead to protein degradation Unlike traditional enzyme inhibitors, these molecular glue degraders act substoichiometrically to catalyse the rapid depletion of previously inaccessible targets. They are clinically effective and highly sought-after, but have thus far only been discovered serendipitously. Here, through systematically mining databases for correlations between the cytotoxicity of 4,518 clinical and preclinical small molecules and the expression levels of E3 ligase components across hundreds of human cancer cell lines, we identify CR8-a cyclin-dependent kinase (CDK) inhibitor-as a compound that acts as a molecular glue degrader. The CDK-bound form of CR8 has a solvent-exposed pyridyl moiety that induces the formation of a complex between CDK12-cyclin K and the CUL4 adaptor protein DDB1, bypassing the requirement for a substrate receptor and presenting cyclin K for ubiquitination and degradation. Our studies demonstrate that chemical alteration of surface-exposed moieties can confer gain-of-function glue properties to an inhibitor, and we propose this as a broader strategy through which target-binding molecules could be converted into molecular glues.

Track: Protein Interactions and Assemblies
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 491 | A New Look at the Iconic Catalytic Antioxidant, Cu/Zn Superoxide Dismutase, by Two-dimensional Gel Electrophoresis and Scanning Transmission Electron Microscopy shows the Enzyme as Multimers

Mary Hamilton[1]
[1]*Department of Natural Sciences, Fordham University College at Lincoln Center (NY, USA)*

When "pure" bovine Cu/Zn superoxide dismutase (SOD1) is analyzed by gel electrophoresis in any 1-D system in either the native or denatured state, instead of the expected dimer, ie a 32 kDa band, an array of multimeric bands is obtained which have enzyme activity. In a 2-D system, the first dimension resolves isoforms in a pH gradient and the second by molecular weight in SDS-PAGE. For pure bovine liver SOD1, the pI's of six isoforms ranged from pH 5.5 to 6 in

various amounts. The most prominent one with a pI of 5.9 represented 70 % and had a MW of ca. 100 kDa. The others had molecular weights ca. 70 to 94 kDa. Bovine erythrocyte SOD1 had a different spectrum of spots. Visualization of freeze-dried pure SOD by Scanning Transmission Electron Microscopy showed a distribution of masses greater than 30 kDa. The Negative Stained image showed ca.100 kDa particles with a "dumbbell" shape. Gel analyses of crude extracts of bivalve mussel tissues also show SOD1 as multimers supporting the conclusion that functional SOD1 is an "oligomer" rather than a 30 kDa dimer.

Track: Bioinformatics

Session: Protein Evolution, Design and Selection

ABS# 492 | Toward understanding the origin and evolution of cellular organisms and viruses

Minoru Kanehisa[1]

[1]*Institute for Chemical Research, Kyoto University, Uji, Kyoto, Japan (Kyoto, Japan)*

The KEGG database (https://www.kegg.jp) has been a resource for understanding system-level functions of cellular organisms from genome sequences and other molecular-level datasets. This is accomplished by the procedure called KEGG mapping, whereby the user's dataset is mapped against the KEGG reference knowledge base to reconstruct information systems represented as molecular interaction and reaction networks and responsible for various cellular processes and organism behaviors. As KEGG becomes more mature – it is now 26 years old – we wish to make KEGG a more fundamental resource toward understanding basic principles of biological systems, such as coevolution of genomes and information systems. We are trying to improve the quality of KOs and modules (conserved genes and gene sets), to identify subsystems (such as reaction modules) and to establish relationships to taxonomic groups. Viruses have been treated as perturbants to human molecular networks in the KEGG PATHWAY and NETWORK databases. They are now part of this effort accumulating more viral KOs and their interactions with host KOs.1. Kanehisa, M. (2019) Toward understanding the origin and evolution of cellular organisms. Protein Sci. 28, 1947-1951. https://doi.org/10.1002/pro.37152. Kanehisa M, et al. (2021) KEGG: integrating viruses and cellular organisms. Nucleic Acids Res. 49, D545-D551. https://doi.org/10.1093/nar/gkaa970

Track: Structure (X-Ray/NMR/EM)

Session: Diffraction Methods are Alive and Well

ABS# 494 | Protein structural dynamics revealed by molecular movie analysis

Eriko Nango[1]

[1]*Tohoku University (Miyagi, Japan)*

Serial femtosecond crystallography (SFX) is a new technique for determining a room-temperature protein structure by collecting diffraction images from randomly oriented microcrystals using X-ray free-electron lasers (XFEL). Intense, femtosecond XFEL pulses afford diffraction patterns from protein microcrystals before the onset of radiation damage. Combined with a reaction trigger, time-resolved SFX has enabled structural dynamics and reactions of proteins to be visualized as a molecular movie with high spatial and temporal resolution. We have developed time-resolved SFX techniques at SPring-8 Angstrom Compact free-electron laser (SACLA) since 2012.Light triggering has been used to initiate reactions in protein crystals by rapid excitation. We previously reported a molecular movie of conformational changes in a light-driven proton pump, bacteriorhodopsin from 16 ns to 1.7 ms after photoactivation, which we measured using our experimental setup (Nango, E. et al. Science, 2016). Subsequently, the photoisomerization process of the retinal in bacteriorhodopsin from femtoseconds to picoseconds was observed (Nogly, P. et al. Science, 2018). However, the application of time-resolved SFX was limited to a light-sensitive protein until recently. Mix-and-inject SFX have allowed us to capture enzymatic reactions by the rapid mixing of protein crystals in a substrate solution (Stagno, J.R. et al. Nature 2017). We used the mix-and-inject SFX techniques to observe structural changes in a beta-decarboxylating enzyme. As a result, it was revealed how a substrate binds to the active site with a mixing time. At the session, we will present detailed results from the mix-and-inject SFX experiment and the results related to time-resolved SFX.

Track: Protein Interactions and Assemblies

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 497 | Protein nanocage assembly and stability tuning by a single amino acid

Mantu Kumar[1], Soumyananda Chakraborti[1], Jonathan Heddle[1]

[1]Malopolska Centre of Biotechnology, Jagiellonian University (Krakow, Poland)

Ferritin is a cage-forming protein that possesses a central hollow cavity used for iron storage. Due to robustness, biocompatibility and nano-sized (12 nm diameter) dimensions it is a versatile bio nanomaterial. Ferritin has been used for diverse purposes including for enzyme encapsulation, antigen display and as a therapeutic cargo carrier, MRI contrast agent and biosensor. Canonical ferritin lacks reversible assembly, usually requiring harsh chemical treatment to open the cage, something which could potentially destroy or inactivate the sensitive macromolecular cargoes. A new class of ferritins have been discovered which overcome this limitation: here cage assembly can be controlled by ionic strength and concentration of certain divalent cations. Archeal ferritin from Archeoglobus fulgidus (AfFtn), and bacterial ferritin, from Thermotoga maritima (TmFtn) are two such assemblies known to show such mild assembly and disassembly characteristics. In our study, we worked with TmFtn ferritin as the alternative, AfFtn cage contains four large pores and meaning that cargo molecules can potentially cargo can leak out. We found that the dimeric interface residue E65 in TmFtn controls cage assembly behavior. Furthermore, we found that reliance on metal ions for assembly can be tuned depending on the identity of this residue. In one case (E65R), requirement for metal ions (Mg2+) was abolished; while in another case, the threshold concentration was lowered. Mutations also imparted differential thermal and pH stability to the assembled cage as studied using CD and nanoDSF. We have crystallized and solved the mutant cage structures to gain more details of the role of residue 65 in assembly while cage formation, size and morphology were assessed using Native PAGE, SEC, DLS and transmission electron microscopy (TEM) imaging. Engineered TmFtn nanocage successfully tested for enzyme encapsulation and will be used for designing 3D hybrid nanostructures of DNA and Protein.

Track: Design/engineering

Session: Protein Evolution, Design and Selection

ABS# 498 | Evolving virus-like nucleocapsids from a bacterial enzyme

Donald Hilvert[1]
[1]Laboratory of Organic Chemistry, ETH Zurich (Zurich, Switzerland)

Viruses consist of a protective proteinaceous shell that packages an RNA or DNA genome. The emergence of protein cages that could load, protect, and transfer their own genetic information was therefore likely to be a critical step in the evolution of all primitive viruses. Using a combination of design and directed evolution, this process can now be recapitulated in the laboratory. We have converted a bacterial enzyme called lumazine synthase into an artificial nucleocapsid that efficiently encapsulates its own encoding mRNA, and have elucidated the structural changes in cargo and container that made this transformation possible. In addition to providing insight into the origins of natural viruses, such constructs may serve as non-viral carriers for diverse vaccine and delivery applications.

Track: Transcription/translation/post-translational modifications

Session: New Protein Post-Translational Modifications

ABS# 499 | Rapid detection and purification of glycosylated proteins and their receptors

Jared Edwards[1], Priyanka Kadav[1], Jessica Krycia[1], Purnima Bandyopadhyay[1], Tarun Dam[1]
[1]Michigan Technological University (Michigan, United States)

Glycosylation is an important and ubiquitous process through which glycans are covalentlyattached to proteins during and after their translation. Glycosylated proteins are divided into two major groups: glycoproteins (GPs) and proteoglycans (PGs). Glycan moieties of GP and PG are recognized by specific reader proteins or receptors, broadly known as glycan-binding proteins (GBPs). Interactions of GBPs with specific GP and PG determine the outcome of numerous biological activities. In order to understand the functions of GBPs, GPs and PGs, they must be detected and then purified. Conventional techniques to detect and purify these proteins are elaborate, expensive, time consuming and often suffer from problems caused by non-specific binding. To minimize these limitations, we have developed and validated a new detection/purification technique termed "Capture and Release (CaRe). In this method, a target (GBP, GP or PG) is captured in solution phase by a target capturing agent (TCA). The captured target is then freed from the TCA by competitive ligands and separated by filtration to get the purified form of the target. A target thus purified is then identified by SDS-PAGE and mass-spectrometric analysis. CaRe is a fast and precise method that does not need affinity matrix,antibodies, functionalization of reagents and special equipment. The method can

efficientlyseparate and identify targets even when the sample volume is limited (0.5 ml to 1.0 ml). CaRehas the ability to expedite the discovery of new GBP, GP and PG and thus can help elucidatetheir biological functions.

Track: Structure (X-Ray/NMR/EM)
Session: Measuring Forces of Biological Systems

ABS# 500 | PROTEIN EXPRESSION AND PURIFICATION OF G-PROTEIN COUPLED RECEPTOR KINASE 6 (GRK6)-TARGETED THERAPEUTICS IN MULTIPLE MYELOMA

Srivatsan Iyer[1], Tien Olson[2]
[1]Undergraduate Student, [2]Postdoctoral Researcher (AZ, United States)

Srivatsan Iyer, Tien L. Olson, Debbie Hansen and Petra FrommeMultiple myeloma (MM) is one of hematological malignancies accounting for 2% of all blood cells related cancers. With the current therapy treatments focusing on high dose chemotherapy and therapeutic drugs, the mean survival time from the time of diagnosis is approximately 5 years. G-protein coupled receptor kinase 6 (GRK6) has been shown as a key protein involved in MM. GRK6 inhibition is broadly lethal to multiple myeloma tumor cells but appears tolerated in human epithelial cells, illustrating a wide therapeutic index. The structure determination of the protein in complex with its inhibitor will be extremely important for the development of a novel therapeutic for MM. In this work, we describe the protein expression and purification of two GRK6 constructs, GRK6His/EK and GRK6His/TEV expressed in Sf9 insect cells. GRK6 protein expressed from both constructs were highly homogeneous and monodisperse. The yields of purified GRK6His/EK and GRK6His/TEV was approximately 0.7 mg per liter of cell culture that is suitable for structural studies of GRK6 with an inhibitor.

Track: Structure (X-Ray/NMR/EM)
Session: Allostery & Dynamics in Protein Function

ABS# 501 | Structure of the AMPK complex in its inactive ATP-bound state

Yan Yan[1], Karsten Melcher[2], Somnath Mukherjee[3], Kaleeckal G. Harikumar[4], Timothy S. Strutzenberg[5], X. Edward Zhou[6], Kelly Suino Powell[1], Ting-Hai Xu[1], Ryan D. Sheldon[7], Jared Lamp[8], Joseph S. Brunzelle[9], Katarzyna Radziw
[1]Program for Structural Biology, Center of Cancer and Cell Biology, Van Andel Institute, [2]Laboratory of Structural Biology & Biochemistry Department of Structural Biology Van Andel Institute, [3]Department of Biochemistry and Molecular Biology, [4](MI, USA)

Structure of the AMPK complex in its inactive ATP-bound stateAMP-activated protein kinase (AMPK) is a master regulator of energy homeostasis and a promising drug target for the treatment of metabolic diseases, including diabetes and cancer. Under energy stress, AMP stabilizes the active AMPK conformation, in which the kinase activation loop (AL) is protected from protein phosphatases, thus keeping the AL in its active, phosphorylated state. In the absence of stress, ATP inhibits AMPK by increasing AL dynamics and accessibility, which posts tremendous challenges for structure determination. We developed conformation-specific antibodies to trap ATP-bound AMPK in its fully inactive, highly dynamic state and determined its structure at 3.5 Å resolution. The structure revealed a dramatic 180° rotation and 100 Å displacement of the kinase domain to fully expose the AL, which we confirmed in solution and by and live cell BRET assays. This allowed us to determine a multi-step mechanism of how adenine nucleotides and pharmacological agonists modulate AMPK activity by altering AL phosphorylation and accessibility.

Track: Chaperones
Session: Allostery & Dynamics in Protein Function

ABS# 503 | Allosterically-driven shifting of dynamic Hsp90 conformations by clients and co-chaperones determines the progression of the chaperone cycle

Abraham Lopez[1,2], Vinay Dahiya[1], Maximilian Biebl[1], Florent Delhommel[1,2], Ralf Stehle[1], Sam Asami[1], Johannes Buchner[1], Michael Sattler[1,2]
[1]Technical University of Munich, [2]Helmholtz Center Munich (Bavaria, Germany)

Hsp90 is a molecular chaperone that interacts with partially folded client proteins and assist them in achieving their final mature states. The underlying molecular mechanism is still enigmatic, involving transitions between open conformations and a fully closed, active state of the Hsp90 dimer. Combining NMR, SAXS and biochemical experiments, we have identified an open state of Hsp90 induced by ATP in which the N-terminal domain (NTD) adopts a rotated state and docks with the middle domain. This interdomain association triggers an

allosteric response that affects elements at the client binding sites and prepares the Hsp90 dimer for closing. By analyzing the interactions of four distinct clients, i.e. two steroid hormone receptors (GR and MR), p53 and Tau, we have identified a switch point that senses the rotation of the NTD and that mediates the binding of specific clients. This element acts as a conformational discriminator, allowing certain clients to select the rotated conformation of the NTD and promote Hsp90 dimer closing, and others to bind in a conformation-independent manner. The co-chaperone p23, a core component of the Hsp90 machinery which inhibits the ATPase activity and favors client activation, binds to the rotated state and further stabilize the closed conformation of Hsp90. Our results show that additional p23 stabilization is important for Hsp90-client complexes which do not readily adopt the closed conformation. Unexpectedly, our experiments revealed a conserved helix in the unstructured p23 tail that interacts with clients in the ternary complex. These interactions are important for the stabilization and conformational processing of the client by the Hsp90 machinery as its deletion compromised client activation in vivo. In summary, our study reveals molecular details of the conformational changes, interactions and allostery involved in the function and regulation of the Hsp90 cycle.

Track: Protein Interactions and Assemblies
Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 504 | Signalling by cooperative assembly formation (SCAF) by TIR domains in innate immunity and cell death pathways

Bostjan Kobe[1]
[1]*School of Chemistry and Molecular Biosciences, University of Queensland (Queensland, Australia)*

TIR (Toll/interleukin-1 receptor) domains are key components of innate immunity and cell-death pathways in animals and plants (1). Signaling depends on association of TIR domains. We reconstituted large assemblies of the TLR (Toll-like receptor) adaptor TIR domains and determined the structure of the filamentous assemblies of the TLR adaptor MAL (2) and the TLR4:MAL complex (unpublished) by cryo-electron microscopy (cryoEM), and MyD88 by micro-electron diffraction and serial femtosecond crystallography (3). As an unexpected twist, we found that TIR domains involved in cell-death pathways, including those from the human TLR adaptor SARM1, involved in axon degeneration, and those from plant immune receptors (NLRs), possess self-association-dependent NAD-cleavage activity (4,5). Crystal and cryoEM structures of human SARM1 and plant NLRs shed light on the structural basis of this enzymatic activity. Our studies unify the mechanism of function of TIR domains as "signaling by cooperative assembly formation (SCAF)" with prion-like features that leads to the activation of effector enzymes and show that some TIR domains can themselves function as effector enzymes (6). These systems display shape-shifting from monomeric proteins to open-ended assemblies to carry out their signalling functions. The structures will be useful for therapeutic development against neurodegenerative and inflammatory diseases and for development of improved resistance in agricultural crops. 1. Ve et al (2015) Apoptosis 20, 2502. Ve et al (2017) Nat Struct Mol Biol 24, 7433. Clabbers et al (2021) Nat Commun 12, 25784. Horsefield et al (2019) Science 365, 7935. Figley et al (2021) Neuron 109, 11186. Vajjhala et al (2017)Mol Immunol 86, 23

Track: Membrane Proteins
Session: Cryo-EM: Beyond Single Particle Reconstruction

ABS# 505 | Towards the 3D-structure of adhesion G-protein coupled receptors

Amal Hassan Ali[1], Michal Szczepek[1], Gunnar Kleinau[1], Franziska Wiechert[1], David Speck[1], Brian Bauer[1], Anja Koch[1], Ines Liebscher[1], Torsten Schöneberg[2], Patrick Scheerer[1]
[1]*Institute of Medical Physics and Biophysics, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Charitéplatz 1, D-10551 Berlin, Germany,* [2]*Rudolf Schönheimer Institute of Biochemist (Germany, Germany)*

Adhesion G protein-coupled receptors (aGPCRs) are broadly expressed and thought to play a fundamental role in intra-cellular connections and organogenesis. AGPCRs are classified as members of the GPCR superfamily but are still the most understudied ones. Although aGPCRs combine key features with other GPCRs, such as the canonical 7-transmembrane (7TM) domain structure, they present an exceptionally unique long ectodomains, which extend several hundreds to thousands of amino acids. AGPCRs contain important cell adhesion-related domains, including the GPCR autoproteolysis- inducing (GAIN) domain. The GAIN domain is conserved among the aGPCRs family and is known to play a crucial role in the receptor activation by undergoing an autoproteolytic process to cleave the receptor into two non-covalently

associated parts; N-terminal fragment (NTF) and C-terminal fragment (CTF).However, compared with their potential importance, their function, molecular mechanism and the downstream signal transduction mediated by aGPCRs are poorly understood at the structural level. Therefore, the application of structural skills will provide deep insights into understanding the receptor architecture and physiological functions. A general problem for crystallization experiments is the enormous size of aGPCRs- ectodomain, thus, cryo-electron microscopy (cryo-EM) is an alternative and suitable technique to study the 3D-structure of such membrane proteins with near atomic resolution.Here we are looking at a set of aGPCRs from a structural-functional point of view to understand their molecular mechanism of signaling. Thus, it will pave the way to deeply understand their pathophysiological role in diseases such as breast and brain cancer or synaptic connections.

Track: Protein Interactions and Assemblies

Session: Quaternary Structure: Shape Shifting in the Control of Protein Function

ABS# 507 | PPI3D: A Computational Resource for Searching, Analysis and Modeling Protein-Protein, Protein-Peptide and Protein-Nucleic Acid Interactions

Justas Dapkunas[1], Albertas Timinskas[1], Kliment Olechnovic[1], Migle Tomkuviene[1], Ceslovas Venclovas[1]
[1]*Vilnius University (Vilnius, Lithuania)*

The absolute majority of proteins function not as isolated entities, but through interactions with other macromolecules and/or ligands. To comprehensively understand how these proteins function, it is essential to know three-dimensional (3D) structures of their complexes. Even though the number of experimentally solved structures of protein complexes in PDB is steadily growing, it still represents only a tiny fraction of all known interactions. Moreover, structural data on protein complexes in PDB are noisy and redundant, hindering their effective use. To address these issues we have developed the PPI3D resource, representing non-redundant structurally-resolved protein-protein, protein-peptide interactions. Recently, PPI3D was extended to include protein-nucleic acid complexes. The non-redundant set of interactions is derived from PDB biological assemblies that are clustered according to both protein sequence similarity and structural similarity of interaction interfaces/binding sites. The latter property is quantified using contact surface areas at different levels of granularity. As a result, the clustering procedure effectively derives clusters of non-redundant interaction data at varying levels of detail without the loss of alternative interaction interfaces. PPI3D provides a possibility to search for all interactions of a given protein or a set of proteins as well as for the interactions between two proteins or two protein groups. Identified interactions (including homologous ones) are annotated in detail and can be analyzed interactively. Furthermore, PPI3D allows construction of structural models using identified protein complexes as structural templates. The usefulness of PPI3D as a resource of structural templates for modeling protein complexes has been demonstrated during recent CASP and CAPRI experiments aimed at unbiased testing of computational modeling methods.

Track: Chemical Biology

Session: Allostery & Dynamics in Protein Function

ABS# 508 | Melanoma-Derived L2538R Human DNA Polymerase θ Variant Exhibits Decreased DNA Polymerase Function

Morgan Andrews[1], Corey Thomas[1], Lisbeth Avalos Valiente[1], Jorge Victorino[1], Jamie Towle-Weicksel[1]
[1]*Rhode Island College (RI, USA)*

DNA damage can be caused by a variety of factors including, but not limited to, ultraviolet (UV) radiation. Solar UV light is known to be directly linked to pyrimidine dimer formation in DNA, which is closely associated with the incidence of skin cancer. These bulky DNA lesions can significantly compromise genomic integrity. Furthermore, they are specifically involved in the mutagenic transformation of melanocytes in the human body, which is known to result in melanoma, the most metastatic type of skin cancer. Human cells are capable of halting mutagenic outcomes through a variety of DNA repair pathways, using a series of specialized repair enzymes called DNA polymerases. Interestingly, Human DNA Polymerase Theta (Pol θ, POLQ), a uniquely error prone, A-family DNA polymerase, exhibits translesion bypass activity on bulky DNA adducts. Additionally, it has also been implicated in double strand break repair, through Microhomology Mediated End Joining (MMEJ) and Base Excision Repair (BER). Studies have shown that POLQ deficient cells experience increased sensitivity to UV radiation compared to normal cells, highlighting human Pol θ as a critical DNA repair enzyme. Several POLQ somatic mutations, notably, L2538R, located in the DNA polymerase active-site domain, have been identified in melanoma tumors from various patients at Yale SPORE in Skin Cancer. We hypothesize that this melanoma-derived L2538R variant,

will have altered DNA polymerase activity compared to wild-type (WT) Pol θ, which may interrupt DNA repair. L2538R was generated via site-directed mutagenesis, expressed in E.coli, and purified by affinity chromatography. Nucleotide incorporation and binding affinity assays were performed to kinetically characterize the biochemical activity of L2538R compared to WT. Preliminary studies reveal that L2538R experiences decreased DNA polymerase activity compared to WT Pol θ, suggesting aberrant Pol θ may have altered DNA repair function, which may contribute genomic instability and be a driver for melanoma.

Track: Protein Interactions and Assemblies
Session: Protein Evolution, Design and Selection

ABS# 512 | CowN, A Newly Characterized Auxiliary Protein That Sustains Nitrogenase Turnover in Presence of the Inhibitor Carbon Monoxide

Max Strul[1], Cedric Owens[1]
[1]*Chapman University (CA, United States)*

Nitrogenase is the only enzyme that has the ability to catalyze the production of ammonia (NH3) via the reduction of dinitrogen gas (N2). Nitrogenase is potently inhibited by carbon monoxide (CO). However, diazotrophs are able to protect themselves from this inhibition, using a protein called CowN. The mechanism of how CowN protects diazotrophs from CO inhibition has not been well characterized. Here we describe our work to characterize CowN. We conducted nitrogenase turnover assays with and without CowN. In the presence of 0.001 ATM CO, CowN almost completely restores nitrogenase activity. Furthermore, this CO protection and recovery also shows a hyperbolic relationship to the concentration of CowN. CowN has no catalytic activity on its own, so we hypothesized that CowN and nitrogenase interact. Diazirine-based crosslinkers and MALDI-TOF mass spectrometry experiments confirmed a protein-protein interaction. In conclusion, our studies suggest that CowN plays an important role as an auxiliary protein against CO inhibition for nitrogenase.

Track: Chemical Biology
Session: Novel Approaches to Observe Proteins in Their Natural Environment

ABS# 513 | Expression, Purification and Characterization of Recombinant Human Heteropolymer Ferritin Using a Novel Expression System

Ayush Srivastava[1], Fadi Bou-Abdallah[1]
[1]*SUNY Potsdam (NY, USA)*

Ferritin is a highly conserved supramolecular nanostructure and a key player in iron homeostasis. Eukaryotic ferritins are predominantly heteropolymeric species consisting of 24 structurally similar, but functionally different subunit types named H (for Heavy, ~ 21 kDa) and L (for Light, ~ 19 kDa). These subunits co-assemble in various H to L ratios to form shell-like protein structures (isoferritins) of ~ 8 nm inner cavity diameter able of accommodating thousands of iron atoms in the form of biologically available hydrous ferric oxide mineral core. Despite their discovery more than 8 decades ago, the isoferritin nanostructure-assembly process has not been studied owing to the lack of a good expression system that could produce these nano-assemblies. Earlier in-vitro reconstitution attempts using chemical denaturation and unfolding of recombinant homopolymers H- and L-subunits followed by their renaturation have either largely failed or yielded very low amounts of heteropolymer ferritins that are not representative of those occurring naturally. Here, we developed a novel expression system that allows the synthesis of recombinant human heteropolymer ferritin of any desired H to L composition using the Gibson Assembly cloning technique. The purified heteropolymer ferritins exhibited a single band on native gel confirming protein assembly and a single protein nanostructure, and their H to L subunit composition was confirmed by SDS gel and capillary electrophoresis. Iron oxidation and mobilization kinetics in ferritin provided insights into the complementary roles of H and L subunits into iron core formation and dissolution.