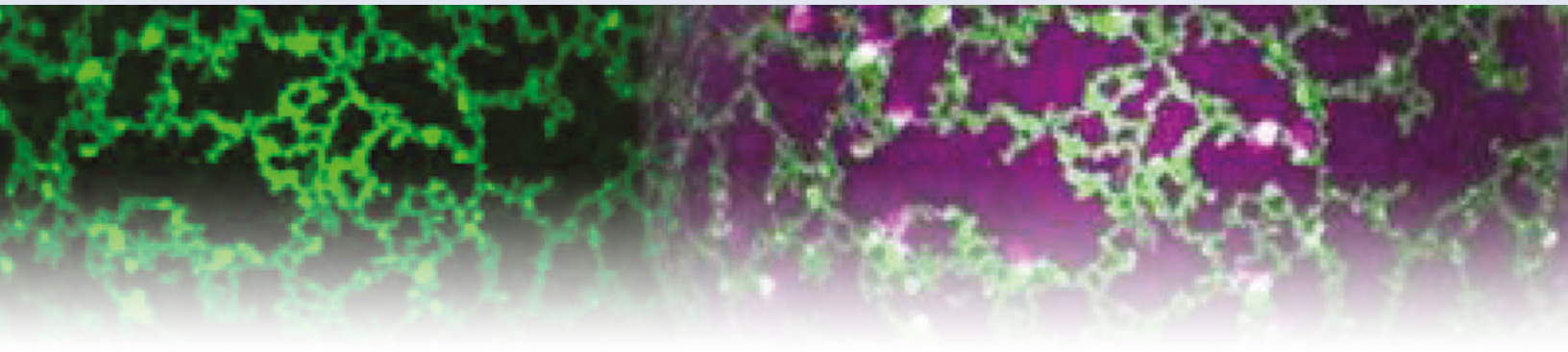
Biophysical Society Conferences



Molecular Biophysics of Membranes

Tahoe, California | Granlibakken | June 5–10, 2022

All scientific sessions and poster sessions will be held at Granlibakken Tahoe, in the Ballroom unless otherwise noted.

Organizing Committee

Linda Columbus, University of Virginia, USA
Merritt Maduke, Stanford University, USA

CHROMA®
nan][on
HAMAMATSU
PHOTON IS OUR BUSINESS
Avanti® POLAR LIPIDS
Part of Croda International Plc
SUTTER INSTRUMENT
NIH National Institutes of Health

Organizing Committee

Linda Columbus, University of Virginia, USA
Merritt Maduke, Stanford University, USA

Thank You to Our Sponsors

Thank you to all sponsors for their support.

June 2022

Dear Colleagues,

We welcome you to the Biophysical Society Conference on *Molecular Biophysics of Membranes*. This conference series is an opportunity for scientists from around the world to gather and exchange ideas. For many, this will be one of the first conferences since before the pandemic and we want to acknowledge the impact of how long we have not had the ability to congregate, present, and engage in science in person and at a destination. We strongly hope that the meeting will not only provide a venue for sharing recent and exciting progress, but also to promote fruitful discussions and to foster future collaborations in the search of our molecular understanding of membranes. We have assembled an exciting program, with talks focusing on different aspects related with the biophysics that investigates what membranes look like, how they function, and their role in biological processes. We have organized a program with 47 talks and 54 posters bringing together 111 attendees from different fields and countries, promising a truly international and multidisciplinary inspiring environment. There are several special-topic lunches focused on career development and panels to provide time and exploration of a variety of topics such as funding and fostering collaborations between experimental and computational investigations.

Our meeting location, Granlibakken, is in the beautiful Tahoe City, California with lots of outdoor activities. The meeting site has activities focused on adventure, family fun, and relaxation. We hope that you take advantage and enjoy the Treetop Adventure Park, tennis courts, hiking trails, bike paths, spa, and any of the other opportunities in this wonderful meeting site. Thank you all for engaging in the program of this meeting, and we look forward to enjoying Biophysics with all of you in Tahoe!

Sincerely,

Merritt Maduke and Linda Columbus
Co-Chairs, Molecular Biophysics of Membranes

Biophysical Society Code of Conduct, Anti-Harassment Policy

The Biophysical Society (BPS) is committed to providing an environment that encourages the free expression and exchange of scientific ideas. As a global, professional Society, the BPS is committed to the philosophy of equal opportunity and respectful treatment for all, regardless of national or ethnic origin, religion or religious belief, gender, gender identity or expression, race, color, age, marital status, sexual orientation, disabilities, veteran status, or any other reason not related to scientific merit.

All BPS meetings and BPS-sponsored activities promote an environment that is free of inappropriate behavior and harassment by or toward all attendees and participants of Society events, including speakers, organizers, students, guests, media, exhibitors, staff, vendors, and other suppliers. BPS expects anyone associated with an official BPS-sponsored event to respect the rules and policies of the Society, the venue, the hotels, and the city.

Definition of Harassment
The term "harassment" includes but is not limited to epithets, unwelcome slurs, jokes, or verbal, graphic or physical conduct relating to an individual's race, color, religious creed, sex, national origin, ancestry, citizenship status, age, gender or sexual orientation that denigrate or show hostility or aversion toward an individual or group.

Sexual harassment refers to unwelcome sexual advances, requests for sexual favors, and other verbal or physical conduct of a sexual nature. Behavior and language that are welcome/acceptable to one person may be unwelcome/offensive to another. Consequently, individuals must use discretion to ensure that their words and actions communicate respect for others. This is especially important for those in positions of authority since individuals with lower rank or status may be reluctant to express their objections or discomfort regarding unwelcome behavior. It does not refer to occasional compliments of a socially acceptable nature. It refers to behavior that is not welcome, is personally offensive, debilitates morale, and therefore, interferes with work effectiveness. The following are examples of behavior that, when unwelcome, may constitute sexual harassment: sexual flirtations, advances, or propositions; verbal comments or physical actions of a sexual nature; sexually degrading words used to describe an individual; a display of sexually suggestive objects or pictures; sexually explicit jokes; unnecessary touching.

Attendees or participants who are asked to stop engaging in harassing behavior are expected to comply immediately. Anyone who feels harassed is encouraged to immediately inform the alleged harasser that the behavior is unwelcome. In many instances, the person is unaware that their conduct is offensive and when so advised can easily and willingly correct the conduct so that it does not reoccur. Anyone who feels harassed is NOT REQUIRED to address the person believed guilty of inappropriate treatment. If the informal discussion with the alleged harasser is unsuccessful in remedying the problem or if the complainant does not feel comfortable with such an approach, they can report the behavior as detailed below.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Reporting a Violation
Violations of this Conduct Policy should be reported immediately. If you feel physically unsafe or believe a crime has been committed, you should report it to the police immediately.

To report a violation to BPS:

- You may do so in person at the Annual Meeting at the BPS Business Office in the convention center.

- You may do so in person to BPS senior staff at Thematic Meetings, BPS Conferences, or other BPS events.

- At any time (during or after an event), you can make a report through http://biophysics.ethicspoint.com or via a dedicated hotline (phone numbers listed on the website) which will collect and relay information in a secure and sensitive manner.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated per the procedure detailed below. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Investigative Procedure

All reports of harassment or sexual harassment will be treated seriously. However, absolute confidentiality cannot be promised nor can it be assured. BPS will conduct an investigation of any complaint of harassment or sexual harassment, which may require limited disclosure of pertinent information to certain parties, including the alleged harasser.

Once a complaint of harassment or sexual harassment is received, BPS will begin a prompt and thorough investigation. Please note, if a complaint is filed anonymously, BPS may be severely limited in our ability to follow-up on the allegation.

- An impartial investigative committee, consisting of the current President, President-Elect, and Executive Officer will be established. If any of these individuals were to be named in an allegation, they would be excluded from the committee.

- The committee will interview the complainant and review the written complaint. If no written complaint exists, one will be requested.

- The committee will speak to the alleged offender and present the complaint.

- The alleged offender will be given the opportunity to address the complaint, with sufficient time to respond to the evidence and bring his/her own evidence.

- If the facts are in dispute, the investigative team may need to interview anyone named as witnesses.

- The investigative committee may seek BPS Counsel's advice.

- Once the investigation is complete, the committee will report their findings and make recommendations to the Society Officers.

- If the severity of the allegation is high, is a possible repeat offense, or is determined to be beyond BPS's capacity to assess claims and views on either side, BPS may refer the case to the alleged offender's home institution (Office of Research Integrity of similar), employer, licensing board, or law enforcement for their investigation and decision.

Disciplinary Actions

Individuals engaging in behavior prohibited by this policy as well as those making allegations of harassment in bad faith will be subject to disciplinary action. Such actions range from a written warning to ejection from the meeting or activity in question without refund of registration fees, being banned from participating in future Society meetings or Society-sponsored activities, being expelled from membership in the Society, and reporting the behavior to their employer or calling the authorities. In the event that the individual is dissatisfied with the results of the investigation, they may appeal to the President of the Society. Any questions regarding this policy should be directed to the BPS Executive Officer or other Society Officer.

Table of Contents

GENERAL INFORMATION

Registration/Information Location and Hours

On Sunday and Monday venue check-in to obtain your room key will be located at the Main Lodge Front Desk at Granlibakken Tahoe, 725 Granlibakken Road, Tahoe City, CA 96145.

An Information Desk to pick up badge and meeting materials will be located at the Ballroom Pre-Function at the following times:

Sunday, June 5	5:00 PM – 6:00 PM
Monday, June 6	8:30 AM - 6:00 PM
Tuesday, June 7	8:30 AM - 6:00 PM
Wednesday, June 8	8:30 AM - 6:00 PM
Thursday, June 9	8:30 AM - 6:00 PM

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in the Ballroom. Speakers are required to bring their own laptops and adaptors. It is recommended to have a backup of the presentation on a USB drive in case of any unforeseen circumstances. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) All poster sessions will be held in the Ballroom.

2) A display board measuring 243 cm wide x 121 cm high (8 feet wide x 4 feet high) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as listed in the e-book.

3) Poster boards require pushpins or thumbtacks for mounting. Authors are expected to bring their own mounting materials.

4) There will be formal poster presentations on Monday, Tuesday, Wednesday, and Thursday. Posters will be available for viewing during their scheduled presentation date only. Presenting authors with odd-numbered poster boards should present from 4:00 PM – 5:00 PM and those with even-numbered poster boards should present from 5:00 PM – 6:00 PM.

5) During the assigned poster presentation sessions, presenters are requested to remain in front of their poster boards to meet with attendees.

6) All posters left uncollected at the end of the meeting will be disposed.

Meals and Coffee Breaks

There will be a one-hour Welcome Reception on Sunday evening from 7:00 PM - 8:00 PM. This reception will be held in the Ballroom.

Breakfasts, Lunch, and Dinner will be served at the Granhall. Coffee Breaks will be held at the Ballroom Pre-Function and Mixers will be held in the Ballroom.

Smoking

Please be advised that smoking is not permitted at Granlibakken Tahoe.

Name Badges

Name badges are required to enter all scientific sessions, poster sessions, and social functions. Please wear your badge throughout the conference.

Internet

Wifi will be provided at the venue.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from June 5-10 during registration hours.

In case of emergency, you may contact the following:

Dorothy Chaconas
Email: dchaconas@biophysics.org

Adam Vincent
Phone : 530.581.7316
Email: adamvincent@granlibakken.com

Molecular Biophysics of Membranes

Tahoe, California, USA
June 5-10, 2022

PROGRAM

Sunday, June 5, 2022

5:00 PM - 6:00 PM	**Registration/Information**	**Ballroom Pre-Function**
6:00 PM - 7:00 PM	**Welcome Reception**	**Ballroom**
7:00 PM - 8:00 PM	**Dinner**	**Granhall/Garden Deck**
8:00 PM - 8:15 PM	Merritt Maduke, Stanford University, USA Linda Columbus, University of Virginia, USA **Opening Remarks**	
8:15 PM - 9:15 PM	Anne Kenworthy, University of Virginia, USA ***Keynote Address*** ***How to Build, Manipulate, and Destroy Functional Nanodomains***	
9:15 PM - 9:45 PM	**Flash Introductions**	

Monday, June 6, 2022

7:00 AM - 8:30 AM	**Breakfast**	**Granhall**
8:30 AM - 6:00 PM	**Registration/Information**	**Ballroom Pre-Function**
Session I	**Mechanotransduction in the Membrane** Miriam Goodman, Stanford University, USA, Chair	
9:00 AM - 9:30 AM	Miriam Goodman, Stanford University, USA ***Deciphering Where and How Touch Happens***	
9:30 AM - 10:00 AM	Bianxiao Cui, Stanford University, USA ***Membrane Curvature Initiated Mechanotransduction in Cells***	
10:00 AM - 10:30 AM	Kate Poole, University of New South Wales, Sydney, Australia ***Multiple Pathways of Mechanoelectrical Transduction in Melanoma Cells***	
10:30 AM - 11:00 AM	**Coffee Break and Group Photo**	**Ballroom Pre-Function**
11:00 AM - 11:30 AM	Medha Pathak, University of California, Irvine, USA ***PIEZO1 on the Move***	
11:30 AM - 11:45 AM	Zheng Shi, Rutgers University, USA * ***Membrane Curvature Mediated Subcellular Distribution of PIEZO1***	
11:45 AM - 12:00 PM	Zhouyang Shen, Memorial Sloan Kettering, USA * ***A Synergy Between Mechanosensitive Calcium- and Membrane-Binding Mediates Tension-Sensing by C2-Like Domains***	

12:00 PM - 1:00 PM	**Special-topic Hosted Lunch Table** Teaching and Mentoring: inclusive practices (Linda Columbus, University of Virginia)	**Granhall/Garden Deck**
1:00 PM - 2:30 PM	**Panel Discussion: Challenges of Diversity and Inclusion in Science and Developing Action-Plans for Overcoming Them** Panelists: Linda Columbus, Karen Fleming, Miriam Goodman, Carlos Villalba-Galea	
2:30 PM - 4:00 PM	**Free Time**	
4:00 PM - 6:00 PM	**Poster Session I**	
6:00 PM - 7:00 PM	**Dinner**	**Granhall/Garden Deck**
Session II	**Membrane Organization** Sergio Grinstein, University of Toronto, Canada, Chair	
7:00 PM - 7:30 PM	Sergio Grinstein, University of Toronto, Canada ***Integration of Mechanosensory Signals by the Spectrin Cytoskeleton in Endothelial Cells***	
7:30 PM - 8:00 PM	Susan Daniel, Cornell University, USA ***Coronavirus Fusion Peptide Interactions with the Host Membrane Leads to Lipid Ordering and Membrane Fusion***	
8:00 PM - 8:30 PM	Syma Khalid, University of Oxford, United Kingdom ***Progress Towards a Computational Bacteriology Approach to Studying Gram-Negative Bacterial Membranes***	
8:30 PM - 8:45 PM	Ting-Sung Hsieh, UT Southwestern, USA * ***Dynamic Remodeling of Host Membranes by Self-Organizing Bacterial Effectors***	
8:45 PM - 9:00 PM	Ivan Castello Serrano, University of Virginia, USA * ***Rafting in a Rush: Membrane Microdomains in Secretory Trafficking***	
9:00 PM - 11:00 PM	**Mixer**	**Ballroom**

Tuesday, June 7, 2022

7:00 AM - 8:30 AM	**Breakfast**	**Granhall**
8:30 AM - 6:00 PM	**Registration/Information**	**Ballroom Pre-Function**
Session III	**Signaling through the Membrane** William Kobertz, University of Massachusetts Medical School, USA, Chair	
9:00 AM - 9:30 AM	William Kobertz, University of Massachusetts Medical School, USA ***Fluorescent Visualization of Cellular Fluxes***	
9:30 AM - 10:00 AM	Anne Carlson, University of Pittsburgh, USA ***Phosphate Position is Key in Mediating the TMEM16A-PI(4,5)P2 Interaction***	
10:00 AM - 10:30 AM	Ming-Feng Tsai, University of Colorado, USA ***Mechanisms and Significance of Tissue-Specific Mitochondrial Calcium Uptake***	

10:30 AM - 10:45 AM	**Coffee Break**	**Ballroom Pre-Function**
10:45 AM - 11:15 AM	Patrick Barth, EPFL, Switzerland ***Uncovering and Reprogramming Allosteric Signal Transductions in GPCRs***	
11:15 AM - 11:45 AM	Carlos Villalba-Galea, University of the Pacific, USA ***New Insights into the Modal Activity of K_V7 Channels***	
11:45 AM - 12:00 PM	Jorge E. Contreras, University of California, Davis, USA * ***Large-Pore Channels as Transporters: Lessons from Connexin, Pannexin and CALHM1 Channels***	
12:00 PM - 1:00 PM	**Special-topic Hosted Lunch Table** Careers: Being a Professor at a Teaching University (Carlos Villalba-Galea, University of the Pacific)	**Granhall/Garden Deck**
1:00 PM - 2:30 PM	**Workshop: Tips for Successful NIH Grant Proposals** Zhongzen Nie, NIH, USA	
2:30 PM - 4:00 PM	**Free Time**	
4:00 PM - 6:00 PM	**Poster Session II**	
6:00 PM - 7:00 PM	**Dinner**	**Granhall/Garden Deck**
Session IV	**Membrane Shape** Dimitrios Stamou, University of Copenhagen, Denmark, Chair	
7:00 PM - 7:30 PM	Dimitrios Stamou, University of Copenhagen, Denmark ***Membrane Curvature as A Regulator of GPCR Organization at the Plasma Membrane***	
7:30 PM - 8:00 PM	Alexey Ladokhin, University of Kansas, USA ***Lipids and Divalent Cations as Regulators of Bilayer Insertion of pHLIP and Bcl-2 Proteins***	
8:00 PM - 8:30 PM	Jeet Kalia, Indian Institute of Science Education and Research Bhopal, India ***Employing the Double Knot Toxin-TRPV1 Ion Channel Complex as a Model System to Interrogate the Roles of Protein-Membrane Interactions in Protein Function***	
8:30 PM - 8:45 PM	Tugba N. Ozturk, Washington University, USA * ***Elimination of Membrane Deformations Drives CLC-ec1 Dimerization***	
8:45 PM - 9:00 PM	Giacomo Fiorin, NIH, USA * ***Potentials of Mean Force of Biomembrane Deformation***	
9:00 PM - 11:00 PM	**Mixer**	**Ballroom**

Wednesday, June 8, 2022

7:00 AM - 8:30 AM	**Breakfast**	**Granhall**
8:30 AM - 6:00 PM	**Registration/Information**	**Ballroom Pre-Function**

Session V	**Ion Channels and Transporters** Joseph Mindell, NINDS, NIH, USA, Chair	
9:00 AM - 9:30 AM	Erkan Karakas, Vanderbilt University, USA ***Structural Basis for Activation and Gating of IP3 Receptor Calcium Channels***	
9:30 AM - 10:00 AM	Joseph Mindell, NINDS, NIH, USA ***Fat Chances: How a Signaling Lipid Influences Lysosomal pH Via the Chloride Transporter ClC-7***	
10:00 AM - 10:30 AM	Emily Liman, University of Southern California, USA ***Structure and Function of the OTOP Proton Channels***	
10:30 AM - 10:45 AM	**Coffee Break**	**Ballroom Pre-Function**
10:45 AM - 11:15 AM	Randy Stockbridge, University of Michigan, USA ***Structural Determinants of Substrate Specificity in Small Multidrug Resistance Transporters***	
11:15 AM - 11:30 AM	Olive E. Burata, University of Michigan, USA * ***Elucidating the Substrate Specificities of the Two Major Functional Subtypes in the Small Multidrug Resistance (SMR) Family***	
11:30 AM - 11:45 AM	Huong T. Kratochvil, University of California, San Francisco, USA * ***Designed Proton Channels Reveal Mechanisms for Proton Channel Selectivity and Conductivity***	
11:45 AM - 12:00 PM	Jun Chen, Genentech, USA * ***TRPA1 – Biased Agonism and Structural Mechanisms of Modulation***	
12:00 PM - 1:00 PM	**Special-topic Hosted Lunch Table** Careers: Research in Biotech (Jun Chen, Genentech)	**Granhall/Garden Deck**
1:00 PM - 2:30 PM	**Panel Discussion: Establishing Expectations and Benchmarks in Collaborations Between Experiment and Computation** Panelists: Linda Columbus, Jessica Swanson, Syma Khalid, and Joseph Mindell	
2:30 PM - 4:00 PM	**Free Time**	
4:00 PM - 6:00 PM	**Poster Session III**	
6:00 PM - 7:00 PM	**Dinner**	**Granhall/Garden Deck**
Session VI	**Membrane Protein Dynamics** Linda Columbus, University of Virginia, USA, Chair	
7:00 PM - 7:30 PM	Linda Columbus, University of Virginia, USA ***Impact of Lipid-Membrane Protein Interactions on Membrane Protein Structure and Dynamics***	
7:30 PM - 8:00 PM	Jessica Swanson, The University of Utah, USA ***Kinetic Selection of Competing Ion Exchange Pathways: When Does the Electrical Versus Chemical Gradient Matter?***	

8:00 PM - 8:30 PM	Lynmarie Thompson, University of Massachusetts, Amherst, USA ***NMR and Hydrogen Exchange Studies of Bacterial Chemotaxis Receptor Complexes Suggest Protein Stabilization is Key to the Signaling Mechanism***
8:30 PM - 8:45 PM	Shwetha Srinivasan, Massachusetts Institute of Technology, USA * ***Lipid Dependence of the Conformational Coupling Across the Membrane Bilayer of Full-Length Epidermal Growth Factor Receptor***
8:45 PM - 9:00 PM	Damien Thévenin, Lehigh University, USA * ***Promoting Receptor Protein Tyrosine Phosphatase Activity by Targeting Transmembrane Domain Interactions***
9:00 PM - 11:00 PM	**Mixer** **Ballroom**

Thursday, June 9, 2022

7:00 AM - 8:30 AM	**Breakfast** **Granhall**
8:30 AM - 6:00 PM	**Registration/Information** **Ballroom Pre-Function**
Session VII	**Membrane Protein Folding** Karen Fleming, Johns Hopkins University, USA, Chair
9:00 AM - 9:30 AM	Karen Fleming, Johns Hopkins University, USA ***SurA: a "Groove-y" Chaperone That Expands Unfolded Outer Membrane Proteins***
9:30 AM - 10:00 AM	Jonathan Schlebach, Indiana University Bloomington, USA ***Coordination of -1 Programmed Ribosomal Frameshifting by the Transcript and Nascent Polypeptide***
10:00 AM - 10:30 AM	Ismael Mingarro, University of Valencia, Spain ***Deciphering an Interfaciality Scale for Proteins at Biological Membranes***
10:30 AM - 10:45 AM	**Coffee Break** **Ballroom Pre-Function**
10:45 AM - 11:15 AM	Joanna Slusky, University of Kansas ***Colicin E1 Opens Its Hinge to Plug TolC***
11:15 AM - 11:30 AM	Kaitlyn V Ledwitch, Vanderbilt University * ***Integrative Structural Modeling of Membrane Proteins Using Sparse Paramagnetic NMR and Neutron Scattering Data***
11:30 AM - 11:45 AM	Zuzana Coculova, University of Oxford, United Kingdom * ***Droplet-On-Hydrogel Bilayer Based Assay for Functional Study of Membrane Proteins***
11:45 AM - 12:00 PM	Shirley Schreier, University of San Paola, Brazil * ***Half a Century Deciphering Membrane Structure, Dynamics and Function Based on a Commentary in Biophysical Reviews, 13:849–852 (2021)***
12:00 PM - 1:00 PM	**Special-topic Hosted Lunch Table** **Granhall/Garden Deck** Publishing: Thoughts from the Perspective of Academic Editors (Dimitrios Stamous, BJ editor; Joseph Mindell, JGP editor; Merritt Maduke, eLife & BJ editor)

1:00 PM - 2:30 PM	**Panel Discussion: Establishing Expectations and Benchmarks in Collaborations Between Experiment and Computation** Panelists: Linda Columbus, Jessica Swanson, Syma Khalid, and Joseph Mindell	
2:30 PM - 4:00 PM	**Free Time**	
4:00 PM - 6:00 PM	**Poster Session IV**	
6:00 PM - 7:00 PM	**Dinner** **Closing Remarks and *Biophysical Journal* Poster Awards**	**Granhall/Garden Deck**
Session VIII	**Membrane Remodeling, Fusion, and Exocytosis** Kelly Lee, University of Washington, USA, Chair	
7:00 PM - 7:30 PM	Kelly Lee, University of Washington, USA ***Dissecting Membrane Structure and Remodeling Using Cryo-Electron Tomography***	
7:30 PM - 8:00 PM	Gregory Voth, University of Chicago, USA ***Membrane Remodeling by Proteins: Insights and Surprises from Multiscale Computer Simulation***	
8:00 PM - 8:30 PM	Peter Kasson, University of Virginia, USA ***Protein Activation and Membrane Deformation in Enveloped Virus Entry***	
8:30 PM - 8:45 PM	Volker Kiessling, University of Virginia, USA * ***Lipid Protein Interactions Guiding Fusion Pore Opening and Expansion During Regulated Exocytosis***	
8:45 PM - 9:00 PM	Joana Paulino, University of California San Francisco, USA * ***Characterization of the Elusive SERINC5-AP2-Nef Complex in the Context of a Lipid Bilayer***	
9:00 PM - 11:00 PM	**Mixer**	**Ballroom**

Friday, June 10, 2022

7:00 AM - 8:30 AM	**Breakfast and Departure**	**Granhall**

**Short talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

HOW TO BUILD, MANIPULATE, AND DESTROY FUNCTIONAL NANODOMAINS

Anne K. Kenworthy;
[1]University of Virginia, Center for Membrane and Cell Physiology, Charlottesville, VA, USA

All membranes share a characteristic bilayer morphology, but their lateral organization can be remarkably complex. In biological membranes, lipids and proteins can self-assemble laterally to generate a variety of compositionally distinct domains that range in size from nanometers to microns, exist over a wide range of time scales, and assume varying curvatures and morphologies. Our group seeks to understand the physicochemical principles that govern the assembly and function of two related yet distinct classes of membrane nanodomains: membrane rafts and caveolae. Both reside within the plasma membrane of cells, form in a cholesterol-dependent manner, regulate multiple cellular processes, and, when defective, contribute to human disease. Yet, they differ substantially in morphology, lifetime, and function. Here, I will discuss recent insights emerging from our work on two major unanswered questions in the field: 1) What is the structural basis for caveolae assembly and function? and 2) Are membrane rafts druggable targets?

DECIPHERING WHERE AND HOW TOUCH HAPPENS

Miriam B. Goodman[1];
[1]Stanford University, Department of Molecular and Cellular Physiology, Stanford, CA, USA

Touch is the first sense to develop and it is often sense the last to fade. Ion channels, the first responders of touch sensation, convert the mechanical energy delivered during touch into electrical signals within milliseconds or faster. At least three classes of proteins form these specialized mechanoelectrical transduction (MeT) complexes: DEG/ENaC/ASIC sodium channels, TMC cation channels, TRP cation channels, and Piezo cation channels. The DEG/ENaC/ASIC and TMC channels are thought to activate via a force-from-filament activation mode, while the others operate in a force-from-lipid mode. Regardless of which force-dependent gating model applies to a given channel, we hypothesize that the subcellular position of MeT channels is tightly regulated and helps to determine the threshold and dynamic range of touch sensation. Despite the importance of the subcellular distribution of MeT channels for touch sensation, however, little is understood about how their positions are established and stabilized within somatosensory neurons. As a first step toward addressing this question, we focused on the junction between somatosensory neurons and surrounding epidermal cells. In many animals, including humans and nematodes, this junction is filled by a specialized extracellular matrix or basal lamina. Using touch receptor neurons in Caenorhabditis elegans, we show that the MeT channel MEC-4 is anchored to stable and punctate mechanosensory complexes in vivo and that these complexes also contain the ancient and conserved basal lamina proteins, laminin and nidogen. All three proteins fail to coalesce into discrete, stable structures in dissociated neurons and in touch-insensitive mec-1, mec-9 and mec-5 mutants lacking secreted ECM proteins. By contrast, only MEC-4, but not laminin or nidogen, is destabilized in animals in which the somatosensory neurons secrete a mutant MEC-1 carrying missense mutations in the C-terminal Kunitz domain. Thus, neuron-epidermal cell interfaces are instrumental in mechanosensory complex assembly and function. Drawing on computational modeling, we propose that these complexes concentrate mechanical stress into discrete foci and they enhance touch sensitivity. Consistent with this idea, loss of nidogen reduces the density of mechanoreceptor complexes, the amplitude of the touch-evoked currents they carry, and touch sensitivity in parallel. These findings imply that somatosensory neurons secrete proteins that actively repurpose the basal lamina to generate special-purpose mechanosensory complexes responsible for touch sensation.

MEMBRANE CURVATURE INITIATED MECHANOTRANSDUCTION IN CELLS

Bianxiao Cui;
[1]Stanford University, Chemistry, Stanford, CA, USA

Membrane curvature in the range of tens to hundreds of nanometers is involved in many essential cellular processes. At the cell-matrix interface, where the cells make physical contact with extracellular matrices, the membrane may be locally deformed by matrix topography or mechanical forces, and this deformation may actively regulate signal transmission through the interface. We explore nanofabrication to engineer vertical nanostructures protruding from a flat surface. These nanostructures deform the plasma membrane to precisely manipulate the location, degree, and sign (positive or negative) of the interface curvature in live cells. We found that these membrane curvatures significantly affect the distribution of curvature-sensitive proteins and modulate mechanotransduction in live cells. Our studies show a strong interplay between membrane curvature and mechanotransduction and reveal molecular mechanisms underlying the connection.

MULTIPLE PATHWAYS OF MECHANOELECTRICAL TRANSDUCTION IN MELANOMA CELLS

Kate Poole[1]; Amrutha Patkunarajah[1]; Surabhi Shrestha[1]; Georgina Sanderson[1]; Lioba Schroeter[1];
[1]University of New South Wales, EMBL Australia Node in Single Molecule Science, School of Medical Sciences, Sydney, Australia

The conversion of mechanical inputs into an electrical signal is an ancient sense, with mechanically gated channels found in all classes of life. The discovery of the PIEZO family of mechanically gated channels has had a profound impact on our understanding mechanoelectrical transduction in mammalian physiology. However, there are still substantial gaps in knowledge about how other molecules influence and mediate mechanoelectrical transduction in mammalian systems. We have recently described a PIEZO1-independent mechanoelectrical transduction pathway that depends on TMEM87a/ELKIN1 and our data suggest that ELKIN1-dependent currents can be mechanically evoked in the cell-substrate and cell-cell interfaces. Deletion of ELKIN1 from melanoma cells was found to modulate cell attachment strengths, cell mechanics, migration speeds and invasive properties, in more than one melanoma cell line. In addition, the impact of deleting ELKIN1 was found to be distinct from the impact of deleting PIEZO1 in a melanoma cell line expressing both molecules. Further work is required to clarify if ELKIN1 is modulating a mechanically activated ion channel or functioning as an ion channel itself. However, our data demonstrate that mechanoelectrical transduction in melanoma cells is not solely dependent on the PIEZO proteins and that signalling via distinct mechanoelectrical transduction pathways can result in different functional outcomes.

PIEZO1 ON THE MOVE

Medha M. Pathak[1];
[1]University of California, Irvine, Dept. of Physiology & Biophysics, Irvine, CA, USA

A major unanswered question in biology is how mechanical forces are generated, detected, and transduced by cells to impact biochemical and genetic programs. Our work is aimed at uncovering the mechanical principles at play in cells and tissues using novel molecular, imaging, and bioengineering tools. Here we present insights gleaned from non-invasive approaches to measure and manipulate mechanotransduction in native cellular conditions. We find that the mechanically-activated ion channel Piezo1 transduces cell-generated traction forces to regulate a variety of biological processes. We show that cellular traction forces generate spatially-restricted Piezo1 Ca^{2+} flickers in the absence of externally-applied mechanical forces. However, Piezo1 channels are widely distributed on the cell surface and are mobile. Single particle tracking reveals a heterogeneity in the mobility behavior of individual channel puncta. We propose that Piezo1 Ca^{2+} flickers allow spatial segregation of mechanotransduction events and that mobility allows channel molecules to efficiently respond to mechanical stimuli.

MEMBRANE CURVATURE MEDIATED SUBCELLULAR DISTRIBUTION OF PIEZO1

Zheng Shi[1];
[1]Rutgers University, Chemistry and Chemical Biology, Piscataway, NJ, USA

Piezo1 is the bona fide mechanosensitive ion channel in mammalian cells, activated by local tension in the plasma membrane. The distribution of Piezo1 within a cell is essential for mechano-transduction, cell division and migration, and wound healing. However, the underlying principle that guides the subcellular distribution of Piezo1 is still unclear. Here, we show that membrane curvature serves as a general regulator of Piezo1 distribution in the plasma membrane of live cells, leading to strong depletion of Piezo1 on membrane protrusions such as filopodia. Quantifying the membrane curvature preference of Piezo1 leads to a direct estimation of the molecular size Piezo1 in live cell membranes. Chemical activation leads to increased density of Piezo1 on filopodia, independent of Ca^{2+}, consistent with a flattened configuration of the channel upon activation. Furthermore, the curvature preference of Piezo1 inhibits filopodia formation and regulates important aspects of cellular development and dynamics.

A SYNERGY BETWEEN MECHANOSENSITIVE CALCIUM- AND MEMBRANE-BINDING MEDIATES TENSION-SENSING BY C2-LIKE DOMAINS

Zhouyang Shen[1,2]; Kalina T Belcheva[3]; Mark Jelcic[1,2]; King L Hui[1]; Anushka Katikaneni[1]; Philipp Niethammer[1];
[1]Memorial Sloan Kettering Cancer Center, Cell Biology, New York, NY, USA
[2]Gerstner Sloan Kettering Graduate School of Biomedical Sciences, New York, NY, USA
[3]Weill Cornell Graduate School of Medical Sciences, Biochemistry, Cellular and Molecular Biology Allied Program, New York, NY, USA

To properly function in a complex physiological environment, a cell must be able to tell whether its surrounding lipid membranes are stretched. Currently, mechanosensitive ion channels (e.g. Piezo1) are the most well studied tension-sensors, but recent work on cytosolic phospholipase A2 ($cPLA_2$) suggests a peripheral enzyme that detects nuclear membrane stretch during cell migration or tissue damage constitutes the second class of mechano-sensors. When nuclear membranes are stretched, $cPLA_2$ binds through its calcium-dependent C2 domain and initiates the biosynthesis of eicosanoids, which participate in numerous physiological processes including immune defense and immune cell motility. However, precisely how membrane tension regulates $cPLA_2$ C2-domain sensing remains poorly understood. Although C2 and C2-like domains are commonly found in various peripheral enzymes involved in protein signal transduction and membrane trafficking, it remains largely unknown how many of them are mechano-sensors and the quantitative relationship between tension and membrane binding, leaving a large knowledge gap in the field of membrane mechano-transduction. In this study, we imaged the mechanosensitive adsorption of $cPLA_2$ and its C2 domains to intact nuclear membranes and artificial bilayers, comparing them to other related C2-like motifs. Membrane stretch enhances Ca^{2+} sensitivity of all tested domains, promoting $cPLA_2$ half-maximal membrane binding at cytoplasmic-resting Ca^{2+} concentrations. In contrast, increasing membrane tension selectively strengthens adsorption affinity of C2 domains that utilize prominent hydrophobic protrusions to insert into the bilayer core (e.g. $cPLA_2$ C2), but it produces no effect or even weakens the affinity of other C2 domains that electrostatically interact with membrane lipids (e.g. Protein Kinase C C2). Overall, our data suggests that a synergy of mechanosensitive Ca^{2+} interactions and deep, hydrophobic membrane insertions contributes to the exceptional mechanosensitivity of $cPLA_2$ C2 domains, providing a quantitative basis for understanding C2 domain membrane mechanotransduction.

INTEGRATION OF MECHANOSENSORY SIGNALS BY THE SPECTRIN CYTOSKELETON IN ENDOTHELIAL CELLS

Sergio Grinstein[1,2]; Sivakami Mylvaganam[1,2]; Spencer A Freeman[1,2];
[1]Hospital for Sick Children, Cell Biology, Toronto, ON, Canada
[2]University of Toronto, Biochemistry, Toronto, ON, Canada

Blood flow induces the secretion of vasoactive compounds, notably nitric oxide (NO), and promotes endothelial cell elongation and reorientation parallel to the direction of applied shear. How shear is sensed and relayed to intracellular effectors is incompletely understood. We demonstrate that an apical spectrin network is essential to convey the force imposed by shear to endothelial mechanosensors. By anchoring CD44, spectrin modulates the cell surface density of hyaluronan, a major component of the glycocalyx that senses and translates shear into changes in plasma membrane tension. Spectrins also regulate the stability of apical caveolae, where the mechanosensitive Piezo1 channels are thought to reside. Accordingly, shear-induced Piezo1 activation and the associated calcium influx were absent in spectrin-deficient cells. As a result, cell realignment and flow-induced stimulation of the NO synthase, eNOS, were similarly dependent on spectrin. We concluded that the apical spectrin network is not only required for shear sensing, but transmits and distributes the resulting tensile forces to mechanosensitive ion channels that elicit protective and vasoactive responses.

CORONAVIRUS FUSION PEPTIDE INTERACTIONS WITH THE HOST MEMBRANE LEADS TO LIPID ORDERING AND MEMBRANE FUSION

Susan Daniel[1];
[1]Cornell University, RF Smith School of Chemical and Biomolecular Engineering, Ithaca, NY, USA

The coronavirus disease 2019 (COVID-19) necessitates develop of effective therapies against the causative agent, SARS-CoV-2, and other pathogenic coronaviruses (CoV) that have yet to emerge. Focusing on the CoV replication cycle, specifically the entry steps involving membrane fusion, is an astute choice because of the conservation of the fusion machinery and mechanism across the CoV family. For coronavirus, entry into a host cell is mediated by a single glycoprotein protruding from its membrane envelope, called spike (S). Within S, the region that directly interacts with the membrane is called the fusion peptide, FP. It is the physico-chemical interactions of the FP with the host membrane that anchors it, enabling the necessary deformations of the membrane leading to delivery of the viral genome into the cell when a fusion pore opens. Thermodynamic, kinetic, and intermolecular interactions are useful to understand molecular level FP interactions with the host membrane. This knowledge can be leveraged to stop the spread of infection. Here, we examine the impact of calcium ions on CoV entry. Using cell infectivity, biophysical assays, and spectroscopic methods, we found that calcium ions stabilize the FP structure during conformational change that then allows its insertion into the host membrane, resulting in increased lipid ordering in the membrane. This lipid ordering precedes membrane fusion and correlates with increased fusion activity and higher levels of infection when calcium in present. As such, depletion of calcium ions leads to structure and activity changes in the fusion peptide that correlate well with in vitro experiments using calcium-chelating agents to block cell infection. In a final set of experiments, we show calcium channel blockers can block virus infection in lung cells.

PROGRESS TOWARDS A COMPUTATIONAL BACTERIOLOGY APPROACH TO STUDYING GRAM-NEGATIVE BACTERIAL MEMBRANES

Syma Khalid[1];
[1]University of Oxford, Oxford, United Kingdom

Bacterial cell envelopes are compositionally complex, crowded and while highly dynamic in some areas their molecular motion is very limited, to the point of being almost static in others, therefore it is no real surprise that studying them at high resolution across a range of temporal and spatial scales requires a number of different techniques. Details at atomistic to molecular scales for up to tens of microseconds is now within range for molecular dynamics simulations. We are using both atomistic and more coarse-grained models to explore (a) the routes via which small molecules and antimicrobial peptides permeate across bacterial membranes and (b) the organisation of bacterial membranes in terms of the arrangement of proteins and lipids. The insights from both of these areas are combined into a continuously developing molecular level picture of the cell envelope of Gram-negative bacteria. Given the threat we face from antibiotic-resistant bacteria, such a picture is important for future development of therapeutic strategies against pathogenic bacteria.

DYNAMIC REMODELING OF HOST MEMBRANES BY SELF-ORGANIZING BACTERIAL EFFECTORS

Ting-Sung Hsieh[1]; Victor A Lopez[1]; Miles H Black[1]; Beatrice Ramm[2]; Adam Osinski[1]; Krzysztof Pawlowski[1,3]; Diana R Tomchick[4,5]; Jen Liou[6]; Vincent S Tagliabracci[1,7];
[1]UT Southwestern Medical Center, Department of Molecular Biology, Dallas, TX, USA
[2]Princeton University, Department of Physics, Princeton, NJ, USA
[3]Warsaw University of Life Sciences, Department of Biochemistry and Microbiology, Institute of Biology, Warsaw, Poland
[4]UT Southwestern Medical Center, Department of Biophysics, Dallas, TX, USA
[5]UT Southwestern Medical Center, Department of Biochemistry, Dallas, TX, USA
[6]UT Southwestern Medical Center, Department of Physiology, Dallas, TX, USA
[7]UT Southwestern Medical Center, Howard Hughes Medical Institute, Dallas, TX, USA

A central theme in cell regulation is phosphorylation and dephosphorylation reactions catalyzed by competing kinases and phosphatases. Particularly, phosphoinositide kinases and phosphatases convert phosphatidylinositol (PI) into various phosphoinositide species that exhibit distinct, dynamic membrane localization to shape compartmental identity and regulate membrane trafficking in eukaryotic cells. Although spatiotemporal regulation of phosphoinositides has been widely studied, it is unclear if phosphoinositide kinases and phosphatases can operate in a self-organized manner to establish order and form structures on the membrane. Here we report a self-organizing system consisting of a bacterial phosphoinositide kinase and its opposing phosphatase that form spatiotemporal patterns, including traveling waves, to remodel host cellular membranes. The Legionella effector MavQ, a PI 3-kinase, is targeted to the host cell's endoplasmic reticulum (ER). MavQ and the Legionella PI 3-phosphatase SidP, even in the absence of other bacterial components, drive rapid PI 3-phosphate turnover on the ER and spontaneously form traveling waves that spread along ER subdomains and induce vesicle/tubule budding. Evidence from in vitro reconstitution strongly suggests that a Turing-like reaction–diffusion mechanism accounts for the behavior of the MavQ/SidP system. Our results not only exemplify the importance of self-organizing behaviors that result from chemically interacting kinases and phosphatases in complex cellular behaviors but also reveal a mechanism that intracellular bacterial pathogens use to remodel host cellular membranes for survival.

RAFTING IN A RUSH: MEMBRANE MICRODOMAINS IN SECRETORY TRAFFICKING

IVAN CASTELLO SERRANO[1]; Ilya Levental[1]; Fred Heberle; Kandice Levental[1]; Rossana Ippolito[1];
[1]University of Virginia, Molecular Physiology and Biological Physics, Charlottesville, VA, USA

Although significant advances have identified a variety of specific motifs responsible for sub-cellular distribution, such motifs are only present on a small subset of membrane proteins. A potential parallel mechanism for organizing membrane protein traffic is sorting small, dynamic membrane domains of preferentially interacting lipids and proteins, known as lipid rafts, that have been widely implicated in some cellular processes. Our lab has recently defined the structural determinants of preferential protein partitioning into these ordered membrane domains and how this affinity is correlated to a plasma membrane distribution. These observations suggested that sorting and trafficking of membrane proteins can be directed by their affinity for a particular membrane environment. To directly assess the role of membrane microdomains in the secretory pathway, we have taken advantage of a robust tool for synchronized protein traffic, known as RUSH (Retention Using Selective Hooks). Here, tagged proteins are retained in specific organelles by a resident "hook", where they can be quickly released upon introduction of biotin, allowing direct and quantitative analysis of trafficking rates and destinations by fluorescence microscopy. We applied this system to a library of transmembrane domain (TMD) constructs to evaluate the role of raft affinity in secretory traffic. We find that while TMD-encoded raft affinity is fully sufficient for PM sorting, it is not sufficient for rapid exit from the endoplasmic reticulum (ER), which requires specific cytosolic sorting motifs. However, we find that Golgi exit rates are highly raft-dependent, with raft preferring proteins exiting ~2.5-fold faster than mutants with perturbed raft affinity. We rationalize these observations with a mechanistic, predictive model of trafficking through the secretory pathway. These observations highlight a central role for lipid rafts in sorting in the secretory pathway. The proposed model helps to understand how TMD proteins migrate from ER to their final post-Golgi destination.

FLUORESCENT VISUALIZATION OF POTASSIUM FLUXES

William R Kobertz[1];
[1]UMASS Medical School, Biochemistry & Molecular Pharm, Worcester, MA, USA

The fluorescent visualization of intracellular ions and metabolites has reimaged our basic understanding of the inner workings of cells, tissues, and living organisms. In contrast, there is a dearth of tools to fluorescently visualize extracellular fluxes. Part of the challenge stems from the fact that cellular egress is contrary to the pervasive intracellular-centric experimental paradigm. Recently, we have been using chemistry to target the cell's glycocalyx, which ideally positions fluorescent sensors within nanometers of the extracellular vestibules of ion channels and membrane transporters. My laboratory's efforts to fluorescently visualize potassium and other cations entering and exiting cells using these technologies will be presented.

PHOSPHATE POSITION IS KEY IN MEDIATING THE TMEM16A-PI(4,5)P2 INTERACTION

Maiwase Tembo[1]; Rachel E Bainbridge[1]; Grant J Daskivich[1]; Jacob D Durrant[1]; Joel C Rosenbaum[1]; **Anne E Carlson**[1];
[1]University of Pittsburgh, Biological Sciences, Pittsburgh, PA, USA

TMEM16A is a Ca2+-activated Cl- channel that plays a critical role in regulating diverse physiologic processes. In addition to Ca2+, TMEM16A activation requires the membrane lipid phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2). Here we interrogated the properties of the lipid that mediate its interaction with the channel using patch- and two-electrode voltage-clamp recordings on cells that endogenously express TMEM16A channels: oocytes from the African clawed frog Xenopus laevis. During continuous application of Ca2+ to excised inside-out patches, we found TMEM16A-conducted currents decayed shortly after patch excision. Following this rundown, the application of synthetic PI(4,5)P2 recovered current. Only lipids that include a phosphate at the 4' position effectively recovered TMEM16A currents; lipids lacking the 4' phosphate had minimal effects on channel gating. Docking PI(4,5)P2 into a homology model of the TMEM16A channel explained why the 4' phosphate is required for this interaction. These findings improve our understanding of how PI(4,5)P2 binds to and potentiates TMEM16A channels.

MECHANISMS AND SIGNIFICANCE OF TISSUE-SPECIFIC MITOCHONDRIAL CALCIUM UPTAKE

Ming-Feng Tsai[1]; Chen-Wei Tsai[1]; Madison Rodriguez[1];
[1]University of Colorado Anschutz Medical Campus, Physiology and Biophysics, Aurora, CO, USA

Mitochondrial calcium uptake, mediated by the mitochondrial Ca^{2+} uniporter, regulates oxidative phosphorylation, apoptosis, and intracellular Ca^{2+} signaling. It is believed that cells exclusively use a MICU1-MICU2 heterodimer to complex with the uniporter to define mitochondrial Ca^{2+} uptake properties. This picture, however, is oversimplified. Here, we demonstrate that skeletal muscle and kidneys equip uniporters with a MICU1 homodimer, and that human/rodent cardiac uniporters are devoid of MICUs. Cells control the population of uniporters with MICU1 homo- and heterodimers by manipulating MICU1/MICU2 importation into mitochondria, and protect correctly-assembled MICU1 dimers by catalyzing an intersubunit disulfide to block proteolysis. For cells, the choice of MICUs presents a trade-off. Using MICU1 homodimer or eliminating MICUs allows mitochondria to more readily import Ca^{2+} to enhance ATP production, but these cells have elevated ROS, impaired basal metabolism, and higher susceptibility to death.

UNCOVERING AND REPROGRAMMING ALLOSTERIC SIGNAL TRANSDUCTIONS IN GPCRS

Patrick Barth
EPFL, Switzerland

No Abstract

NEW INSIGHTS INTO THE MODAL ACTIVITY OF K_V7 CHANNELS

Carlos A. Villalba-Galea;
[1]University of the Pacific, Physiology and Pharmacology, Stockton, CA, USA

Potassium-selective, voltage-gated (K_V) channels of the K_V7 family are commonly found in the nervous, cardiovascular, and gastrointestinal systems. K_V7 channels produce discernible currents at the typical cellular resting potential (about -60 mV). This property sets them apart from other K_V channels which are typically closed at voltages below -40 mV. This property also makes them critically important in the regulation of electrical excitability in cells. In fact, disrupted K_V7 channel activity can lead to disorders such as long QT syndrome, arrhythmias, multiple epileptic disorders and peripheral nerve hyper-excitability. In general, K_V7 channels contribute to the cell's basal K^+ conductance due to their activity at the resting potentials. Therefore, we argue that the stability of their open state is critical for the physiological function of K_V7 channels. To assess open channel stability, we have focused our studies on the deactivation of K_V7 channels. We have found that deactivation becomes slower as K_V7 channels are kept activated under voltage clamp. This indicates that the open K_V7 channel becomes more stable as they remain activated. Noteworthy, the stabilization of the open channel is not caused by strong depolarization. In fact, this occurs even at negative potentials. This strongly suggests that K_V7 channels undergo further rearrangements into a more stable open conformation following activation. We have shown that changes in voltage dependence, deactivation kinetics, and sensitivity to pharmacological agents are correlated with changes in the deactivation rate. We have proposed that, following activation, the open K_V7 channels dwells on a set of states and eventually transitions into a second set of states from which deactivation is slower. Accordingly, K_V7 channels' behavior have at least two modes of activity. Here, we are showing that modal activity is unaltered by changes in the voltage sensing machinery, suggesting that the pore domain is responsible for modal activity.

LARGE-PORE CHANNELS AS TRANSPORTERS: LESSONS FROM CONNEXIN, PANNEXIN AND CALHM1 CHANNELS

Jorge E. Contreras[1]; Pablo S Gaete[1]; Cynthia I Fernandez[1]; Wenjuan Jiang[2]; Yun Luo[2];
[1]University of California Davis , Physiology & Membrane Biology, Davis, CA, USA
[2]Western University of Health Sciences, Pharmaceutical Sciences, Pomona, CA, USA

Large-pore channels are a diverse group of non-conventional membrane channels characterized by their permeability to atomic ions and a broad repertoire of small molecules. Examples of vertebrate large-pore channels include those formed by connexins (Cxs), pannexins, calcium homeostasis modulators (CALHMs), and LRRC8 (also known as SWELL channels). Although they are currently emerging as important regulators in health and disease, mechanisms for molecular permeation and selectivity remain largely unexplored. Accordingly, we have recently developed a methodology that allows us to simultaneously examine the flux of atomic ions and molecules through large-pore channels. We found that connexin hemichannels, pannexin, and CALHM-1 channels display molecular transport that can be described by Michaelis-Menten kinetics, with apparent K_M and V_{max}. The observed V_{max}, but not K_M values, were sensitive to channel gating modulators (e.g., voltage and extracellular calcium). To mechanistically examine these properties, we performed molecular dynamics using equilibrated Cx26 and Cx30 hemichannels. Simulations confirmed molecular permselectivity and identified discrete binding sites and energy barriers for molecules within the pore. Calculation of the nonbonded interaction between molecules and protein residues showed that permeants form strong interactions with the N-terminal (NT) region of connexin hemichannels. Mutagenesis analysis and functional assays revealed that the NT is a critical player in the mechanisms of permselectivity. Consistently, human disease-associated mutations at the N-terminal region of Cx26 and Cx30 significantly affected kinetics of molecular transport and selectivity. Our results reveal that large-pore channels are not free diffusion pores for molecules and suggest that the presence of binding sites in the permeation pathway could also determine selectivity, similarly to transporters/carriers.

MEMBRANE CURVATURE AS A REGULATOR OF GPCR ORGANIZATION AT THE PLASMA MEMBRANE

Dimitrios Stamou[1];
[1]University of Copenhagen, Copenhagen, Denmark

The plasma membrane (PM) is constantly subject to forces exerted by the cell and the surrounding tissue environment. However, intriguingly most of the PM surface area remains nearly flat, displaying only gentle undulations of extremely shallow curvature (< 1μm-1). Nevertheless, this prevalent spatial PM phenotype and its biological role have been disregarded to date. Here we used a combination of 1) super-resolved 3D live-cell imaging, 2) high throughput mechanical cell-curvature modulation, and 3) molecular field theory to investigate the influence of extremely shallow curvature on the spatial organization of G protein-coupled receptors (GPCRs) at the PM.Our work revealed GPCR energetic coupling to extremely shallow PM curvature (< 1 μm-1) as the dominant, necessary, and sufficient molecular mechanism of GPCR spatiotemporal organization. Shallow-curvature coupling is GPCR-specific and cell-specific and is regulated by ligands. However, it is based on universal physicochemical principles and should influence the spatial organization of PM-associated proteins in general. This novel mechanism elucidates why and how any (biomechanical or biochemical) stimulus that modulates PM-morphology can regulate protein organization and thus likely signaling at the PM.

LIPIDS AND DIVALENT CATIONS AS REGULATORS OF BILAYER INSERTION OF PHLIP AND BCL-2 PROTEINS

Alexey Ladokhin[1];
[1]University of Kansas, Kansas City, KS, USA

Every tissue, cell, and organelle has specific electrochemical properties resulting from differences in concentrations of protons, small ions, and charged macromolecules. Delivering proteins to these different environments can trigger functionally relevant structural rearrangements, which we refer to as conformational switching. A notable example is the transition of specific soluble proteins into lipid membranes. This phenomenon is prominent in many physiological and pathogenic processes, including the regulation of apoptosis by the Bcl-2 family of proteins, critical for cancer treatment. Our ability to target or manipulate these processes can be beneficial for human health. For example, membrane insertion of the pH Low Insertion Peptide (pHLIP) has recently been used to target drugs to cancer cells. While these processes are of fundamental biomedical importance, basic knowledge of the mechanism of conformational switching is often lacking, impeding our ability to predict protein-lipid interactions under physiological conditions. Here we present our progress applying various experimental and computational approaches in deciphering complex interactions on membrane interfaces and establishing the role of lipid composition and divalent cations (i.e, Ca2+ and Mg2+) in modulating important physiological processes.

EMPLOYING THE DOUBLE-KNOT TOXIN-TRPV1 ION CHANNEL COMPLEX AS A MODEL SYSTEM TO INTERROGATE THE ROLES OF PROTEIN-MEMBRANE INTERACTIONS IN PROTEIN FUNCTION

Jeet Kalia;
[1]Indian Institute of Science Education and Research (IISER) Bhopal, Biological Sciences, Bhopal, India

Elucidating the roles of membrane lipids in the function of ion channel proteins is a long-cherished goal of ion channel biologists. These efforts have been tremendously facilitated by the recent success in obtaining high-resolution structures of ion channels complexed with membrane lipids. In this regard, the structure of the transient receptor potential vanilloid-1 (TRPV1) ion channel bound to its potent bi-lobed peptide agonist, the double-knot spider toxin (DkTx), is particularly insightful as it depicts the toxin, the channel, and membrane lipids forming an intimate tripartite complex. In my talk, I will describe how using this structure as a blueprint, we have investigated the role of the membrane in DkTx-activation of TRPV1. In one class of experiments, we generated a series of site-directed variants of DkTx at its membrane and channel-interacting sites and characterized their TRPV1-activation and membrane partitioning properties. These experiments demonstrated that the lipid-interacting residues of DkTx play a profound role in endowing it with both its high TRPV1-activating potency and its extremely slow wash-off rates, and that different toxin-membrane interfaces contribute in different ways to the mechanism of toxin-mediated channel activation. Additionally, experiments on a series of DkTx variants possessing altered lengths and rigidity of the peptide linker between the two lobes of the toxin revealed that the wild-type linker ensures optimal partitioning of the two lobes of the toxin into the membrane. I will also describe our studies that involved the creation of diverse DkTx-based toxin constructs possessing a range of valences and membrane affinities, that enables us to tease apart the contributions of the toxin's bivalency and its high membrane affinity to its TRPV1-activation properties. Taken together, these studies establish that the membrane plays a crucial role in the activation of TRPV1 by DkTx.

ELIMINATION OF MEMBRANE DEFORMATIONS DRIVES CLC-EC1 DIMERIZATION

Tugba N. Ozturk[1,2]; Rahul Chadda[1]; Nathan Bernhardt[2]; José D Faraldo-Gómez[2]; Janice L Robertson[1];
[1]Washington University, Department of Biochemistry and Molecular Biophysics, St. Louis, MO, USA
[2]National Institutes of Health, National Heart, Lung, and Blood Institute (NHLBI), Bethesda, MD, USA

Most membrane proteins are found as oligomers. These hydrophobic proteins interact with each other instead of their hydrophobic solvent, the membrane. The physical forces driving membrane protein assemblies in the context of the membrane are not well understood. The fundamental understanding of the driving forces involved in self-assembly reactions of proteins in the membrane is essential for physiological and pharmacological modulation of membrane proteins. What causes the high affinity of membrane protein complexes in the membrane? In order to answer this question, we carried out coarse-grained molecular dynamics simulations combined with umbrella sampling and studied the dimerization of a bacterial chloride/proton antiporter, CLC-ec1. CLC-ec1 forms high affinity homodimers in the membrane in which two, large membrane-embedded and hydrophobic surfaces associate with each other. Our results show that the membrane alone can drive the assembly of two CLC-ec1 protomers to eliminate local deformations caused by the exposed dimerization interface of each subunit. The dimerization is driven by the membrane via a directional driving force that favors association at the native interface over other non-specific interfaces, prior to the formation of direct protein-protein contacts. The dimerization is governed by the structure of CLC-ec1 dimerization interface and the perturbation it imparts onto the surrounding membrane when exposed. Our physical model describes how this dimerization is initially driven by the energetics of the membrane solvating the CLC-ec1's monomeric state and how changes in lipid composition impact the free energy of dimerization, presenting a way of quantifying lipid regulation in membranes.

POTENTIALS OF MEAN FORCE OF BIOMEMBRANE DEFORMATION

Giacomo Fiorin[1,2]; José D Faraldo-Gómez[1];
[1]National Institutes of Health, National Heart, Lung and Blood Institute, Bethesda, MD, USA
[2]National Institutes of Health, National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA

Lipid membranes are known to undergo macroscopic deformations as the cell's morphology changes, as well as microscopic ones caused by individual proteins. It is also increasingly recognized that a membrane's shape actively contributes to the function of the proteins bound to it. However, quantifying the extent of this coupling requires connecting different length scales and physical effects. Toward this goal, we introduced a method to measure the free-energy of lipid bilayer deformations from enhanced-sampling molecular dynamics simulations. The bilayer need not be homogeneous or symmetric, and may be atomically detailed or coarse-grained. A set of three-dimensional density maps is used to define a “reaction pathway” for the membrane leaflets, and the associated free-energy profile is computed directly from intermolecular forces and configurational sampling. While the likelihood of certain membrane configurations is artificially enhanced, no further restraints are placed on the internal motions of the membrane, thus allowing for fully-reversible thermodynamic sampling. This method, called Multi-Map, was applied alongside standard equilibrium MD to atomistic bilayers of different compositions and sizes. A diversity of microscopic effects is shown to contribute to membrane bending, and thus the same stiffness constant can account for different effects in different lipids. This notion can rationalize otherwise surprising changes in the membrane's stiffness between synthetic compositions and more physiological ones, for example in phospholipid-cholesterol mixtures. The data gathered also shed light on a recent controversy regarding the effect of cholesterol on membrane mechanics (Ashkar et al, PNAS 2021; Nagle et al, PNAS 2021). Given the increasing significance of biomembrane energetics at the molecular scale, techniques based on direct simulation are in a unique position to complement theory and experiment precisely in those cases where quantification is most challenging.

STRUCTURAL BASIS FOR ACTIVATION AND GATING OF IP3 RECEPTOR CALCIUM CHANNELS

Erkan Karakas
Vanderbilt University, USA

No Abstract

FAT CHANCES: HOW A SIGNALING LIPID INFLUENCES LYSOSOMAL PH VIA THE CHLORIDE TRANSPORTER CLC-7

Joseph A. Mindell[1];
[1]NINDS, NIH, Bethesda, MD, USA

The acidic luminal pH of lysosomes, maintained within a narrow range, is essential for proper degrative function of the organelle and is generated by the action of a V-type H+ ATPase, but other pathways for ion movement are required to dissipate the voltage generated by this process. ClC-7, a Cl-/H+ antiporter responsible for lysosomal Cl- permeability, is a candidate to contribute to the acidification process as part of this “counterion pathway”. The signaling lipid PI(3,5)P2 modulates lysosomal dynamics, including by regulating lysosomal ion channels, raising the possibility that it could contribute to lysosomal pH regulation. Here we demonstrate that depleting PI(3,5)P2 by inhibiting the PIKfyve kinase causes lysosomal hyperacidification, primarily via an effect on ClC-7. We further show that PI(3,5)P2 directly inhibits ClC-7 transport and that this inhibition is eliminated in a disease-causing gain-of-function ClC-7 mutation. Together these observations suggest an intimate role for ClC-7 in lysosomal pH regulation.

STRUCTURE AND FUNCTION OF THE OTOP PROTON CHANNELS

Emily Liman
University of Southern California, USA

No Abstract

STRUCTURAL DETERMINANTS OF SUBSTRATE SPECIFICITY IN SMALL MULTIDRUG RESISTANCE TRANSPORTERS

Randy Stockbridge;
[1]University of Michigan, Molecular, Cellular, and Developmental Biology, Ann Arbor, MI, USA

By providing broad resistance to ubiquitous disinfectants and antiseptics and other environmental biocides, transporters from the small multidrug resistance (SMR) family drive the spread of multidrug resistance cassettes among bacterial populations. A fundamental understanding of substrate selectivity by SMR transporters is needed to identify the types of selective pressures that contribute to this process. This talk addresses the molecular basis for the recognition of diverse substrates by two representative SMR proteins with different substrate specificities, revealed through a combination of x-ray crystallography and electrophysiological approaches.

ELUCIDATING THE SUBSTRATE SPECIFICITIES OF THE TWO MAJOR FUNCTIONAL SUBTYPES IN THE SMALL MULTIDRUG RESISTANCE (SMR) FAMILY

Olive E. Burata[1,2]; Randy B Stockbridge[1,2,3];
[1]University of Michigan, Program in Chemical Biology, Ann Arbor, MI, USA
[2]University of Michigan, Molecular, Cellular, and Developmental Biology, Ann Arbor, MI, USA
[3]University of Michigan, Biophyics, Ann Arbor, MI, USA

The small multidrug resistance (SMR) family contains the smallest membrane transport proteins found in both bacteria and archaea. The family is functionally dominated by two major subtypes: the quaternary ammonium compound subtype (Qac) which transports a chemically diverse range of biocides, and the guanidinium exporter subtype (Gdx) which selectively transports the essential bacterial metabolite, guanidinium. Here, we 1) determined an x-ray crystal structure of a representative Qac protein, EmrE and 2) using high-throughput mutagenesis, directed evolution, electrophysiology, and cell resistance assays, identified key molecular determinants of the specificity differences between the Gdx and Qac subtypes.

DESIGNED PROTON CHANNELS REVEAL MECHANISMS FOR PROTON CHANNEL SELECTIVITY AND CONDUCTIVITY

Huong T. Kratochvil; William F DeGrado[1];
[1]University of California-San Francisco, Pharmaceutical Chemistry, San Francisco, CA, USA

Selective and fine-tuned proton conductance is critical for many biocatalytic and bioenergetic processes. Proton channel proteins precisely control proton transport through proton conduction pathways comprised of hydrogen-bonding networks of nanoconfined water, polar sidechains and backbone carbonyls that are interspersed in well-packed hydrophobic segments that would appear to be barriers to conduction. We hypothesize that these apolar constrictions can transiently expand to accommodate hydrogen-bonding chains of water molecules, and it is the fleeting nature of these water wires that ultimately facilitate highly selective conduction of protons over other ions. To critically test our hypothesis, we turn to protein design. Starting from a minimalist membrane-spanning pentameric bundle with a dry pore, we systematically introduce polar residues, such as Gln, to key pore-lining positions to modulate the lengths of these apolar tracts. From X-ray crystallographic structures of these designs, these polar residues appear to facilitate the formation of water wires within the pore. Furthermore, liposomal proton flux assays reveal that these designs are indeed conductive for protons with selectivity of $> 10^6$ over K^+ and Na^+. Molecular dynamics simulations reveal water permeation with the introduction of the Gln mutations, further corroborating our hypothesis. Through this combination of design, experiment, and simulation, we are able to dissect the physical principles that underlie the formation of these hydrogen bonding networks and their roles in proton selectivity and conduction.

TRPA1 – BIASED AGONISM AND STRUCTURAL MECHANISMS OF MODULATION

Jun Chen[1];
[1]Genentech, Biochemical Cellular Pharmacology, South San Francisco, CA, USA

TRPA1, also known as wasabi receptor, is a chemonociceptor implicated in pain, neurogenic inflammation and respiratory diseases. Electrophilic agonists activate the channel through covalent modification of cysteine residues, but how non-covalent ligands activate and how antagonists block the channel remain poorly understood. By using electrophysiology, mutagenesis, and cryo-EM, we have determined binding sites and interaction mechanisms for several classes of TRPA1 agonists and antagonists. Furthermore, we demonstrate that agonists targeting the same channel protein, or even the same structural domains, can exert distinct biophysical, physiological and pharmacological consequences. Hence, we reveal biased agonism and modulation mechanisms, shedding insights on understanding TRPA1 function and guiding therapeutic development.

IMPACT OF LIPID-MEMBRANE PROTEIN INTERACTIONS ON MEMBRANE PROTEIN STRUCTURE AND DYNAMICS

Linda Columbus[1];
[1]University of Virginia, Chemistry, Charlottesville, VA, USA

The role of lipids and detergents in stabilizing membrane protein structure and dynamics as a solvent and through specific binding is not well understood. Much is now known about the partitioning of amino acids within the membrane in the context of membrane structure and synthesis, however, the energetics and molecular determinants of interactions between proteins and lipids (or membrane mimics) are largely unknown A few binding motifs for specific interactions such as a CARC or CRAC domain have been proposed and there is additional evidence for other specific interactions. Yet, a unifying principle (similar to soluble proteins and water) of how membrane proteins interact with and evolve with their solvent does not currently exist. Our laboratory aims to develop an understanding of how lipids stabilize membrane protein structure using membrane mimics that allow interactions to be controlled and titrated. Our current systems include a beta-barrel outer membrane protein, Opa, an inner membrane enzyme, LspA, a two transmembrane alpha-helical membrane protein, TM0026, and the alpha-helical leucine transporter, LeuT. I will present our latest work in determining how lipids and membrane mimics interact with these proteins to stabilize their structure, dynamics, and function.

KINETIC SELECTION OF COMPETING ION EXCHANGE PATHWAYS: WHEN DOES THE ELECTRICAL VERSUS CHEMICAL GRADIENT MATTER?

Jessica MJ Swanson[1];
[1]The University of Utah, Department of Chemistry, Salt Lake City, UT, USA

It is increasingly apparent that membrane transport mechanisms can involve kinetic selection between competing pathways, each with a different sequence of transitions between intermediates during the transport process. Moreover, the dominance of these pathways can shift under different reaction conditions. In this talk the mechanistic reaction network for coupled Cl–/H+ exchange in ClC antiporters will be described. Using a multiscale kinetic modeling approach that integrates experimental and simulation data the dominance of different pathways will be shown to shift as a function of pH. This highlights the importance of the lower H+ binding site, which has had a questionable role in the transport cycle to date, in maintaining the ion exchange ratio over a range of pH values. The flux will also be shown to shift in response to transmembrane electrochemical potentials. Interestingly, these effects will be shown to be different for an electrical gradient vs chemical gradient of the same magnitude, pointing to a more nuanced influence of electrochemical potentials than commonly assumed by the Nernst relationship.

NMR AND HYDROGEN EXCHANGE STUDIES OF BACTERIAL CHEMOTAXIS RECEPTOR COMPLEXES SUGGEST PROTEIN STABILIZATION IS KEY TO THE SIGNALING MECHANISM

Lynmarie K Thompson[1];
[1]University of Massachusetts Amherst, Chemistry, Amherst, MA, USA

Bacterial chemotaxis proteins are both potential targets for novel antibiotics and a key model system for understanding transmembrane signaling mechanisms. A remarkable sensor array of membrane-bound chemoreceptors, CheW, and CheA detect molecules in the environment and control swimming direction. Signaling through this complex begins as a ligand-induced 2 Å displacement of a receptor alpha helix that extends from the periplasm through the membrane. The objective of this study is to compare the structure and dynamics of signaling states to discover how the signal is propagated an additional ~200 Å from the membrane to the cytoplasmic tip of the receptor and how this signal controls the activity of CheA. Our approach begins with assembly of homogeneous, native-like functional complexes of an E coli Asp receptor cytoplasmic fragment (CF) with the kinase CheA and coupling protein CheW. Hydrogen exchange mass spectrometry (HDX-MS) results indicate that the CF is partially disordered within functional complexes and has a small, well-ordered protein interaction region. Slower hydrogen exchange throughout the kinase-on state suggests signaling inputs modulate the disorder of the cytoplasmic domain to control the kinase activity. HDX-MS of CheA alone and in these functional complexes indicate the domain-domain interactions and changes in domain stability that occur during kinase activation and inhibition. NMR spectra of both the highly flexible and rigid regions of these proteins are mapping how and where dynamics change with signaling state, and should enable us to obtain structural information on the CF/CheA/CheW interactions. This study reveals the mechanistic role of protein disorder and stabilization in signaling and control of catalysis. This research supported by National Institutes of Health Grant R01-GM120195.

LIPID DEPENDENCE OF THE CONFORMATIONAL COUPLING ACROSS THE MEMBRANE BILAYER OF FULL-LENGTH EPIDERMAL GROWTH FACTOR RECEPTOR

Shwetha Srinivasan[1]; Raju Regmi[1]; Xingcheng Lin[1]; Courtney A Dreyer[2]; Xuyan Chen[1]; Steven D Quinn[1]; Wei He[4]; Matthew Coleman[3,4]; Kermit L Carraway III[2]; Bin Zhang[1]; Gabriela S Schalu-Cohen[1];
[1]Massachusetts Institute of Technology, Chemistry, Cambridge, MA, USA
[2]University of California Davis School of Medicine, Biochemistry and Molecular Medicine, Sacramento, CA, USA
[3]University of California Davis School of Medicine, Radiation Oncology, Sacramento, CA, USA
[4]Lawrence Livermore National Laboratory, Livermore, CA, USA

Membrane proteins regulate several vital cellular processes, establishing them as major drug targets. A main function of membrane proteins is to transduce extracellular signal across the membrane bilayer. One such protein is the epidermal growth factor receptor (EGFR), which plays a crucial role in cell signaling and is implicated in cancer. While the structures of the extracellular and intracellular regions of this protein have been well-elucidated individually, conformational coupling connecting these two regions during signal transduction is challenging to probe due to the mixture of hydrophobic and hydrophilic domains intrinsic to membrane proteins. Here, I will discuss the signal transduction mechanism across full-length EGFR. We combine two biochemical tools, cell-free protein expression and nanodiscs, to isolate full-length, functional EGFR in a near-physiological environment. Using a multidisciplinary approach involving single-molecule Förster resonance energy transfer, mutagenesis, and molecular dynamics simulations, we observe a compaction in the intracellular domain of EGFR upon extracellular ligand binding in a neutral lipid environment. The ligand-induced extracellular/intracellular conformational coupling is reversed in the presence of anionic lipids and is robust around the physiological 15-30% anionic content. Our findings show how the extracellular and intracellular domains are coupled to each other in this critical receptor and the impact of the lipid composition in this conformational coupling. Our results could be universal to other membrane receptors which share the same structural homology and perform other significant functions.

PROMOTING RECEPTOR PROTEIN TYROSINE PHOSPHATASE ACTIVITY BY TARGETING TRANSMEMBRANE DOMAIN INTERACTIONS

Eden Sikorski[1]; Sophia Rizzo[1]; Will Hart[2]; Jacqueline Gerritsen[3]; Forest White[3]; Matthew Lazzara[2]; **Damien Thévenin**[1];
[1]Lehigh University, Chemistry, Bathlehem, PA, USA
[2]University of Virginia, Chemical Engineering, Charlottesville, VA, USA
[3]Massachusetts Institute of Technology, Biological Engineering, Cambridge, MA, USA

Receptor Protein Tyrosine Phosphatases (RPTPs) are one of the most important regulators of receptor tyrosine kinases (RTKs) and therefore play a crucial role in mammalian signal transduction. But our still-incomplete understanding of the structural aspects of their regulation and the lack of selective agonists have hampered efforts to understand their roles in downstream signaling regulation, and to pursue them as potential therapeutic targets. However, the reported ability of RPTP homodimerization to antagonize their catalytic activity presents potential opportunities to develop unique strategies to modulate the activity of RPTPs. We recently reported that the homodimerization of a representative member of the RPTP family (protein tyrosine phosphatase receptor J or PTPRJ; also known as DEP1) is regulated by specific transmembrane (TM) residues and that these interactions are essential in regulating its enzymatic activity and substrate access in cells. Building on these new insights and in response to the lack of selective agonists, we report here the design and testing of a tumor-selective peptide capable of binding to the TM domain of PTPRJ and disrupting its homodimerization. By doing so, it promotes PTPRJ TM-mediated access to EGFR (a known substrate), reduces EGFR phosphorylation and other downstream signaling effectors, and antagonizes EGFR-driven cancer cell phenotypes. This peptide represents a novel allosteric and a possibly orthogonal way to target the activity of PTPRJ and RTKs phosphorylation. It could therefore be used not only as probes to tease out PTPRJ regulating mechanisms but also for therapeutic purposes via the attenuation of signaling by dysregulated RTKs in cancers. We also expect that the basic framework developed here can be extended to other RPTPs.

SURA: A "GROOVE-Y" CHAPERONE THAT EXPANDS UNFOLDED OUTER MEMBRANE PROTEINS

Karen G. Fleming[1];
[1]Johns Hopkins University, Dept. Biophysics, Baltimore, MD, USA

The periplasmic chaperone network ensures the biogenesis of bacterial outer membrane proteins (OMPs) and has recently been identified as a promising target for antibiotics. SurA is the most important member of this network both due to its genetic interaction with the β-barrel assembly machinery complex as well as its ability to prevent unfolded OMP (uOMP) aggregation. Using only binding energy, the mechanism by which SurA carries out these two functions is not well understood. Here we use a combination of photo-crosslinking, mass spectrometry, solution scattering, and molecular modeling techniques to elucidate the key structural features that define how SurA solubilizes uOMPs. Our experimental data support a model in which SurA binds uOMPs in a groove formed between the core and P1 domains. This binding event results in a drastic expansion of the rest of the uOMP, which has many biological implications. Using these experimental data as restraints, we adopted an integrative modeling approach to create a sparse ensemble of models of a SurA•uOMP complex. We validated key structural features of the SurA•uOMP ensemble using independent scattering and chemical crosslinking data. Our data suggest that SurA utilizes three distinct binding modes to interact with uOMPs and that more than one SurA can bind a uOMP at a time. This work demonstrates that SurA operates in a distinct fashion compared to other chaperones in the OMP biogenesis network.

COORDINATION OF -1 PROGRAMMED RIBOSOMAL FRAMESHIFTING BY THE TRANSCRIPT AND NASCENT POLYPEPTIDE

Patrick Carmody[1]; Matthew Zimmer; Francis J Roushar[1]; Dyotima Dyotima[1]; Haley R Harrington[1]; Wesley D Penn[1]; Thomas F Miller III; Suchetana Mukhopadhyay[1]; **Jonathan Schlebach**[1];
[1]Indiana University, Bloomington, Chemistry, Bloomington, IN, USA

Programmed ribosomal frameshifting (PRF) is a translational recoding mechanism involved in the regulation of protein biosynthesis. PRF is stimulated by RNA structures that pause the ribosome during the decoding of a “slippery” sequence that allows the tRNA to slip into alternative reading frames. We recently showed that the cotranslational folding of the alphavirus structural polyprotein at the endoplasmic reticulum membrane generates pulling forces on the ribosome that enhance the efficiency of -1PRF. To explore this mechanochemical feedback, we measured the effects of 4,530 mutations on the efficiency of -1PRF by deep mutational scanning. Mutational trends near the slip-site reveal the sequence constraints within stimulatory RNA structures while trends upstream of the site suggest frameshifting can be tuned by the interaction of the nascent chain with the ribosome and translocon. Molecular modeling and coarse-grained molecular dynamics simulations suggest forces are generated by the formation of a translocation intermediate that forms within the translocon. These findings demonstrate that the activity of the ribosome can respond to cotranslational folding in real time. As an extension of these studies, we show that similar -1PRF sites can be engineered into other transcripts and carry out an informatic analysis to identify putative frameshift motifs in the human transcriptome. Finally, we validate a novel -1PRF site within the cystic fibrosis transmembrane conductance regulator (CFTR) and provide preliminary evidence to suggest that it may function as a quality control checkpoint during translation. Together these findings reveal how structural features within the transcript and nascent chain coordinate translational regulation.

DECIPHERING AN INTERFACIALITY SCALE FOR PROTEINS AT BIOLOGICAL MEMBRANES

Ismael Mingarro[1]; Brayan Grau[1]; Maria J Garcia-Murria[1]; Manuel M Sanche del Pino[1]; Luis Martinez-Gil[1];
[1]University of Valencia, Biochemistry and Molecular Biology, Valencia, Spain

Biological membranes are formed by a hydrophobic core surrounded by two interfacial regions that together are comparable in size to the former. The insertion and folding of most α-helical membrane proteins in this heterogeneous environment require the assistance of protein-conducting channels termed translocons. Accumulated precise functional and structural knowledge about how translocons work helped to understand the insertion of hydrophobic sequences into biological membranes and to develop translocon-based hydrophobicity scales. However, an equivalent analysis of the interfacial propensity of a particular sequence is not yet available. By challenging the ER Sec61 translocon with a large set of designed interfacial polypeptide sequences, we have determined the propensity of all 20 amino acids to achieve a membrane interfacial disposition. These estimated interfacial propensities can be converted to apparent free energies for direct comparison with previous biophysical data.

COLICIN E1 OPENS ITS HINGE TO PLUG TOLC

Joanna S.G. Slusky[1,2]; Jimmy Budiardjo[1]; Jacqueline J Stevens[2]; Anna Calkins[3]; Ayotunde P Ikujuni[2]; Virangika K Wimalasena[2]; Emre Firlar[4]; David A Case[5]; Julie S Biteen[3]; Jason T Kaelber[4];
[1]University of Kansas, Center for Computational Biology, Lawrence, KS, USA
[2]University of Kansas, Department of Molecular Biosciences, Lawrence, KS, USA
[3]University of Michigan, Department of Chemistry, Ann Arbor , MI, USA
[4]Rutgers University, Rutgers CryoEM & Nanoimaging Facility and Institute for Quantitative Biomedicine, Piscataway, NJ, USA
[5]Rutgers University, Department of Chemistry and Chemical Biology, Piscataway, NJ, USA

The double membrane architecture of Gram-negative bacteria forms a barrier that is impermeable to most extracellular threats. Bacteriocin proteins evolved to exploit the accessible, surface-exposed proteins embedded in the outer membrane to deliver cytotoxic cargo. Colicin E1 is a bacteriocin produced by, and lethal to, Escherichia coli that hijacks the outer membrane proteins TolC and BtuB to enter the cell. Here we capture the colicin E1 translocation domain inside its membrane receptor, TolC, by high-resolution cryoEM to obtain the first reported structure of a bacteriocin bound to TolC. Colicin E1 binds stably to TolC as an open hinge through the TolC pore—an architectural rearrangement from colicin E1's unbound conformation. This binding is stable in live E. coli cells as indicated by single-molecule fluorescence microscopy. Finally, colicin E1 fragments binding to TolC plug the channel, inhibiting its native efflux function as an antibiotic efflux pump and heightening susceptibility to three antibiotic classes. In addition to demonstrating that these protein fragments are useful starting points for developing novel antibiotic potentiators, this method could be expanded to other colicins to inhibit other outer membrane protein functions.

INTEGRATIVE STRUCTURAL MODELING OF MEMBRANE PROTEINS USING SPARSE PARAMAGNETIC NMR AND NEUTRON SCATTERING DATA

Kaitlyn V Ledwitch[1]; Georg Kuenze[3]; Elleansar Okwei[1]; Katherine Larochelle[1]; Shuo Qian[2]; Hugh O'Neill[2]; Jens Meiler[1,3];
[1]Vanderbilt University, Center for Structural Biology, Nashville, TN, USA
[2]Oak Ridge National Lab, Oak Ridge, TN, USA
[3]University of Leipzig, Institute of Drug Discovery, Leipzig, Germany

Combining different types of experimental information with computation is a powerful approach to investigate complex biological systems. Here, we combine experimentally-derived nuclear magnetic resonance (NMR) restraints and small-angle neutron scattering (SANS) data to guide membrane protein structural modeling in Rosetta. We demonstrate this integrative modeling strategy using the disulfide bond formation protein B (DsbB) in a nanodisc as a model membrane protein system. In this work, we developed two new technology platforms: 1) an unnatural amino acid-based paramagnetic NMR tagging strategy for membrane proteins and 2) a SANS deuteration strategy for producing 'stealth'-like circularized nanodiscs (cNW9). NMR restraints from paramagnetic labeling gives structural information in the form of long-range distance and angular restraints. We complement these atomic details with SANS-derived envelopes to probe the overall geometric shape of DsbB in the nanodisc. These datasets are used as experimental input to evaluate how the membrane mimetic perturbs the overall structure and architecture of DsbB. We carried out these experiments for DsbB in dodecylphosphocholine (DPC) detergent micelles in parallel to compare differences between lipid-loaded nanodiscs and micelles. We demonstrate this approach for the model membrane protein system DsbB but our integrated biophysical framework is equally applicable to other complex membrane protein systems.

DROPLET-ON-HYDROGEL BILAYER BASED ASSAY FOR FUNCTIONAL STUDY OF MEMBRANE PROTEINS

Zuzana Coculova[1]; Richard Berry[1];
[1]University of Oxford, Department of Physics, Oxford, United Kingdom

Functional study of membrane proteins, especially when transmembrane potential is required, is notoriously difficult. Various creative approaches - using either liposomes, lipid nanodiscs, or a wide range of different planar biomembrane systems - did contribute to building our understanding of these molecular machines, but the picture is not complete yet. Here we introduce a system, which adapts the droplet-on-hydrogel technique [1], for high resolution optical studies of membrane proteins with independent control of the electrical and chemical transmembrane potentials. The experiment is performed by embedding a membrane protein of interest into a lipid bilayer formed between a 200 nl water droplet and a supporting thin layer of agarose. This guarantees stability, hydration and accessibility of the membrane. The protein is delivered in positively charged proteoliposomes, which fuse with the negatively charged lipid bilayer [2]. Sample delivery into the water droplet, and continuous control over the proteins' environment, is achieved through two glass micropipettes of our custom built electronically controlled perfusion system.We chose to demonstrate the capabilities of our assay by studying the Esterichia coli F_1F_o. This complex of two molecular motors - membrane embedded F_o and water soluble F_1 - catalyses the synthesis of ATP molecules, the basic energy units of life, using the transmembrane proton gradient created during respiration or photosynthesis. Our assay will allow us to mimic native energized membranes, study activation potential at different conditions, as well as observe single-molecule rotation steps of F_1F_o at the time scale of a few microseconds. We would also like to use this opportunity to get in contact with scientists who may find our assay helpful for studying other biological systems in energized biological membranes. [1] Leptihn, S. et al. Nature Protocol. 8 (6): 1048-10572 (2013) [2] Ishmukhametov, R. et al. Nature Communications. 7: 13025 (2016)

HALF A CENTURY DECIPHERING MEMBRANE STRUCTURE, DYNAMICS AND FUNCTION BASED ON A COMMENTARY IN BIOPHYSICAL REVIEWS, 13:849–852 (2021)

Shirley Schreier[1];
[1]University of Sao Paulo, Biochemistry, Sao Paulo, Brazil

My research on membranes started in 1970, at the National Research Council of Canada. Research during the 70's revealed the dynamical properties of membranes, and spin labeling EPR contributed greatly to these findings. We studied the effects of cholesterol, ions, and pH on the order and mobility of lipid bilayers and designed a novel experiment to monitor membrane permeability. The lab was among the first to introduce deuterium NMR, providing molecular details of local anesthetics-membrane interactions. My laboratory started in 1973. We were the first to work with liposomes and other phospholipid membranes and to use EPR for this purpose in Brazil. We examined membrane effects on apparent pK of ionizable compounds and on reaction kinetics of membrane-partitioning compounds. Methods were designed to determine partition coefficients. We investigated hemin-membrane interaction and obtained detailed information about aggregation and autoxidation of the polyene antibiotic amphotericin B. Our studies also focused on detergent micelles. The amino acid 2,2,6,6-tetramethyl-N-oxyl-4-amino-4- carboxylic acid (TOAC) is useful to analyze peptide conformational properties. TOAC can be introduced via a peptide bond at any position in the chain. In angiotensin II and bradykinin, insertion in the middle led to a bend, inactivating the compounds. Since the 1990's, we mostly focused on peptide-membrane interaction, making additional use of circular dichroism and fluorescence. Our studies with GPCR fragments showed that the peptides tend to acquire a similar conformation to that in the protein. Moreover, they oriented as expected, parallel or perpendicular to the membrane. Sea anemone toxins, and their N-terminal peptides were investigated to understand their leakage-promoting mechanism. Another relevant question is understanding antimicrobial peptide mechanism of action and toxicity. TOAC-labeled analogues of tritrpticin presented a higher therapeutic index than the parent compound, making them interesting drug candidates. I keep working, and I am still fascinated by the wonder of membranes.

DISSECTING MEMBRANE STRUCTURE AND REMODELING USING CRYO-ELECTRON TOMOGRAPHY

Kelly K. Lee;
[1]University of Washington, Seattle, WA, USA

Membranes are lipidic structures that define biological compartments in cells, organelles, and enveloped viruses. Because membranes serve such fundamental roles, essential protein machinery has evolved whose function is to manipulate membrane bilayers and remodel them during key biological processes. Until recently, it has been challenging to image membrane structure with resolution of leaflets, local curvature, and non-bilayer organizations, and it has been even more challenging to capture the protein machinery in the functional intermediate states that drive membrane remodeling. Cryo-electron microscopy provides a means to flash freeze liposomes, vesicles, and proteoliposomes, capturing the biological systems with native hydration and structures intact. From axial angular tilt images of the specimens, it is possible to reconstruct three-dimensional tomograms that provide sub-nanometer resolution of the membranes and associated proteins. With this approach, one can trigger a reaction and then capture and reconstruct 3-dimensional images of the reaction at intermediate stages. By preparing cryo-EM specimens over a time course, we capture snapshots across the population of reaction intermediates, allowing us to trace the reaction pathways for protein-mediated membrane remodeling. Our investigations of lipid microdomains and protein-mediated membrane fusion by enveloped viruses highlight the versatility of cryo-ET in capturing the fine structure of membranes and their interplay with membrane-remodeling protein machinery. From this, we gain new structural and mechanistic insights into complex biological processes.

MEMBRANE REMODELING BY PROTEINS: INSIGHTS AND SURPRISES FROM MULTISCALE COMPUTER SIMULATION

Gregory A Voth[1];
[1]University of Chicago, Department of Chemistry, Institute for Biophysical Dynamics, and James Franck Institute, Chicago, IL, USA

The protein-driven remodeling of biological membranes spans from the molecular to mesoscopic length and time scales. To access the full range of such complex processes, we employ a multiscale theoretical and computational methodology. The advantage of our approach is that it systematically connects all-atom molecular dynamics, coarse-grained (CG) modeling, and mesoscopic phenomena. We especially develop and apply methods for deriving CG models from protein structures and their underlying atomic-scale interactions, yielding accurate CG molecular dynamics (MD) simulation force fields. As a result, we can simulate large-scale biological process using information from the molecular level, which are inaccessible by standard atomistic MD simulation techniques. With this approach, we then can carefully study membrane remodeling mediated by various proteins. For example, with large scale CG molecular dynamics simulations, we investigate the way proteins interact with one another on the membrane and the underlying physics driving their assembly and generation of membrane curvature. A critical component of our multiscale approach is also its connection to experimental data, such as cryo-EM, cryo-ET, fluorescence, and optical tweezers. As time allows, specific applications of this simulation approach will be given for membrane remodeling by BAR domain proteins, HIV-1 virion budding and scission, and lipid droplet biogenesis.

PROTEIN ACTIVATION AND MEMBRANE DEFORMATION IN ENVELOPED VIRUS ENTRY

Peter Kasson[1,2];
[1]University of Virginia, Departments of Molecular Physiology and Biological Physics, Charlottesville, VA, USA
[2]Uppsala University, Department of Cell and Molecular Biology, Uppsala, Sweden

Enveloped viruses infect cells via a process of membrane fusion between the viral membrane and a cellular membrane, mediated by viral fusion proteins. In many cases, the fusion proteins also perform receptor-binding functions, guiding the virus to particular cellular membranes where the protein is activated for fusion and entry occurs. We utilize a biophysical toolbox to dissect the requirements for fusion protein activation, the protein-membrane interactions involved in fusion, and the host membrane requirements for viral entry. I will present recent results from our group in these areas: how receptor binding affects the conformational equilibria controlling fusion protein activation and how host membrane deformability is a critical parameter controlling viral entry.

LIPID PROTEIN INTERACTIONS GUIDING FUSION PORE OPENING AND EXPANSION DURING REGULATED EXOCYTOSIS

Volker Kiessling[1,2]; Lukas K Tamm[1,2];
[1]University of Virginia, Molecular Physiology and Biological Physics, Charlottesville, VA, USA
[2]University of Virginia, Center for Membrane and Cell Physiology, Charlottesville, VA, USA

It has long been known that synatpotagmin-1 (Syt1) is the sensor that triggers fast, evoked release of neurotransmitter by the fusion of synaptic vesicles to the presynaptic membrane in response to calcium. It is also well established that the SNAREs syntaxin-1a, SNAP-25, and synaptobrevin-2/VAMP-2 form the core of the membrane fusion machinery that drives calcium-triggered neuronal exocytosis. We recently proposed a mechanism where the lipid bilayer is intimately involved in coupling calcium sensing to fusion. Using TIRF- (total internal reflection fluorescence) and sd-FLIC (site-directed fluorescence interference contrast) microscopy, we demonstrate that fusion of purified dense core vesicles and insulin granules with supported membranes containing syntaxin-1a and SNAP-25 is strongly linked to the tilt angle of the cytoplasmic domain of the nascent SNARE complex with respect to the plane of the target membrane. As the tilt angle increases, force is exerted on the SNARE transmembrane domains to drive the merger of the two bilayers as the trans-SNARE complex completes folding into the cis-SNARE complex. The tilt angle can be modulated by the order of the lipid bilayer, and the order of the bilayer is changed by Ca^{2+} dependent binding of the two C2 domains of Syt1. The strong dependency of vesicle fusion efficiency on membrane order is further confirmed in live INS1 cell experiments where the plasma membrane is acutely enriched with lipids of defined acyl-chain saturation. In addition to fusion efficiencies and fusion kinetics, TIRF data from single vesicle fusion events contains information about how the fluorescent content is released. Of particular interest is, how fast the content is released. We present data that show how interactions between PIP2 and conserved arginine residues of Syt1's C2B domains regulate fusion pore expansion.

CHARACTERIZATION OF THE ELUSIVE SERINC5-AP2-NEF COMPLEX IN THE CONTEXT OF A LIPID BILAYER

Joana Paulino[1]; Alisa Bowen[1]; Phuong Nguyen[1]; Janet Finer-Moore[1]; Robert M Stroud[1]; [1]University of California San Francisco, Dept Biochemistry and Biophysics, San Francisco, CA, USA

The human Serine Incorporator 5 (Serinc5) is an anti-HIV restriction factor that actively inhibits the fusion of the virion with a new host cell. HIV antagonizes this intrinsic immune response by targeting Serinc5 for degradation utilizing its accessory protein, Nef. Nef hijacks the host's clathrin adaptor protein complex 2 (AP2), directing clathrin mediated endocytosis to target many of the host cell anti-HIV lines of defense. Previous structural studies of Nef-AP2-host protein complexes have shown that although the Nef-AP2 interaction is constant, the interaction between Nef and the host protein is variable. In Serinc5 a large intracellular loop devoid of a canonical AP2 cargo motif is essential for Nef antagonism. This loop remains uncharacterized by structural efforts due to its dynamics. We have assembled the complex between Nef-AP2 and Serinc5 incorporated into lipid nanodiscs and our preliminary cryo-EM density (res.10Å) suggests that binding of AP2 to the lipid bilayer is a contributing factor to the formation of the complex. Interestingly, the interaction of AP2 with lipids alters the conformation of AP2 in comparison with other AP2-Nef complex structures without changing how Nef and AP2 interact. Currently, we are optimizing our cryo-EM structural characterization pipeline to account for the sample dynamics and sensitivity to the blotting/freezing process. Simultaneously, we are investigating the role of the native N-terminal myristoylation of Nef on complex formation and possibly on directly interacting with Serinc5, as well as measuring binding affinities of AP2-Nef to Serinc5 in the context of the lipid bilayer and its lipidic composition.

POSTER ABSTRACTS

Monday, June 6
POSTER SESSION I
4:00 PM – 6:00 PM
Ballroom

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. Presenting authors with odd-numbered poster boards should present from 4:00 PM – 5:00 PM and those with even-numbered poster boards should present from 5:00 PM – 6:00 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 4:00 PM – 5:00 PM | Even-Numbered Boards 5:00 PM – 6:00 PM

Al Faouri, Radwan	**1-POS**	**Board 1**
Baryiames, Christopher	**5-POS**	**Board 2**
Clowes, Katherine	**9-POS**	**Board 3**
Dastvan, Reza	**13-POS**	**Board 4**
Goldberg, Andrew	**17-POS**	**Board 5**
Honerkamp-Smith, Aurelia	**21-POS**	**Board 6**
Kumar, Deepak	**25-POS**	**Board 7**
Lin, Yie Chang	**29-POS**	**Board 8**
Ong, Kyrstyn	**33-POS**	**Board 9**
Reagle, Tyler	**37-POS**	**Board 10**
Sanders, Charles	**41-POS**	**Board 11**
Stefanski, Katherine	**45-POS**	**Board 12**
Wang, Qi	**49-POS**	**Board 13**
Zimmer, Stephanie	**53-POS**	**Board 14**
Liang, Ziyu	**57-POS**	**Board 15**

Posters should be set up on the morning of Monday, June 6 and removed by 11:00 PM on Monday, June 6. All uncollected posters will be discarded.

1-POS Board 1

AN EFFECTIVE ELECTRIC DIPOLE MODEL FOR VOLTAGE-INDUCED GATING OF LYSENIN

Radwan Al Faouri[1,2]; Daniel Fologea[5]; Ralph L Henry[3]; David Straub[6]; Mahmoud Moradi[4]; Qusay Alfaori[7]; Hanan Alismail[8]; Gregory J Salamo[2];
[1]University of the Ozarks, Physics, Clarksville, AR, USA
[2]University of Arkansas, Physics, Fayetteville, AR, USA
[3]University of Arkansas, Biological Sciences, Fayetteville, AR, USA
[4]University of Arkansas, Biochemistry, Fayetteville, AR, USA
[5]Boise State University, Physics, Boise, ID, USA
[6]University of Arkansas for Medical Sciences, Department of Biochemistry and Molecular Biology, Little Rock, AR, USA
[7]Texas Tech University/Health Science Center/El Paso, Medical School/Education, El Paso, TX, USA
[8]King Saud bin Abdulaziz University for Health Sciences, Health Sciences, Al Riyadh, Saudi Arabia

Lysenin is a pore-forming toxin, which self-inserts open channels into Sphingomyelin containing membranes and is known to be voltage regulated. The mechanistic details of its voltage gating mechanism, however, remains elusive despite much recent efforts. The study's objective is to understand the mechanism of lysenin voltage-gating. Here, we have employed a novel experimental technique to examine a model for voltage gating, that is based on the existence of an "effective electric dipole" inspired by recent reported structures of lysenin. We support this mechanism by the observations that (i) the charge-reversal and neutralization substitutions in lysenin result in changing its electrical gating properties by modifying the strength of the dipole, and (ii) an increase in the viscosity of the solvent increases the drag force and slows down the gating. Experiments were conducted by using bilayer lipid membrane (BLM) to host lysenin channels. Lipid was made of Asolectin from soybean, Sphingomyelin, and Cholesterol all were dissolved in n-Decane. The BLM was built across a small hole that is made in a Teflon partition and bathed in NaCl electrolyte solution. A protein named lysenin was used to make channels in the BLM structure, and by electrophysiology, we were able to measure the electrical activities across the BLM. An electrostatic surface representation of the lysenin monomer showed a distribution of a fixed charges revealing additional physical features of the channel structure. Our results support a model in which an internal effective electric dipole within lysenin pore forming module interacts with an applied electric field across the pore. This interaction induces a torque on the dipole that aligns it the direction of the applied field, causing motion that closes the pore. The lysenin voltage-gating mechanism depends on the negatively charged glutamates at positions Glu84 and Glu85 which create an effective electric diploe.

5-POS Board 2

BICELLE SEGREGATION MEASURED AS A FUNCTION OF COMPOSITION-DEPENDENT VARIATIONS

Christopher P. Baryiames; Matthew Necelis[1]; Linda Columbus[1];
[1]The University of Virginina, Chemistry, Charlottesville, VA, USA

Despite being essential for understanding the structure and function of cells and proteins, the energetics of specific lipid-lipid interactions remain elusive. This is in part due to the extreme heterogeneity of the lipid bilayer, which contains thousands of lipid species, necessitating the use of membrane models. Bicelles are attractive options for membrane studies as they combine the small scattering profile of micelles with the bilayer-like environment of vesicles. The lipid-rich core provides the bilayer-like structure, while the detergent “rim” stabilizes the lipid hydrophobic tails. However, the energetics that dictate this phase segregation are unknown. We measure the composition-dependent changes in bicelle structure by titrating different detergents into the bicelle and observing the effects of headgroup structure, tail length, and number of tails on bicelle morphology. Using scattering techniques in conjunction with NMR methods, the aggregation structure of each detergent/lipid mixture, as well as the extent of lipid segregation, has been determined. We have used these structural measurements to calculate the chemical potential of mixing lipids and detergents, demonstrating the value of bicelles as a platform with which lipid-lipid interactions may be measured.

9-POS Board 3

INVESTIGATING KCNQ1 MISTRAFFICKING IN LONG QT SYNDROME

Katherine R. Clowes; Hui Huang[1]; Alfred L George[2]; Charles Sanders[1];
[1]Vanderbilt University, Biochemistry, Nashville, TN, USA
[2]Northwestern University, Pharmacology, Chicago, IL, USA

It is estimated that 1 in 2500 individuals suffer from congenital long QT syndrome (LQTS), a cardiac disorder that can cause syncope, cardiac arrythmia, and cardiac arrest, which can be fatal. Loss of function mutations in the voltage gated potassium channel protein KCNQ1 cause 30-50% of cases of congenital LQTS, and over 250 LQTS-associated mutations in KCNQ1 have been identified. These mutations are distributed throughout the protein and result in variable severity of symptoms, leading to uncertainty about how these mutations cause loss of function. We have characterized 51 mutations in the KCNQ1 voltage sensing domain (VSD) by their impact on KCNQ1 expression, trafficking, stability, and function. We found that reduced trafficking to the plasma membrane (mistrafficking) was a common cause of protein dysfunction in the KCNQ1 VSD. This led to the hypothesis that mistrafficking is a common mechanism of protein dysfunction across domains in KCNQ1, and an interest in characterizing the impact of additional mutations on trafficking. The trafficking assay used in prior studies only allows for analysis of one mutant at a time, requiring use of a more high throughput method. Here, we describe a deep mutational scanning approach to determine the impact of a library of KCNQ1 variants on KCNQ1 cell surface trafficking. KCNQ1 variants are stably expressed in cells, which are sorted into “trafficking competent” and “trafficking deficient” populations based on their cell surface expression of KCNQ1 via flow cytometry. The proportion of each mutant in the two populations is then determined to identify mutations that increase or decrease KCNQ1 trafficking. This allows us to determine residues and regions that are important for KCNQ1 trafficking. Using this method, we have begun characterizing trafficking phenotypes of mutations in KCNQ1 in a high-throughput fashion, providing additional information on the mechanisms of KCNQ1 loss of function in long-QT syndrome.

13-POS Board 4

PROTON-DRIVEN ALTERNATING ACCESS IN A SPINSTER TRANSPORTER, AN EMERGING FAMILY OF BROAD-SPECIFICITY EFFLUX PUMPS

Reza Dastvan[1]; Ali Rasouli[2,3]; Sepehr Dehghani-Ghahnaviyeh[2,3]; Samantha Gies[1]; Emad Tajkhorshid[2,3];
[1]Saint Louis University School of Medicine, Biochemistry and Molecular Biology, Saint Louis, MO, USA
[2]University of Illinois at Urbana-Champaign, NIH Center for Macromolecular Modeling and Bioinformatics, Urbana, IL, USA
[3]University of Illinois at Urbana-Champaign, Biochemistry, and Center for Biophysics and Quantitative Biology, Urbana, IL, USA

Spinster (Spns) lipid transporters are critical for transporting sphingosine-1-phosphate (S1P) across cellular membranes. S1P regulates growth, survival and migration of cells with profound proangiogenic effects. In humans, Spns2 acts as the main S1P transporter in endothelial cells, making it a potential drug target for modulating S1P export and signaling. Using an integrated approach in lipid membranes, we combined double electron-electron resonance spectroscopy with molecular dynamics simulations to characterize major conformational states of a close bacterial homolog of the Spns proteins from Hyphomonas neptunium (HnSpns) and to define its proton- and substrate-coupled conformational dynamics. Our systematic study reveals conserved residues critical for the protonation step of the cycle and its regulation, and how sequential protonation of these proton switches coordinates the conformational transitions in the context of a noncanonical, ligand-dependent alternating access, in the absence of an obvious outward-facing conformational state. While our inward-facing model is similar to the X-ray structure, we have detected and generated a novel occluded state of HnSpns in the membrane. A conserved periplasmic salt bridge ($Asp60^{TM2}$-$Arg289^{TM7}$) keeps the transporter in a closed conformation, while proton-dependent conformational dynamics are significantly enhanced on the periplasmic side, providing a pathway for ligand exchange. Decoupled transmembrane proton-dependent conformational changes highlight a distinctive feature of HnSpns, and potentially other Spns proteins, that may be facilitated by more flexible transmembrane region particularly the gating helices. Accordingly, a considerably lower periplasmic pK of conformational changes compared with the intracellular side implies the functional necessity of an inwardly directed proton gradient across the membrane. Furthermore, our resistance assays reveal substrate polyspecificity and HnSpns multidrug resistance (MDR) activity, underscoring a previously unknown role for Spns proteins in MDR, beyond their activity in sphingolipid transport and signaling.

17-POS Board 5

TETRASPANIN SELF-ASSEMBLY GENERATES MEMBRANE CURVATURE IN VERTEBRATE PHOTORECEPTORS

Andrew F. Goldberg[1]; Michelle L Milstein[1]; Breyanna Cavanaugh[1]; Stefanie Volland[2]; David S Williams[2];
[1]Oakland University, Eye Research Institute, Rochester, MI, USA
[2]UCLA School of Medicine, Stein Eye Institute, Los Angeles, MI, USA

Membrane curvature is an essential aspect of cellular structure; it functions in a broad range of processes including endocytosis, exocytosis, vesicular trafficking, protein localization and others. Highly curved (< 40 nm diameter) biological membranes can be generated and stabilized in multiple ways. Several different protein-based mechanisms have been identified to date, and multiple mechanisms often participate in particular instances. The elegant architecture of vertebrate rod and cone photoreceptor outer segment (OS) organelles is characterized by abundant (negative) membrane curvature, but the molecular mechanisms responsible for its generation and stabilization have remained obscure. OS organelle biogenesis includes the production of many hundreds of pita bread-shaped membranous disks that are bounded by high-curvature (~26 nm diameter) rims. Disruption of disk rim membrane curvature generation in vertebrate photoreceptors causes dysmorphic OSs, vision loss, and a variety of progressive retinal diseases.Our studies utilize in vitro, in cellulo, and vertebrate expression systems, in conjunction with biochemical, biophysical, molecular genetic, and imaging approaches to investigate how OS disk rim membrane curvature is generated and propagated. Our findings demonstrate that the photoreceptor-specific tetraspanin, peripherin-2 (prph2; TSPAN22), can directly generate membrane curvature in cellulo and in vivo. Further, they suggest a mechanistic model in which V-shaped transmembrane tetramers create an initial membrane deformation (dimple), followed by disulfide-mediated tetramer polymerization, which organizes that deformation into extended high curvature bends. This work advances understanding of peripherin-2 function for OS biogenesis and architecture, and clarifies how pathogenic PRPH2 mutations trigger progressive retinal degenerations. It also introduces the possibility that other tetraspanins may generate or sense membrane curvature in support of their diverse biological functions.

21-POS Board 6

SLIDING, EXPANDING, AND COMPRESSING: HOW MEMBRANES RESPOND TO FLUID FLOW

Aurelia R. Honerkamp-Smith[1]; Sreeja Sasidharan[1]; Autumn R Anthony[1]; Amanda Ratajczak[1]; [1]Lehigh University, Physics, Bethlehem, PA, USA

A remarkable feature of lipid membranes is their fluidity, which allows them to self-heal, bend, and flow. Unsupported membranes circulate in response to flows in the water surrounding them, but cell plasma membranes are reinforced by a cytoskeletal network of protein filaments which alters lipid and protein mobility. We use supported model membranes, microfluidics and microscopy to investigate how flow can transport membrane-linked proteins and modify lipid properties. We apply piconewton-sized forces to membranes and proteins in order to investigate frictional coefficients, phase boundaries, and protein concentration gradients in membranes. We also investigate a membrane protein advection-based mechanism for flow mechanosensing. Understanding how membrane lipids and proteins move in response to flow will help us understand how cells use flow responses to regulate important biological functions such as blood pressure and immune responses.

25-POS Board 7

AMMONIA SELECTIVITY THROUGH AQUAPORIN ATTIP2;1 IN GEL PHASE LIPID BILAYER

Deepak Kumar[1]; Tomas Kubar[1];
[1]Karlsruhe Institute of Technology, Institute of Physical Chemistry (IPC), Karlsruhe, Germany

Aquaporins are highly efficient transmembrane protein channels that selectively, yet efficiently allow water and other small solutes to pass through lipid bilayer. Aquaporin AtTIP2;1 facilitates the permeation of water as well as ammonia across the vacuolar membrane of plants. Experimentally, it has been suggested that the selective filter region plays a pivotal role in the permeation of ammonia across the aquaporin. The main objective of this work is to study the permeabilities of water and ammonia through AtTIP2;1 in liquid and gel phase lipid by means of microsecond molecular dynamics simulation. Our simulations shows that all channels are open in either ways, in both phases of the lipid. The passage of ammonia across the channels are in the direction of the flow of the water. Whereas ammonia permeates through the channels in liquid phase lipid, that process is largely blocked in gel phase lipid, in agreement with experimental observations. Additionally, potentials of the mean force generated by means of extended sampling simulations for the permeation of water along the pore of the aquaporins in both lipid phases revealed a low barrier to the permeation, corroborating with the observed frequent permeation events.

29-POS Board 8

CHARACTERIZING THE LIPID FINGERPRINT OF THE MECHANOSENSITIVE ION CHANNEL PIEZO2

Yie Chang Lin[1]; Amanda Buyan[1]; Ben Corry[1];
[1]Australian National University, Research School of Biology, Canberra, Australia

Piezo2 is a membrane-embedded mechanosensitive ion channel which mediates gentle touch sensations, tactile pain and proprioception. Ubiquitous depletion of Piezo2 in mice leads to early postnatal death, likely due to respiratory insufficiency. Additionally, malfunction of Piezo2 is known to cause mechanically induced pain syndromes and respiratory issues. Activated by mechanical stimuli such as membrane stretch and fluid flow, Piezo2 opens to allow cations to enter the cell. The cryo-EM structures of Piezo2 and its homologue Piezo1 shows that they consist of three subunits, forming a triskelion shape with curved propellers extending out from a central pore towards the extracellular space. In detergent and when reconstituted into liposomes, Piezo1 adopts a bowl-shaped conformation which induces local curvature within the bilayer, a feature thought to be critical for force sensing. As membrane proteins, Piezo channels affect and are affected by the surrounding lipid bilayer. In particular, Piezo channel function is known to be regulated by phosphoinositide lipids, cholesterol and dietary fatty acids. Recently, several studies have used molecular dynamics simulations to explore the interactions between Piezo1 and its lipid environment, revealing functionally important PIP2 and cholesterol binding sites on the protein. Given the recent elucidation of the Piezo2 cryo-EM structure and its similarities to Piezo1, molecular dynamics simulations of Piezo2 could yield interesting insights into its relationship with the bilayer and show whether it also induces local membrane curvature. Here, we present the characteristic lipid fingerprint of PIezo2 in a complex mammalian membrane, revealing key lipid-protein binding sites and highlighting its similarities and differences to the published Piezo1 lipid fingerprint.

33-POS Board 9

STRAIN RESPONSE OF A SIMULATED LIPID BILAYER TO OSCILLATING PRESSURE IN THE MHZ RANGE

Kyrstyn S. Ong[1]; Merritt Maduke[2]; Evan Reed[1]; Stephen Baccus[3];
[1]Stanford University, Department of Materials Science and Engineering, Stanford, CA, USA
[2]Stanford University, Department of Molecular and Cellular Physiology, Stanford, CA, USA
[3]Stanford University, Department of Neurobiology, Stanford, CA, USA

Ultrasound has been shown in many species to modulate neural activity by either exciting or inhibiting neurons, but ultrasonic effects at the molecular level are unclear[1]. Because ultrasound delivers acoustic energy in the form of a pressure wave, it may act in part through a mechanical mechanism. Previous research has distinguished between two major mechanical effects of ultrasound: cavitation, which refers to the formation and collapse of bubbles, and radiation force, which refers to the transfer of momentum from the ultrasound wave to the tissue that can result in mechanical strain[2]. However, it is still unknown which of these effects dominates the response of the membrane to ultrasound and how that response depends on ultrasound parameters such as frequency and power. Moreover, both of these effects occur as a function of the amplitude of the ultrasound pressure integrated over time, and little is known about effects at high frequencies in the MHz range of oscillating acoustic pressure. We have investigated the direct effect of oscillating pressure on membrane strain with atomistic molecular dynamics simulations. We constructed a lipid bilayer patch of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) lipids solvated in water. After equilibrating the system at physiological conditions, we applied sinusoidal pressure over a range of frequencies from 1 to 43 MHz. Because we are interested in atomic and molecular effects, we computed strain tensors for individual lipid molecules from the coordinate trajectories of every atom with a kinematical algorithm[3]. Our simulation results indicate that (1) strain changes in the membrane were time-locked with the frequency of the pressure wave and (2) increasing the peak pressure increased its effect on strain. These strain effects on the membrane are independent of the effects of cavitation and radiation force and must be included in molecular scale mechanistic explanations of ultrasonic neuromodulation. [1]Blackmore, J., Shrivastava, S., Sallet, J., Butler, C.R., & Cleveland, R.O. Ultrasound Neuromodulation: A Review of Results, Mechanisms, and Safety. Ultrasound Med. Biol. 45(7), 1509-1536 (2019). [2]Menz, M.D., Oralkan, Ö., Khuri-Yakub, P.T., & Baccus, S.A. Precise Neural Stimulation in the Retina Using Focused Ultrasound. J. Neurosci. 33(10), 4550-4560 (2013). [3]Gullet, P.M., Horstemeyer, M.F., Baskes, M.I., & Fang, H. A deformation gradient tensor and strain tensors for atomistic simulations. Modelling Simul. Mater. Sci. Eng. 16, (2008).

37-POS Board 10

INVESTIGATION INTO THE MECHANOCHEMISTRY OF NUMBER ASYMMETRY GENERATION IN MODEL MEMBRANES: A COMBINED MASS-ACTION AND MICROMECHANICAL STUDY

Tyler R Reagle[1]; Yuxin Xie[1]; Tobias Baumgart[1];
[1]University of Pennsylvania, Dept. of Chemistry, Philadlephia, PA, USA

Nonvesicular lipid transport is a mechanism employed by lipid trafficking machinery to regulate compositional inhomogeneities of biomembranes. Globally, lipid transfer proteins (LTPs) catalyze nonvesicular lipid exchange between different organelles to efficiently pool the cellular lipidome. Locally, transmembrane flippases and scramblases catalyze the nonvesicular exchange of lipids between the two leaflets of a single bilayer. Flippases catalyze active lipid transport in which the energetically-costly process of membrane lipid asymmetry generation is coupled to ATP hydrolysis. Contrarily, scramblases catalyze passive transport between both leaflets in which transport proceeds downhill to relax gradients from membrane lipid asymmetry. Membrane lipid asymmetry refers to both 1) dissimilar leaflet-specific lipid compositions across the bilayer (I.e. compositional asymmetry) and 2) unequal numbers of lipid molecules composing both leaflets across the bilayer (I.e. number asymmetry). While it is recognized that a bilayer's asymmetry is a result of the regulated activities of flippases/scramblases, whether lipid flux catalyzed by LTPs could also be influenced by the specific compositional/number asymmetries of the exchanging bilayers is an open question. To consider this, we have used the lipid chelator, methyl-β-cyclodextrin (mbCD), to probe the physical chemistry and micromechanics of number asymmetry relevant for lipid transport in vivo. We scrutinized the equilibrium binding model of mbCD-lipid complexation under conditions when unbound mbCD/mbCD-lipid inclusion complexes in the aqueous phase were in coexistence with membranes with nonzero number asymmetry. In doing so, we identified whether number asymmetry contributed detectably to lipid mass-action. We also performed micromechanical experiments upon giant unilamellar vesicles with number asymmetry using optical trapping/micropipette aspiration. We observed that, at some threshold mechanical tension, spontaneous lipid exchange between both leaflets occurred to relax initial number asymmetry within the membrane. Our results highlight our ongoing efforts to study the mechanochemistry of number asymmetry and propose an interplay between flippases and LTPs in lipid trafficking.

41-POS Board 11

DISCOVERY OF VERY IMPORTANT MEMBRANE PROTEINS (VIMPS) THAT HAVE ESCAPED PREVIOUS ATTENTION

Charles R. Sanders[1]; Tucker L Apgar[1]; Adam L Sanders[1]; Jake Hermanson[1];
[1]Vanderbilt University School of Medicine - Basic Sciences, Biochemistry, Nashville, TN, USA

Bioinformatic studies were conducted to identify membrane proteins of great biological and/or biomedical relevance that have previously eluded notice as "very important membrane proteins" (VIMPs). Here we pursued two different approaches to generate a list of VIMPs that can be used, among other applications, by new investigators seeking to establish new projects involving membrane proteins of high significance. Our first approach was to explore Mendelian diseases and their causative proteins. "Simple" Mendelian diseases are genetic disorders that are caused by pathogenic mutations in a single gene. Although these disorders are considered promising drug targets due to their simple etiology, they typically are classified as rare disorders, which has historically discouraged drug discovery efforts to target that disease by the pharmaceutical industry. However, a number or these diseases are not that rare. Here we systematically explored the several thousand known Mendelian disorders and compiled as list of all those 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 gene mutations. We found that there are 74 such "common rare" disorders and compiled a list of the causative genes and their encoded proteins, some of which are understudied membrane proteins. In a second approach we also explored the entire human proteome by applying missense intolerance analysis to all possible 31 amino acid segments of all human proteins to determine whether each segment is intolerant to mutation in the gnomAD collection of human genes. For the roughly 20,000 human proteins it was found that 290 proteins are so important that they contain at least one 31 residue segment in which amino acid mutations are absolutely not seen in the $>10^5$ human gene sequences currently deposited in gnomAD. Such proteins are deemed to be subject to "purifying selection" by evolution. Of these zero-tolerance proteins, 37 were integral membrane proteins. While some of these proteins are well known, such as the ryanodine receptor, some have received almost no attention to date despite the fact that natural selection finds them to be VIMPs. It is hoped that the list of VIMPs generated here based both on analysis of Mendelian disorders and on genetic intolerance analysis will be useful to those investigators, especially new investigators, looking to establish projects that focus on a VIMP and its associated biology and disease relatedness.

45-POS Board 12

HIGH-THROUGHPUT SCREENING TO IDENTIFY MODULATORS OF RAFT FORMATION AND MEMBRANE PROTEIN RAFT AFFINITY IN GIANT PLASMA MEMBRANE VESICLES

Katherine M Stefanski[1]; Hui Huang[1]; Joshua A Bauer[1]; Anne K Kenworthy[2]; Charles R Sanders[1];
[1]Vanderbilt University, Biochemistry, Nashville, TN, USA
[2]University of Virginia, Charlottesville, VA, USA

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

49-POS Board 13

INTERPLAY OF CHOLESTEROL AND HEADGROUP EFFECTS ON MEMBRANE OXYGEN PERMEABILITY

Qi Wang[1]; Sally Pias[1];
[1]New Mexico Institute of Mining and Technology, Chemistry, Socorro, NM, USA

Diffusive transport of oxygen (O_2) at the level of cell membranes is essential for energy metabolism. Prior studies by our group and others have indicated that cholesterol and increased saturated tail chain length can reduce the permeability of phosphocholine (PC) bilayers by 10-25% or more. Here, we investigate the interplay of lipid headgroup variations and cholesterol content. Breast tumor lipidomics data indicating that a particular lipid incorporating PE(18:0/18:1) is abnormally high in the cell membranes of aggressive breast tumors. Our hypothesis is that lipid changes promoting increased membrane order, including the smaller phosphatidylethanolamine (PE) headgroup, the longer 18:0 tail, and increased cholesterol content will reduce oxygen permeability and may tend to promote tumor cell hypoxia—O_2 deficiency. We have used atomistic molecular dynamics simulations at 37°C to investigate the combined influence of headgroup and cholesterol on oxygen permeability in double-component bilayer systems. Relative to the oxygen permeability of PC(16:0/18:1) or POPC at 16.5 ± 0.6 cm/s, exchanging the headgroup with PE or introducing 50 mol% cholesterol gives a drop in permeability to ~12cm/s. Increasing the saturated chain length to 18:0 gives a permeability of 10 to 11 cm/s, regardless of the presence or absence of cholesterol at 50 mol%. Among bilayer physical properties, the best established predictor of membrane permeability for a given solute is area-per-lipid, an indicator of lipid lateral packing density. Our findings are consistent with the dominance of area per lipid as a determinant of oxygen permeability, though cholesterol complicates the analysis somewhat. In particular, cholesterol increases the lateral packing density of PC lipids but cannot further increase the tightness of packing (or reduce the permeability) for PE lipids. Further, the longer 18:0 saturated tail reduces the area-per-lipid and the oxygen permeability, relative to the 16:0 tail, diminishing the effect of the smaller PE headgroup.

53-POS Board 14

THE DESMOGLEIN TRANSMEMBRANE DOMAIN DETERMINES ASSOCIATION WITH ORDERED LIPID DOMAINS TO ASSEMBLE ROBUST DESMOSOMES

Stephanie E Zimmer[1]; Mateusz Sikora[3]; Ilya Levental[4]; Andrew P Kowalczyk[1,2];
[1]Penn State College of Medicine, Dermatology, Hershey, PA, USA
[2]Penn State College of Medicine, Cellular and Molecular Physiology, Hershey, PA, USA
[3]Max Planck Institute of Biophysics, Frankfurt, Germany
[4]University of Virginia, Molecular Physiology and Biological Physics, Charlottesville, VA, USA

Cholesterol- and sphingolipid-enriched ordered lipid domains, or "lipid rafts," selectively recruit proteins for specific functions. However, mechanisms driving certain membrane proteins into such domains while excluding others are not fully understood. Intercellular adhesive junctions called desmosomes are ordered mesoscale lipid domains that represent unique models for understanding these mechanisms. Desmosomes confer robust mechanical strength to tissues by linking intermediate filament networks of adjacent cells through a series of adaptor proteins assembled at cell borders by adhesive desmogleins (DSG) and desmocollins. A unique disease-causing mutation resulting in a glycine to arginine substitution in the DSG1 transmembrane domain (TMD) abrogates association with ordered lipid domains to disrupt desmosome assembly and adhesive function, suggesting that the DSG TMD supports these processes through partitioning to ordered lipid environments. TMD physical properties, including length, exposed surface area, and palmitoylation, have been shown to drive raft association of single-pass transmembrane proteins like DSG. We hypothesized that TMD physical properties drive DSG raft association to promote desmosome assembly and function. We created a panel of GFP-tagged $DSG1_{TMD}$ variants by individually modifying each of the above properties and stably expressed these $DSG1_{TMD}$-GFP variants in DSG-null cells. Sucrose gradient fractionations showed reduced raft partitioning for variants with altered TMD length or exposed surface area while loss of palmitoylation had no effect. Super-resolution imaging and functional assays revealed a linear relationship between $DSG1_{TMD}$ lipid raft association and the assembly of morphologically and functionally robust desmosomes. These findings suggest that DSG raft association is an important nucleation step during desmosome assembly and that strong desmosome adhesion requires raft association. We propose that reduced association with ordered lipid domains is an underlying disease mechanism in some inherited human diseases.

57-POS Board 15

FLOW-SENSITIVE ACTIVATION OF MURINE OTOP CHANNELS BY NH4CL

Ziyu Liang[1]; Bochuan Teng[1]; Emily Liman[1];
[1]University of Southern California, Neurobiology, Los Angeles, CA, USA

The otopetrin proteins (OTOPs) constitute a family of proton-selective ion channels found in diverse tissues, one of which (OTOP1) functions as a sour taste receptor. NH4Cl, which exists in equilibrium with the membrane permeable gas NH3, is used commonly as a means to alkalize the cytosol of cells. We therefore wondered whether currents through OTOP1 and other OTOP channels could be induced by exposure to NH4Cl. To test this possibility, murine OTOP channels were expressed HEK-293T cells and currents were measure by whole-cell patch-clamp recording. We found that large currents were induced in OTOP1 and OTOP2 but not OTOP3 expressing cells. Interestingly, the responses to NH4Cl, but not to HCl, showed a markedly flow-sensitivity. We considered two possible explanations for this phenomenon: first, the cell cytosol is similarly alkalinized by NH4Cl for all flow rates and OTOP1/2 are gated in a flow-sensitive manner. Second, the degree of alkalization of the cell cytosol is flow-sensitive and the gating of the channels is unaffected by varying flow rate. To distinguish between these possibilities, we combined whole-cell patch clamp recording with pH imaging using the pH indicator, pHlourin. We found that the degree of alkalization was sensitive to flow rate and correlated with the magnitude of the currents for both mOTOP1 and mOTOP2. Thus, flow-sensitivity is conferred by the rate of diffusion of NH4Cl across the membrane and not by mechanical gating of OTOP channels. The response of OTOP channels to NH4Cl is likely to have physiologically significance: for example, NH4Cl is a potent activator of the gustatory system and is used routinely as a control in gustatory nerve recordings. Future work will be needed to determine whether OTOP channels are required for this and other physiological responses to NH4Cl.

Tuesday, June 7
POSTER SESSION II
4:00 PM – 6:00 PM
Ballroom

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. Presenting authors with odd-numbered poster boards should present from 4:00 PM – 5:00 PM and those with even-numbered poster boards should present from 5:00 PM – 6:00 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 4:00 PM – 5:00 PM | Even-Numbered Boards 5:00 PM – 6:00 PM

Al Tekreeti, Abdullah	**2-POS**	**Board 1**
Cook, Leslie	**6-POS**	**Board 2**
Coffman, Robert	**10-POS**	**Board 3**
Diederichs, Kathryn	**14-POS**	**Board 4**
Gonthier, Alyse	**18-POS**	**Board 5**
Hryc, Jakub	**22-POS**	**Board 6**
Li, Shisheng	**26-POS**	**Board 7**
Lynch, D.	**30-POS**	**Board 8**
Paratore, Trevor	**34-POS**	**Board 9**
Reddy, Krishna	**38-POS**	**Board 10**
Selvasingh, Jazlyn	**42-POS**	**Board 11**
Tariq, Anam	**46-POS**	**Board 12**
Wilm, Matthias	**50-POS**	**Board 13**
Jurisic, Nikola	**54-POS**	**Board 14**

Posters should be set up on the morning of Tuesday, June 7 and removed by 11:00 PM on Tuesday, June 7. All uncollected posters will be discarded.

2-POS Board 1

CALCIUM INHIBITION OF CALCIUM ACTIVATED TRPM4 CURRENT

Abdullah A. Al Tekreeti[1]; Jie Zheng[1];
[1]University of California, Davis, Department of Physiology and Membrane Biology, Davis, CA, USA

The TRPM4 channel is a voltage-dependent calcium-activated channel capable of sustaining membrane depolarization during cell activation. Paradoxically, TRPM4 current diminishes as calcium concentration continues to increases. We observed that, at the millimolar concentrations range, calcium application and wash-off produce two slow (in seconds) ON/OFF peaks that flank the calcium-activated current. We found that the prominent OFF response is caused by a rapid recovery from calcium inhibition in combination of a slow decline of TRPM4 current. This study examines the mechanistic and functional properties behind this calcium inhibitory effect, which may improve current understanding of the TRPM4 channel gating behavior during different cellular conditions.

6-POS Board 2

NON-EQUILIBRIUM ENTROPY CALCULATIONS OF MEMBRANE PROTEINS DURING THE AGGREGATION PROCESS

Leslie Cook[1]; Isabella Makelaar[1]; Garrett Baughman[1]; Preet Sharma[1];
[1]Midwestern State University, Physics and Chemistry, Wichita Falls, TX, USA

Aggregation of membrane proteins is a complex biological phenomena with potentially detrimental consequences. It is recognized as the hallmark of neurodegenerative diseases, suffered by millions of people each year. Abnormal deposits of amyloid fibrils accumulate causing irreparable damage that results in the deterioration of brain tissue and the death of vital neuron cells. This leads to severe impairment in cognitive functioning that progresses at an exponential rate. Membrane protein aggregation is known to be a highly dynamic, irreversible process which is the source of its difficulty to understand and develop new technologies for therapeutic intervention and early detection of neurodegenerative diseases. The design of our study is to interpret the mechanics of membrane proteins that misfold and self-assemble into highly structured fibrils. The aim is to gain a deeper understanding of protein-membrane interactions and the misfolding mechanisms that attribute to the aggregation process. The complexity this biophysical process cannot be accurately modeled using statistical physics and statistical thermodynamics of equilibrium processes. Which suggests, according to numerous studies, that membrane protein aggregation is a non-equilibrium process. Based on non-equilibrium physics, one of the best ways to understand aggregation is through the Langevin equations and the Fokker-Planck equations. Langevin equations describe the stochastic dynamics of non-equilibrium processes and the Fokker Planck equation is used to calculate the probability distribution that reveals the trend in entropy of a model independent protein aggregation process.

10-POS Board 3

DO MEMBRANES OR SNARE PROTEINS MEDIATE THE EFFECT OF ETHANOL ON VESICLE FUSION?

Robert E Coffman[1]; Alex J Kreutzberger[2]; Volker Kiessling[3]; Lukas K Tamm[3]; Dixon J Woodbury[1];
[1]Brigham Young University, Neuroscience Center, Provo, UT, USA
[2]Harvard University, Cell Biology, Boston, MA, USA
[3]University of Virginia Health System, Molec Physiol/Biol Phys, Charlottesville, VA, USA

In neurons, neurotransmitter release is highly regulated and sensitive to alcohol. Release of neurotransmitters occurs when synaptic vesicles undergo membrane fusion with the plasma membrane. The SNARE proteins catalyze this reaction by zippering together. The SNARE proteins Syntaxin-1A and SNAP-25 are associated with the plasma membrane and Synaptobrevin-2 is associated with the vesicle membrane. We hypothesize that alcohol may act directly on the fusion machinery or on the fusing membranes, separately from any upstream or downstream process. We used a reconstituted single particle SNARE driven fusion assay (Domanska et al., 2009 JBC. 284:32158-66) to measure vesicle fusion probability with increasing amounts of methanol and ethanol. We also used Circular Dichroism Spectrometry (CD) and site directed fluorescence interference contrast microscopy (FLIC) to measure the effect of these alcohols on neuronal SNARE proteins and their interaction with membranes. We show that fusion probability increases significantly with a physiologically relevant concentration of ethanol (0.4% v/v, 68 mM), but methanol requires a 20-fold higher concentration to see a similar effect. We also show that SNAREs are only affected by alcohol concentrations above 2%. Previously, we reported that ethanol significantly increases fusion rates at 0.4%, in the absence of proteins (Paxman, et al., 2017 Biophys J. 112:121-132). These combined data show that the effect of ethanol on SNARE driven fusion is mediated through its interaction with membranes and not SNARE proteins.

14-POS Board 4

TOWARDS MECHANISTIC UNDERSTANDING OF MITOCHONDRIAL BETA-BARREL BIOGENESIS: FUNCTIONAL STUDIES OF THE SORTING AND ASSEMBLY MACHINERY COMPLEX

Kathryn A Diederichs[1]; Sarah E Rollauer[1]; Martin S King[3]; Edmund R.S. Kunji[3]; Joseph A Mindell[2]; Susan K Buchanan[1];
[1]NIH, NIDDK, Bethesda, MD, USA
[2]NIH, NINDS, Bethesda, MD, USA
[3]University of Cambridge, MRC Mitochondrial Biology Unit, Cambridge, United Kingdom

The mitochondrial outer membrane of yeast contains four types of beta-barrel proteins (Sam50, Tom40, Mdm10, and VDAC), all of which are translated in the cytosol and imported into the mitochondria as unfolded precursors. The sorting and assembly machinery (SAM) complex recognizes beta-barrel precursors by a conserved C-terminal sequence motif, the beta-signal, and facilitates their folding and insertion into the outer membrane. The SAM complex is composed of three subunits: Sam50, Sam35, and Sam37. Sam50 and Sam35 are essential for cell viability and beta-barrel biogenesis, and have been demonstrated to specifically interact with precursor beta-signal. However, the molecular mechanisms of SAM complex beta-signal recognition, precursor folding, and membrane insertion are currently unknown. We previously solved a high-resolution cryo-electron microscopy structures of the SAM complex from the fungi Thermothelomyces thermophilus. Current work uses these structures to design experiments that will characterize the interaction of the SAM complex subunits with beta-signal and beta-barrel precursors as they fold using a variety of biochemical and biophysical techniques. Ultimately, this work will improve mechanistic understanding of SAM complex function and mitochondrial outer membrane beta-barrel biogenesis.

18-POS Board 5

MEMBRANE TENSION DRIVES CELL RESPONSE TO POROUS SUBSTRATES: A COMPUTATIONAL STUDY

Alyse R Gonthier[1]; Anna Grosberg[2,3]; Elliot Botvinick[2]; Ali Mohraz[1,3];
[1]University of California, Irvine, Materials Science & Engineering, Irvine, CA, USA
[2]University of California, Irvine, Biomedical Engineering, Irvine, CA, USA
[3]University of California, Irvine, Chemical & Biomolecular Engineering, Irvine, CA, USA

Understanding physical cell-substrate interactions is critical to improving the long-term stability of biomedical implants. Many studies have examined the impact of biochemical cues on immune cell response, but the role of physical and geometrical cues such as local curvature is not as well understood. Recent studies have suggested that local curvatures within a porous material have a significant impact on immune cell behavior, such as macrophage polarization. Specifically, a recently developed porous implant with a unique interconnected and uniform pore structure, called a bijel-templated material or BTM, demonstrated improved vascularization and significantly increased presence of pro-healing macrophages in comparison to the current state-of-the-art. Because of their spontaneous thermodynamic formation process, BTMs have a unique predominance of negative Gaussian curvatures, which can be thought of as saddles, along their internal surfaces. Initial studies suggest that this specific curvature and its impact on membrane tension sensing directly contribute to the increase in pro-healing macrophage presence, and downstream immune benefits. Utilizing a computational modeling approach, this work aims to provide a mechanistic understanding of this special substrate curvature as a direct factor contributing to improved immune response. The model is necessarily developed to emphasize the contribution of membrane tension to overall cell motility and behavior, significantly improving its ability to replicate cell behavior observed in vitro. This combination of computational modeling and in vitro validation predicts a strong, characteristic relationship between unique BTM curvature and cell behavior, in terms of shape and motility. Continuing work will illuminate a mechanistic relationship between this effect of local curvature and the increased pro-healing macrophage population. This would in turn inform improvements to implant success on a broad scale, and significantly contribute to understanding how implantable devices can be optimized for success and longevity in the body without biochemical intervention.

22-POS Board 6

LIPID/WATER INTERFACE OF GALACTOLIPID BILAYERS IN DIFFERENT LYOTROPIC LIQUID-CRYSTALLINE PHASES

Jakub Hryc[1]; Michal Markiewicz[1]; Marta Pasenkiewicz-Gierula[1];
[1]Jagiellonian University, Department of Computational Biophysics and Bioinformatics, Kraków, Poland

Model membranes are hydrated lipid bilayers of a controlled lipid composition typical for the specific biomembrane. Each bilayer has three distinct regions, the water phase, the polar interface and the nonpolar hydrocarbon chain region. Of the regions, the lipid/water interface is the most structurally and dynamically complex. It consists of various lipid polar groups and water molecules; the polar groups are in constant motions and the interfacial water molecules, even though they are predominantly bound to the polar groups, exchange with bulk water fast.In this study, the organisation of the lipid/water interface of bilayers composed of galactolipids with both α-linolenoyl (di-18:3, cis) acyl chains, in three different lyotropic liquid-crystalline phases, is analysed and compared. The studied systems include monogalactosyldiglyceride (MGDG) and digalactosyldiglyceride (DGDG) bilayers in the lamellar phase, MGDG double bilayer in the stalk structure and the inverse hexagonal MGDG phase (H_{II}). For each system, lipid-water as well as direct and water mediated lipid-lipid interactions are identified and their lifetimes assessed. The analyses concern interactions among lipids belonging to the same bilayer leaflet and among lipids belonging to the opposite leaflets at the onset of the new phase formation.

26-POS Board 7

DISTINCT OPEN STATES OF TRPV1 AND ENERGETICS OF RTX ACTIVATION

Shisheng Li[1]; Bo Hyun Lee[1]; Jie Zheng[1];
[1]UC Davis, Physiology and Membrane Biology, Davis, CA, USA

TRPV1 is a major nociceptor for plant toxins such as capsaicin and resiniferatoxin (RTX), protons and heat. Activation of the multi-subunit TRPV1 channel is known to be allosteric. However, most ligands including capsaicin activate TRPV1 rapidly, making it difficult to capture transition states between the initial closed state and the fully liganded open state by functional or structural methods. RTX activates TRPV1 slowly but irreversibly, offering an opportunity to obtain a glimpse of the transition states from patch-clamp recordings. We found the Y512A mutation makes RTX binding reversible. Incorporating Y512A mutation into concatemers allowed fixing RTX binding in wildtype subunits and removing RTX from mutant subunits, hence gaining control of the exact number of bound RTX to investigate transition states from steady current recordings. Using this system, we showed that binding of RTX to each of the four subunits yields an equal stabilization energy, which we estimated to be 1.78 kcal/mol. We further confirmed that a different number of RTX binding changes only the open probability but not the single-channel conductance, as predicted by a classic allosteric model. However, using different ligands (capsaicin, 2-APB) or activation methods (heat or proton), we did observe distinct single-channel conductance levels as well as ion-ion interactions, suggesting the existence of distinct open states.

30-POS Board 8

EXPLORING THE MECHANISM OF PROTEIN TRANSLOCATION ACROSS THE INNER MITOCHONDRIAL MEMBRANE

D. L Lynch[1]; S. I Sim[2,3]; Y. Chen[2,3]; E. Park[2,3]; J. C Gumbart[1];
[1]Georgia Institute of Technology, School of Physics, Atlanta, GA, USA
[2]University of California, Department of Molecular and Cell Biology, Berkeley, CA, USA
[3]University of California, California Institute for Quantitative Biosciences, Berkeley, CA, USA

The majority of mitochondrial proteins are cytosolically translated and, therefore, require import across the outer and, depending on the destination, inner mitochondrial membranes (IMM). Protein translocases, multi-subunit complexes, recognize, import, and sort these precursor proteins. The TIM23 complex mediates import across the IMM into the matrix, as well as the IMM itself, and is comprised of the essential proteins Tim23, Tim17, and Tim44. Protein precursors are typically synthesized with a targeting signal, in the case of matrix proteins this is often a N-terminal basic, amphipathic stretch of amino-acids. As such the import of matrix proteins is thought to be partially driven by an electrophoretic force due to the inner membrane potential, which must be maintained for the respiration activity of mitochondria. Although the process of protein import across the IMM has been studied by a variety of biophysical approaches, the structural data necessary to parse the detailed translocation mechanism has been lacking. Moreover, it has recently been recognized that membrane thinning contributes to the process of retrotranslocation across the ER, suggesting an active role for the membrane itself in protein translocation processes. Recently, utilizing cryo-EM of the Tim23-Tim17-Tim44 complex and molecular dynamics simulations employing a realistic IMM membrane composition, we have begun to explore the roles that the membrane potential as well as membrane thinning play in the process of import across the IMM.

34-POS Board 9

PHOSPHOINOSITIDE DOMAIN FORMATION AND PROPERTIES IN ASYMMETRIC LIPID BILAYERS

Trevor A. Paratore[1]; Alonzo H Ross[1]; Arne Gericke[1];
[1]Worcester Polytechnic Institute, Department of Chemistry and Biochemistry, Worcester, MA, USA

Phosphoinositides (PIPs) have been shown to mediate a broad range of cellular events by attracting proteins to specific cellular sites. This spatiotemporal control of protein function is often associated with the formation of phosphoinositide enriched patches (domains) or gradients. The plasma membrane lipid composition is different (asymmetric) in the two leaflets of the bilayer. It has been suggested that outer leaflet lipid raft domains affect inner leaflet phosphoinositide domains and hence PIP mediated signaling events. Phosphoinositides exhibit predominantly a stearoyl-arachidonoyl acyl chain composition and PIP accumulation in a liquid-ordered membrane environment is expected to be unfavorable. Therefore, the question arises how outer leaflet lipid rafts can affect inner leaflet phosphoinositide distribution and function. To address this question, we adapted the hemifusion method introduced by Enoki and Feigenson (Biophys. J. 117 (2019) 103) to fabricate asymmetric giant unilamellar vesicles (aGUVs). This process involves the Ca^{2+} initiated fusion of a symmetric GUV with a solid supported lipid bilayer (SLB), which contains in our case the phosphoinositide component. We find a large variability of the PIP concentration in the resulting aGUV population. We attribute this to the formation of micro-sized domains in the SLB, leading to the fusion of the sGUVs with regions rich in PIPs and other areas that lack them. To overcome this issue, we use 1 mM Mg^{2+} instead of Ca^{2+} as the initiator for the fusion process since in the presence of Mg^{2+} PIPs don't form macroscopic domains. We obtain a narrow PIP concentration distribution across the aGUV population. In the next step we investigate PIP domains in the presence of a lipid raft mixture in the opposing leaflet.

38-POS Board 10

USING ANCESTRAL SEQUENCE RECONSTRUCTION TO UNDERSTAND MEMBRANE TRANSPORTER STRUCTURE, FUNCTION, AND MECHANISM

Krishna Reddy[1]; Olga Boudker[1];
[1]Weill Cornell Medical College, New York, NY, USA

Through evolution, membrane transporters adapt their functional properties to environmental changes and physiological needs. Phylogenetic analyses can reconstruct the evolutionary history of a protein family, inferring ancestral protein sequences before and after the emergence of novel functionality. Biochemical characterization of these sequences can reveal subtle changes in protein properties required for functional diversification that are not evident in traditional sequence comparisons of extant proteins. Ancestral sequence reconstruction (ASR) is a powerful approach to understanding evolution, but its applications to membrane proteins remain limited. We used ASR to uncover the mechanism of functional diversification in glutamate transporters, which have subfamilies coupling substrate import to different ion gradients. Specifically, we recapitulated an evolutionary transition from Na+- to H+-coupling using phylogenetics to reveal the architectural differences between transporters with divergent ion-coupling mechanisms. We then purified an ancestral Na+-coupled transporter to homogeneity, enabling functional characterization in reconstituted proteoliposomes and structural analysis by cryo-EM. The ancestral transporter displays unique structural and functional properties not observed in extant transporters thus far, providing a potential mechanistic basis for functional diversification. Our results provide a framework in which evolution-guided analyses uncover novel structure-function relationships in membrane proteins.

42-POS Board 11

ULTRA-DARK NANODISCS AS A MEMBRANE MIMETIC FOR ASSESSING MEMBRANE PROTEIN STABILITY BY NANO DIFFERENTIAL SCANNING FLUORIMETRY (NANODSF)

Jazlyn Selvasingh[1]; Jake Mckinney[1]; Jens Meiler[1,2]; Kaitlyn Ledwitch[1];
[1]Vanderbilt University, Nashville, TN, USA
[2]Leipzig University, Leipzig, Germany

Membrane proteins are particularly challenging targets to study because they associate with unique, asymmetric lipid environments and therefore, require the presence of a lipid membrane mimetic for in vitro studies. Here, we developed a new method that leverages nano Differential Scanning Fluorimetry (nanoDSF) coupled with an engineered non-fluorescent 'ultra-dark' nanodisc (replaces both tryptophan and tyrosine residues with non-fluorescent sidechains) to measure membrane protein thermostability. In this work, we demonstrate the utility of this approach using the disulfide bond formation protein B (DsbB) as a model membrane protein system. This new technology platform allows for the evaluation of membrane protein stability in the presence of lipids without signal interference from the lipid-loaded nanodisc mimetic. NanoDSF experiments for DsbB measured in ultra-dark nanodiscs resulted in a thermal unfolding curve with two distinct inflection points corresponding to a melting temperature of 70.5 and 77.5 °C respectively. We compared this to DsbB in a panel of detergent micelles, which all resulted in a single inflection point. DsbB in DDM (n-dodecyl-β-D-maltoside) micelles showed the highest melting temperature of 55.5 °C. This new technology platform facilitates high-throughput evaluation of membrane protein thermostability in the context of a lipid-based membrane mimetic. The application of this method is broadly applicable to determining the change in stability associated with a range of membrane proteins in complex lipid environments.

46-POS Board 12

BIOPHYSICAL CHARACTERIZATION OF MAJOR FACILITATOR SUPERFAMILY TRANSPORTER STY4874 FROM SALMONELLA ENTERICA SEROVAR TYPHI

Anam Tariq[1,4]; Aqsa Shaheen[2]; Randy Stockbridge[4]; Moazur Rahman[1,3];
[1]National Institute for Biotechnology and Genetic Engineering (NIBGE), PIEAS, Faisalabad, Pakistan
[2]University of Gujrat, Department of Biochemistry and Biotechnology, Gujrat, Pakistan
[3]University of the Punjab, School of Biological Sciences, Lahore, Pakistan
[4]University of Michigan, Department of Molecular, Cellular and Developmental Biology, Ann Arbor, MI, USA

Salmonellae are foodborne pathogens and the major cause of gastroenteritis in humans. Salmonellae express multidrug efflux transporters that play a key role in their drug resistance, which is becoming an increasing problem for therapeutic intervention. Despite their biomedical importance, the mechanisms underlying substrate transport by multidrug efflux transporters remain poorly understood. Here, we describe the biochemical characterization of a multidrug transporter belonging to the major facilitator superfamily from Salmonella enterica serovar Typhi. This transporter was found to be constitutively expressed in clinical isolates of Salmonella Typhi making it a potent drug target. To identify its role in multidrug resistance, a combination of cell-based functional assays, protein expression and purification, substrate binding analysis, and secondary structural analysis were conducted. We showed that this transporter is proton-dependent nature with broad-spectrum substrate specificity including fluoroquinolones and aminoglycosides. We further showed that it exists as structural monomer in DDM, and used CD spectroscopy and FTIR to show that, like its close homologues MdtM and MdfA from E. coli, STY4874 is rich in alpha helices. Efforts to crystallize and solve a high-resolution structure are ongoing. The structure elucidation of this transporter would greatly contribute in understanding the efflux mediated drug resistance mechanism in genus Salmonellae and in the structure-based drug design.

50-POS Board 13

SYNTHESIS OF LARGE, TRANSPORTABLE, BIO-MEMBRANES INCLUDING MEMBRANE PROTEINS FROM GAS PHASE BY MOLECULAR BEAM DEPOSITION

Matthias Wilm[1];
[1]University College Dublin, Conway Institute, Dublin, Ireland

The aim was to synthesise transportable protein containing membranes of an inherently unlimited size using a nano-electrospray ion source. A nano-electrospray was directed towards the liquid meniscus of a 3 mm wide reservoir holding an aqueous buffer and some SM-2 Biobeads for detergent extraction. In several steps bipolar lipids, glycerol and detergent solubilised membrane proteins were sprayed onto the surface and incubated to remove the detergent from the surface layer. Finally, the correct conditions were found to allow for self-assembly of a single lipid bio-membrane containing the proteins. The layer covered the entire surface as verified by transmission electron microscopy. No denatured protein was found. Instead, the images correspond to a protein filled membrane. The protein initially used was the integral membrane protein ompG, a mono-molecular pore. Additionally, I used listeriolysin O to investigate whether the procedure is gentle enough to allow for non-covalent complex formation. Listeriolysin O assembles on membranes into ring shaped complexes of 30 or more units. These rings insert themselves into membranes and form large pores. Using this method the pores formed and their assembly process from monomers could be directly visualised in the electron microscope. With the help of nano-electrospray it is possible to synthesise large, transportable bio-membranes that contain intact membrane proteins. These membranes allow the study of bio-membranes of defined composition using electron- and light microscopy. In addition the membranes can be incorporated into technical devices to benefit from the unique biochemical properties of specific membrane proteins. References: M. Wilm, “Synthesis of Extended, Self-Assembled Biological Membranes containing Membrane Proteins from Gas Phase,” bioRxiv, vol. 23, pp. 661215, 2019M. Wilm, “Evidence for Membrane Complex Assembly in Nanoelectrospray Generated Lipid Bilayers,” bioRxiv, vol. 42, pp. 661231, 2019

54-POS Board 14

QUANTUM MECHANICS, UNIVERSAL SCALING AND FERROELECTRIC HYSTERESIS REGIMES IN THE GIANT SQUID AXON PROPAGATING ACTION POTENTIAL: A PHASE SPACE APPROACH

Nikola Jurisic[1]; Fred Cooper[2,3];
[1]Independent researcher, Los Angeles, CA, USA
[2]The Santa Fe Institute, Santa Fe, NM, USA
[3]Los Alamos National Laboratory, Theoretical Division and Center for Nonlinear Studies, Los Alamos, NM, USA

Experiments done 70 years ago on patches of the squid axon membrane describe sodium and potassium currents with parameters of limited physical meaning. The shape of the nerve impulse thus reconstructed is somewhat inaccurate, especially in the recovery region. We have analyzed a simpler experiment, the steady propagation of the fully functioning nerve impulse and have described the ionic currents in terms of known physical phenomena and constants.
Displaying currents as functions of potential in the recovery region of the nerve impulse reveals the presence of outgoing overlapping potassium and sodium currents with quasilinear segments. Similarly, the partial overlap of the incoming sodium current and the sodium polarization current is revealed in the rising edge segment. Our fits reveal two different lattices of sodium channels separated by continuous phase changes, one in the rising edge region of the impulse from its inception to its peak and the other in the recovery region. Fits of fractions of open channels using ionic time rates during the nerve impulse reveal the universal role of the dimensionless and temperature independent fine-structure constant from quantum mechanics. We find that the activation energies of ionic currents are of the same order as the rate-limiting metabolic biochemical reactions.
Our results will motivate research of the: role of the continuous phase change in the inception of the nerve impulse; role of sodium channel lattices in creation and storage of memories and their pharmacological implications; role of the fine-structure constant in ions traversing ionic channels.

Wednesday, June 8
POSTER SESSION III
4:00 PM – 6:00 PM
Ballroom

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. Presenting authors with odd-numbered poster boards should present from 4:00 PM – 5:00 PM and those with even-numbered poster boards should present from 5:00 PM – 6:00 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

dd-Numbered Boards 4:00 PM – 5:00 PM | Even-Numbered Boards 5:00 PM – 6:00 PM

Barrera, Francisco	**3-POS**	**Board 1**
Beaven, Andrew	**7-POS**	**Board 2**
Baughman, Garrett	**11-POS**	**Board 3**
Edelmaier, Christopher	**15-POS**	**Board 4**
Hilton, Jacob	**19-POS**	**Board 5**
Huang, Yun	**23-POS**	**Board 6**
Liang, Ruibin	**27-POS**	**Board 7**
Makelaar, Isabella	**31-POS**	**Board 8**
Park, Yein	**35-POS**	**Board 9**
Russell, Charles	**39-POS**	**Board 10**
Sharma, Preet	**43-POS**	**Board 11**
Van Horn, Wade	**47-POS**	**Board 12**
Woods, Hope	**51-POS**	**Board 13**
Kaplan, Joshua	**55-POS**	**Board 14**

Posters should be set up on the morning of Wednesday, June 8, and removed by 11:00 PM on Wednesday, June 8. All uncollected posters will be discarded.

3-POS Board 1

PIP2 PROMOTES SELECTIVE DIMERIZATION OF THE MEMBRANE REGION OF THE EPHA2 RECEPTOR

Katherine M Stefanski[1]; Westerfield M Justin[1]; Charles M Russell [1]; Rajan Lamichhane[1]; **Francisco N. Barrera**[1];
[1]University of Tennessee, Knoxville, TN, USA

The influence of the EphA2 receptor on cancer malignancy hinges on the two different ways it can be activated. EphA2 induces anti-oncogenic signaling after ligand binding. However, alternate activation of EphA2 by kinases in the absence of ligand generates a metastatic phenotype. The transmembrane (TM) domain of EphA2 adopts two alternate conformations in the ligand-dependent and the ligand-independent states. However, it is poorly understood how the different TM helical crossing angles that characterize the two states impact the activity and regulation of EphA2. We devised a method that uses bilayer hydrophobic matching to stabilize these two conformations for a sequence comprising the TM domain and a portion of the intracellular juxtamembrane (JM) segment. The two conformations have different TM crossing angles, as expected for the ligand-dependent and ligand-independent states of EphA2. We devised a single-molecule approach that uses SMALP native nanodiscs to measure dimerization in membranes. Using this method we observed that the signaling lipid PIP_2 (phosphatidylinositol 4,5-bisphosphate) promotes TM dimerization, but only in the conformation with a small crossing angle, which we propose corresponds to the ligand-independent state. In this conformation the two TM are almost parallel, and the positively charged JM segments are close to each other and cause electrostatic repulsion that reduces dimerization. We propose that that PIP_2 uses its high density of negative charges to alleviate JM repulsion, promoting EphA2 dimerization. The single-molecule results indicate a conformational coupling between the TM and JM regions of EphA2. Our work additionally suggests that PIP_2 directly exerts a regulatory effect on EphA2 activation in cells that is specific to the pro-oncogenic, ligand-independent state of the receptor. These data suggest that modulation of the lipid make-up around the EphA2 receptor could achieve an anti-tumor effect.

7-POS Board 2

CHOLESTEROL DISTRIBUTIONS AND INTERACTIONS IN EARLY FUSION PORES

Andrew H Beaven[1,2]; Kayla C Sapp[1]; Jefferson D Knight[3]; Alexander J Sodt[1];
[1]NIH, NICHD, Bethesda, MD, USA
[2]NIH, NIGMS, Bethesda, MD, USA
[3]University of Colorado Denver, Chemistry, Denver, CO, USA

We have used all-atom molecular dynamics (MD) simulations to study cholesterol's preferred distributions in early lipid-only fusion pores. These MD simulations demonstrate that cholesterol is excluded from early fusion pores that have strong negative curvature (i.e., lipids bend toward their head groups). Previous experimental and computational data have demonstrated that cholesterol has a strong negative curvature preference, so why is cholesterol excluded from this region? Guided by complementary evidence, we propose that cholesterol is excluded from the pore for two interrelated reasons. (1) Gaussian (saddle) curvature thins the pore's bilayer interior (i.e., the region between the two leaflets' neutral surfaces) and cholesterol prefers thicker bilayers. (2) Cholesterol has a strong Gaussian modulus that is incompatible with this pore's saddle curvature. By adding saddle curvature considerations into a theoretical model, we produce predicted lipid distributions in good agreement with simulation. Recent work from the Francis lab at Univ. South Dakota has demonstrated that sterols lower the energetic barriers for clathrin-mediated endocytosis (Anderson et al. Cell Reports. 2021.). Therefore, cholesterol redistribution at early steps of fusion could shift a pore's geometry and be a critical part of endocytosis. We also detail an imperative fusion protein, synaptotagmin 7 (Syt7). We demonstrate that Syt7's tandem domains (C2A and C2B) induce drastically different membrane curvature. We then show that these domains sequester anionic lipids (e.g., PIP_2 and POPS) and cholesterol. We use geometric and physical modeling to show why cholesterol is sequestered. We posit that these sequestrations will affect the local lipid populations, thereby influencing fusion pore opening/closure.

11-POS Board 3

DOES PROTEIN MEMBRANE OSCILLATIONS FOLLOW A NON-POLYNOMIAL TYPE OSCILLATOR POTENTIAL?

Preet Sharma; **Garrett Baughman**;
[1]Midwestern State University, Chemistry & Physics, Wichita Falls, TX, USA

The molecular dynamics and interactions of protein is very interesting and has a great level of complexity to it. There are numerous results which show that proteins oscillate. This oscillatory behavior of proteins gives deep insights into the functioning and properties of proteins. In this study we are applying a non-polynomial type potential which can be a fundamental mechanism in understanding as to how the proteins oscillate. We have solved the Schrodinger's equation for this non-polynomial type harmonic oscillator.

15-POS Board 4

SIMULATIONS OF MICRON-SCALE CURVATURE SENSING

Christopher Edelmaier[1]; Wenzheng Shi[1]; Brandy Curtis[2]; Amy Gladfelter[2,3]; Ehssan Nazockdast[1];
[1]UNC Chapel Hill, Applied Physics, Chapel Hill, NC, USA
[2]UNC Chapel Hill, Biology, Chapel Hill, NC, USA
[3]Marine Biological Laboratory, Woods Hole, MA, USA

Cells use their cytoskeletal elements to perform many functions, including sensing and adapting to their environment. One such class of proteins are septins, which can sense and communicate details about micron-scale curvature based on their binding to cellular membranes. Amphipathic helix (AH) domains are thought to be responsible for the ability of these nanoscale proteins to probe micron-scale curvatures by sensing local curvature effects. Previous studies have investigated similar domains in other contexts (other AH-domains and BAR-domains), however, much of the understanding relies on kinetic models that effectively capture the phenomenology at the micron-scale of this interaction but cannot give a description of the underlying mechanical interactions that lead to these kinetic descriptions. One barrier to understanding these mechanical effects from simulations is the difference in time scale(s) between atomistic, coarse-grained, and continuum simulations when compared to in vivo or in vitro septin binding rates. Here, we present preliminary research into how AH-domains might interact with lipid bilayer membranes at both the atomistic and coarse-grained level to understand how these domains associate with membranes, change local membrane properties, and result in the correct binding-rate and curvature specificity seen in cells.

19-POS Board 5

TONIC INHIBITION OF THE CHLORIDE/PROTON ANTIPORTER CLC-7 BY PI(3,5)P2 IS CRUCIAL FOR LYSOSOMAL PH MAINTENANCE

Jacob K Hilton[1]; Xavier Leray[1]; Joseph A Mindell[1];
[1]National Institutes of Health, National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA

The acidic luminal pH of lysosomes, maintained within a narrow range, is essential for proper degrative function of the organelle and is generated by the action of a V-type H^+ ATPase, but other pathways for ion movement are required to dissipate the voltage generated by this process. ClC-7, a Cl^-/H^+ antiporter responsible for lysosomal Cl^- permeability, is a candidate to contribute to the acidification process as part of this "counterion pathway". The signaling lipid PI(3,5)P2 modulates lysosomal dynamics, including regulating lysosomal ion channels, raising the possibility that it could contribute to lysosomal pH regulation. Here we demonstrate that depleting PI(3,5)P2 by inhibiting the PIKfyve kinase causes lysosomal hyperacidification, primarily via an effect on ClC-7. We further show that PI(3,5)P2 directly inhibits ClC-7 transport and that this inhibition is eliminated in a disease-causing gain-of-function ClC-7 mutation. These observations suggest an intimate role for ClC-7 in lysosomal pH regulation.

23-POS Board 6

AN ENVIRONMENTALLY ULTRASENSITIVE MONOFLUOROETHYL NMR PROBE FOR MONITORING MULTI-CONFORMATIONAL EQUILIBRIA IN MEMBRANE PROTEIN

Yun Huang[1,2]; Krishna Reddy[3]; Clay Bracken[3]; Wenhu Zhang[4]; David Eliezer[3]; Olga Boudker[1,2];
[1]Weill Cornell Medicine, Department of Physiology & Biophysics, New York, NY, USA
[2]Howard Hughes Medical Institute, Chevy Chase, MD, USA
[3]Weill Cornell Medicine, Department of Biochemistry, New York, NY, USA
[4]Weill Cornell Medicine, Department of Microbiology & Immunology, New York, NY, USA

Membrane proteins often undergo multiple conformational transitions to mediate solute transport or signal transduction. 19F NMR is an important tool for detecting and monitoring conformational transitions in membrane proteins. However, current 19F labels have a narrow chemical shift dispersion, limiting their ability to differentiate multiple conformations. Here, we report a new 19F NMR label which shows ultra-high sensitivity to conformational changes and achieves chemical shift dispersion reaching 9 ppm. Using the new probe, we show that at least 6 states comprise the conformational ensemble of a glutamate transporter homolog, GltPh. Guided by the 19F NMR spectra, we have used cryo-EM to determine the structures of these conformations and propose an ensemble description of the functional states of the transporter. This work demonstrated that combining the new 19F NMR and cryo-EM is powerful in studying the dynamic mechanisms of membrane proteins.

27-POS Board 7

MULTISCALE SIMULATION OF THE LIGHT-ACTIVATION MECHANISM OF CHANNELRHODOPSIN 2

Ruibin Liang[1]; Jimmy K Yu[2]; Jan Meisner[4]; Fang Liu[3]; Todd J Martinez[2];
[1]Texas Tech University, Chemistry and Biochemistry, Lubbock, TX, USA
[2]Stanford University, Chemistry, Stanford, CA, USA
[3]Emory University, Chemistry, Atlanta, GA, USA
[4]Heinrich-Heine University Düsseldorf, Düsseldorf, Germany

The channelrhodopsin 2 (ChR2) is a light-gated ion channel and a widely used tool in optogenetics. The photoisomerization of the retinal protonated Schiff base (RPSB) in ChR2 triggers the channel opening and firing of neuronal signals. Despite the importance of the ChR2, its light activation mechanism is still not fully understood in atomistic detail. To this end, in this work we combine quantum dynamics, classical dynamics, electronic structure, and free energy calculations to comprehensively characterize the light activation mechanism of ChR2. Nonadiabatic dynamics simulations of both the wild type (WT) ChR2 and its E123T mutant were carried out using the ab initio multiple spawning (AIMS) method in a QM/MM setting, where spin-restricted ensemble-referenced Kohn-Sham (REKS) method is used to describe the QM region. Our simulations agree well with the experiments and highlight the interplay between the photochemical reaction and the surrounding protein environment: (1) the E123T mutation changes the protein's electrostatic environment around the RPSB, and significantly slows down its photoisomerization; (2) the photoisomerization facilitates its subsequent deprotonation and the hydration of the ion channel. This work presents the first simulation of the photodynamics of ChR2 with a correlated first-principles electronic structure method and provides design principles for new optogenetic tools.

31-POS Board 8

ELECTROMAGNETIC FIELD EFFECTS ON MEMBRANE PROTEIN OSCILLATIONS AND AGGREGATION BY NON-EQUILIBRIUM ENTROPY CALCULATIONS

Isabella Makelaar[1]; Leslie Cook[1]; Preet Sharma[1];
[1]Midwestern State University, Chemistry and Physics, Wichita Falls, TX, USA

The dynamics of membrane proteins involve oscillations, interactions with the environment, conformational changes, and aggregation to name a few important ones. The behavior of membrane proteins is affected by external factors or the protein-protein/protein-membrane interactions which may affect the dynamics at a more fundamental level. There are studies that suggest protein oscillations attribute to the aggregation mechanism or vice versa. This could have an affect on the properties of the individual amino acids in the peptide, the protein as a whole, or the membrane attached to the protein that influence protein misfolding. In this study we begin by applying an external independent electric field, an independent magnetic field, and then an electromagnetic field to study the oscillations that occur in membrane proteins. We know that the fields mentioned can make changes to oscillations, so we aim to apply them to the aggregation process as a possible way to disrupt toxic protein accumulation associated with neurodegenerative diseases. We explain this connection with non-equilibrium calculations of the entropy of membrane protein aggregation by its self and compare it with the application of an independent electric field, an independent magnetic field, and an electromagnetic field. Since oscillations are linked to aggregation, the application of one or more of these fields could affect the aggregation process by slowing it down, delaying the response to disease formation, or inhibit membrane protein aggregation altogether.

35-POS Board 9

STATE-DEPENDENT MORPHOLOGICAL DEFORMATIONS OF THE LIPID BILAYER EXPLAIN MECHANOSENSITIVE GATING OF MSCS CHANNEL

Yein Park[1]; Eduardo Perozo[2]; Jose Faraldo-Gomez[1];
[1]NIH, Bethesda, MD, USA
[2]University of Chicago, Chicago, IL, USA

Mechanosensitive ion channels are paradigmatic examples of the interdependence between membrane protein structures and the morphology and physical state of the lipid bilayer. Here, we investigate the molecular basis of this interplay by studying the MscS channel from Escherichia coli as well as one of its eukaryotic homologs, namely MSL1 from Arabidopsis thaliana. First, we use single-particle cryo-EM to determine the structure of a seemingly open state of wild-type MscS in a lipid nanodisc. Unlike existing models of this state, this structure does not result from mutation of the channel or detergent solubilization, but it is induced through a modest degree of membrane thinning. Based on this new structure, and on that of a closed state reported recently, we examine how the morphology of the lipid bilayer is altered upon channel gating, using molecular dynamics simulations. The simulations reveal that closed-state MscS causes drastic deformations in the lipid bilayer, which develop to provide adequate solvation to the features of the protein surface, but clearly reflect a high energy state in terms of membrane shape. Strikingly, these deformations are almost entirely eradicated in the open conformation of the channel. An analogous comparison for open and closed states of MSL1, based on existing experimental structures, corroborates these findings. Taken together, these observations strongly suggest that the gating mechanism of MscS and its homologs is dictated by opposing conformational preferences, namely those of the lipid membrane and of the protein structure, and that this coupling ultimately explains why this class of channels are mechanosensitive. Specifically, we theorize that any condition that increases the energetic cost of the membrane deformations required to stabilize the closed state will shift the gating equilibrium towards the conductive form, as this state perturbs the membrane minimally; possible stimuli with such an effect include membrane tension and membrane thinning.

39-POS Board 10

THE CANDIDA ALBICANS VIRULENCE FACTOR CANDIDALYSIN POLYMERIZES IN SOLUTION TO FORM MEMBRANE PORES AND DAMAGE EPITHELIAL CELLS

Charles Russell[1]; Katherine Schaeffer[2]; Jordan Pyron[1]; Amber Gray[3]; Thanh Do[3]; Francisco N Barrera[1]; Gavin King[2];
[1]University of Tennessee, Biochemistry & Cellular and Molecular Biology, Knoxville, TN, USA
[2]University of Missouri, Physics and Astronomy, Columbia, MO, USA
[3]University of Tennessee, Chemistry, Knoxville, TN, USA

Pathogenic Candida albicans is responsible for infections resulting in high mortality to vulnerable populations. Recently, a novel virulent peptide named Candidalysin (CL) was found to be required for infection. It was proposed that CL promotes infection by permeabilizing cell membranes. However, the specific mechanism by which this occurs is unknown. Atomic force microscopy (AFM) imaging of lipid bilayers treated with CL revealed various pore populations, suggesting that membrane disruption occurs through pore formation. We observed the existence of two types of pores that seem to exist as structural intermediates to efficiently confer cellular toxicity. In the absence of a bilayer, however, CL readily oligomerizes into long chains that form closed-loop structures. Biophysical techniques such as mass photometry, analytical ultracentrifugation, ion-mobility mass spectrometry, and fluorescent dye-release assays suggest that oligomerization in buffer is a prerequisite for pore formation. We tested this by conducting scanning mutagenesis on CL to identify residues necessary for self-assembly and membrane disruption. The effects of these gain and loss-of-function variants in vitro are recapitulated in epithelial cells. Our approach led us to propose a novel mechanism of pore formation that deviates from the mechanisms of other pore forming proteins. CL octamers oligomerize into long chains that eventually close into loops. CL loops insert into the membrane and undergo a conformational change to form a toxic pore structure. Characterizing this model of pore formation has provided insight into unique protein-membrane interactions that will inform innovative avenues to treat C. albicans infections.

43-POS Board 11

ENTROPIC STUDY OF THE EFFECT OF MEMBRANE FLUCTUATIONS IN PROTEIN OSCILLATION AND AGGREGATION

Preet Sharma;
[1]Midwestern State University, Department of Chemistry & Physics, Wichita Falls, TX, USA

Entropy is a fundamental property of any process, be it physical, chemical or biological. The study of entropy can reveal deeper understanding of systems which may not be possible using other methods. In this study we have used fluctuations in the membrane of proteins as they oscillate and aggregate. Since protein aggregation and even oscillation is considered to be a non-equilibrium process, we have used the dynamics of non-equilibrium physics to explain the entropy of a protein aggregation and oscillation. Fluctuations in the membrane of proteins contain vital information of how proteins behave. The information of physical dynamics such as protein oscillations and aggregation depend on the fluctuations in the membrane. The membranes can be of various structural identity. We have assumed a smooth protein membrane and applied fluctuations which are spontaneous in nature and might affect or even give rise to protein oscillations and other dynamics which happen in proteins. The fluctuations we have applied are mechanical in description and the proteins are model independent.

47-POS Board 12

DECONSTRUCTING THE POLYMODAL FUNCTION OF THE TRPV1 HEAT-SENSOR FROM BIOPHYSICAL STUDIES

Wade D. Van Horn; Dustin D Luu[1]; Mubark D Mebrat[1]; Aerial M Owens[1]; Karan H Shah[1]; Yu-Tzu Chang[1];
[1]Arizona State University, School of Molecular Sciences, Tempe, AZ, USA

The ability to sense temperature is crucial. The TRPV1 ion channel is the canonical heat sensor in eukaryotes, where it acts as a molecular thermometer. Beyond temperature-sensing, TRPV1 is also activated by protons (low pH), and chemical ligands, like capsaicin the pungent compound from chili peppers. Heat, protons, and ligands gate TRPV1. This polymodal regulation by diverse stimuli provides a platform to probe fundamental aspects in membrane proteins, including transmembrane allostery and cooperativity. Building on variable temperature NMR and whole-cell patch-clamp electrophysiology experiments, we have identified the minimal domains and regions required to detect heat, ligands, and protons. Our biophysical results indicate that temperature-sensing is isolated to the transmembrane domain and for the first time quantifies contributions from distinct regions. Additionally, our studies identify allosteric networks that form the molecular basis for cross-talk between activation mechanisms, including that these allosteric networks remain intact in isolated transmembrane domains. In an effort to validate these outcomes, we've adapted a recently developed method where recombinantly expressed and purified membrane proteins can be delivered to mammalian cells for functional characterization. Patch-clamp electrophysiology characterization of a bacterially purified biophysical construct validates that a minimal TRPV1 domain is sufficient to recapitulate polymodal functional behavior that was identified from NMR and other biophysical studies.

51-POS Board 13

GENERALIZING MEMBRANE GEOMETRIES FOR IMPLICIT MODELING OF MEMBRANE PROTEIN STRUCTURES

Hope Woods[1]; Julia Koehler[2]; Jens Meiler[1,3,4];
[1]Vanderbilt University, Chemical and Physical Biology Program, Nashville, TN, USA
[2]Flatiron Institute, Simons Foundation, Computational Biology, New York, NY, USA
[3]Vanderbilt University, Chemistry Department, Nashville, TN, USA
[4]Leipzig University, Institute for Drug Discovery , Leipzig, Germany

Interactions between membrane proteins (MPs) and lipid bilayers are critical for many cellular functions and each can influence one another. Some MPs are shown to change the membrane they are in, either by changing thickness, curvature, lipid composition or recruiting specific lipids to their location. In other cases, different membrane environments can affect MP function or conformational stability. Implicit membrane energy functions are generally based on a "slab" model, which represent the membrane as a flat surface. Curvature of membrane environments can impact stability and structure of MPs. For classic structure determination techniques, MPs must be reconstituted in membrane mimetics, which can also impact stability and structure. Therefore, it is important computational methods to model MPs be able to simulate membrane systems with different geometries. We have modified existing membrane energy potentials within the Rosetta MP Framework to allow users to model MPs in different membrane geometries. These geometries include ellipsoids that can mimic the shape of micelles, bicelles, and nanodiscs and vesicles with varying degrees of curvature. We show that these modifications can be utilized in core applications within Rosetta, such as structure refinement and protein design.

55-POS Board 14

APICAL LOCALIZATION OF THE SOUR RECEPTOR OTOP1 IN TASTE CELLS

Joshua P Kaplan[1,2]; Anne Tran[1,2]; Paul Cohen[3]; Emily R Liman[1,2];
[1]University of Southern California , Section of Neurobiology, Department of Biological Sciences, Los Angeles, CA, USA
[2]University of Southern California, Program in Neuroscience , Los Angeles, CA, USA
[3]The Rockefeller University, Laboratory of Molecular Metabolism, New York, NY, USA

Sour taste is evoked by acid stimuli that signal through a proton-selective ion channel Otopetrin-1 (OTOP1). Within taste buds, OTOP1 expression is mostly restricted to Type III taste receptor cells that mediate sour taste. The subcellular localization of OTOP1 within those cells is not known. In particular, it is not known if OTOP1 is on the apical surface, like the sweet and bitter receptors, where it would be accessible to compounds that could modulate its activity or if it is on the basolateral surface, like the taste transduction channel TRPM5, hidden from such compounds. Previous work from our lab using a commercially available antibody did not detect membrane localization of OTOP1 in taste cells or when expressed in HEK-293 cells, in contradiction of electrophysiological evidence for OTOP1 expression on the cell membrane in these cell types. This suggests that the antibody detected only protein in the secretory pathway, where the epitope was exposed. To resolve this discrepancy and determine the subcellular localization of the correctly folded protein, we took advantage of the CRISPR-Cas9 system to engineer a mouse line in which a 2x-HA epitope tag was introduced at the N terminus of OTOP1. Confocal microscopy showed strong HA-immunoreactivity that was highly enriched at the apical surface of taste buds in microvilli. Interestingly, HA-OTOP1 did not appear to be restricted to the microvilli of taste cells but was also detected in a sub-apical zone. Localization of OTOP1 in the apical region of taste cells is consistent with its function as a sour receptor and suggests that it may be accessible to taste-modifying compounds.

Thursday, June 9
POSTER SESSION IV
4:00 PM – 6:00 PM
Ballroom

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. Presenting authors with odd-numbered poster boards should present from 4:00 PM – 5:00 PM and those with even-numbered poster boards should present from 5:00 PM – 6:00 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 4:00 PM – 5:00 PM | Even-Numbered Boards 5:00 PM – 6:00 PM

Barreto, Yan	**4-POS**	**Board 1**
Campbell-Kruger, Nicholas	**8-POS**	**Board 2**
Curtis , Brandy	**12-POS**	**Board 3**
Fan, Zixing	**16-POS**	**Board 4**
Hoffman, Mikaila	**20-POS**	**Board 5**
Moller, Elissa	**32-POS**	**Board 8**
Rawle, Robert	**36-POS**	**Board 9**
Samanta, Rituparna	**40-POS**	**Board 10**
Siegel, David	**44-POS**	**Board 11**
Wang, Hongyin	**48-POS**	**Board 12**
Wu, Qianyi	**52-POS**	**Board 13**

Posters should be set up on the morning of Thursday, June 9, and removed by 11:00 PM on Thursday, June 9. All uncollected posters will be discarded.

4-POS Board 1

RANDOM-WALK MODEL OF COTRANSPORT

Yan B. Barreto[1]; Adriano M Alencar[1];
[1]University of São Paulo (USP), Institute of Physics, São Paulo, Brazil

Cotransporters are transmembrane carrier proteins that facilitate the movement of nutrients, ions, drugs and waste across the cell membrane. In this work, we present a statistical mechanical model for the dynamics of these carrier proteins. Our starting point was the alternating-access model, which suggests the existence of six states for the cotransport cycle. Then we determined the transition probability matrix between these states and used it to write a set of master equations for describing the time evolution of the system. The asymptotic behavior of this set of equations is in agreement with the equilibrium condition obtained by other authors from thermodynamics. Next, the predictions of our model were compared with the experimental data for the lactose-H^+ symport catalyzed by LacY. In addition to satisfactorily describing the time course of lactose transport, we paved the way to solve an open question related to this symporter. We discovered that the importance of protonation is in the fact that the H^+ binding causes a large free-energy drop, reducing the rate of reverse protonation, and allowing relatively slow lactose binding to occur. In future works, our model can be used to study the mechanosensitivity of cotransporters, which is a poorly studied problem on the interface between physics and biology with possible applications to heart disease therapies.

8-POS Board 2

THE LIPOPROTEIN LPRG TRANSPORTS AN UNKNOWN LIPID THAT IS REQUIRED FOR MYCOMEMBRANE IMPERMEABILITY IN THE PATHOGENIC MYCOBACTERIUM M. ABSCESSUS

Nicholas Campbell-Kruger[1]; Sarah Stanley[1,2];
[1]University of California Berkeley, Molecular and Cell Biology, Berkeley, CA, USA
[2]University of California Berkeley, School of Public Health, Berkeley, CA, USA

Mycobacterium abscessus and other mycobacterial pathogens are characterized by the presence of an atypical outer membrane called the mycomembrane. The mycomembrane represents a formidable permeability barrier, giving M. abscessus high innate resistance to many classes of antibiotics. Historically, the impermeability of the mycomembrane has been attributed primarily to the α-branched, β-hydroxylated extremely long-chain fatty acids called mycolic acids that make up the inner leaflet and part of the outer leaflet of the mycomembrane. However, recent research has highlighted a variety of non-mycolic acid lipid species that contribute significantly to the impermeability of the mycomembrane. One such lipid species is the substrate of LprG, a lipoprotein, and its operonic partner, a Major Facilitator Superfamily transporter, both of which are widely conserved in mycobacteria. In M. tuberculosis and M. smegmatis, LprG has been shown to bind to and transport two components of the mycomembrane – triacylglycerides (TAGs) and phosphatidylinositol dimannosides (PIMs). However, the M. abscessus LprG aligns poorly with both the M. tuberculosis and the M. smegmatis LprG and structural models predict a significantly smaller binding pocket, suggesting that LprG transports different substrates in this species. To test the contribution of LprG to pathogenesis, antibiotic resistance, and lipid transport in M. abscessus we generated a mutant lacking the LprG-MFS operon. We found that the LprG-MFS deletion mutant is impaired in pathogenesis in a macrophage infection model and shows increased susceptibility to a wide range of antibiotics as assayed through minimum inhibitory concentration. However, we found through lipid extraction and thin layer chromatography that TAGs are more accessible to solvent extraction in the mutant, suggesting that LprG transports an as yet unidentified substrate to the mycomembrane in M. abscessus, or that the direction of LprG transport is retrograde rather than anterograde.

12-POS Board 3

AN INVESTIGATION OF HOW LIPID PACKING IMPACTS SEPTIN ASSOCIATION AND ASSEMBLY

Brandy Curtis[1,2]; Christopher Edelmaier[3]; Amy Gladfelter[1];
[1]University of North Carolina-Chapel Hill, Biology, Chapel Hill, NC, USA
[2]University of North Carolina-Chapel Hill, Biochemistry and Biophysics, Chapel Hill, NC, USA
[3]University of North Carolina-Chapel Hill, Applied Physical Science, Chapel Hill, NC, USA

Throughout the lifecycle of most cells, they must be able to sense, generate, and react to changes in their shape. Cell shape can be described in terms of membrane curvature which may span nanometer to micrometer length scales. While nanometer-scale curvature sensors are well-documented in processes such as in endocytosis and cell trafficking, there are few known sensors of micrometer-scale curvature. Septins are filament-forming proteins necessary for cell processes involving major changes to cell shape, such as cytokinesis and polarized growth, and are intrinsically sensitive to micrometer-scale membrane curvature. Interestingly, they possess an amphipathic helix (AH) that is necessary and sufficient for septin curvature sensitivity but is structurally similar to AH domains specific to nanometer-scale curvature. How do these small helical domains give rise to micrometer curvature sensitivity? Previous work has shown that changes to lipid composition that alter lipid packing may mimic membrane curvature, and this may be especially relevant for septins which are capable of localizing to many different geometries, both in vitro and in vivo. This work focuses on revealing the impact of lipid packing on septin association and assembly by changing the physicochemical properties of the membrane that may help septins “sense” curvature, such as the identity of the head group and the degree of lipid packing. Using a combination of molecular dynamics simulations and the membrane tension dye, FLIPT-R, we have assembled lipid compositions that span from loosely packed to tightly packed. We measure septin association rates and observe septin assembly over time on these different compositions using in vitro reconstitution assays on supported lipid bilayers to assess how modulating membrane properties may alter septin assembly independently of curvature. As lipid packing becomes tighter, septin association and assembly rates decrease, signifying both membrane geometry and lipid packing may be regulating spatio-specific localization of septins.

16-POS Board 4

COMPARING THE DYNAMICS OF THE BAM COMPLEX BETWEEN TWO SPECIES

Zixing Fan[1]; Zijian Zhang[2]; Evan Billings[3]; Nicholas Noinaj[3]; James C Gumbart[2,4];
[1]Georgia Institute of Technology, Bioengineering, Atlanta, GA, USA
[2]Georgia Institute of Technology, Physics, Atlanta, GA, USA
[3]Purdue University, Department of Biological Science, West Lafette, IN, USA
[4]Georgia Institute of Technology, School of Chemistry and Biochemistry, Atlanta, GA, USA

Outer membrane proteins (OMPs) in Gram-negative bacteria are transmembrane β-barrel proteins that are involved in important biological activities such as nutrient transport, signal transduction and the export of virulence factors. While it's known that the protein complex responsible for assembly and insertion of OMPs into the outer membrane is the β-barrel assembly machinery (BAM), the detailed mechanisms for OMP insertion by BAM are only recently coming into focus. Nonetheless, to date, the only complete high-resolution structures of BAM are from Escherichia coli, leaving open the question of the degree of conservation of mechanisms across species. To shed light on these issues, we have carried out microsecond-scale molecular dynamics simulations of a newly resolved structure of the BAM complex from Neisseria gonorrheae to identify key interactions between BAM components relevant for the insertion process. The structure of BAM from N. gonorrheae, solved by cryo-electron microscopy, shares many similarities with that from E. coli, although the former species lacks the accessory protein, BamB, which may play a stabilizing role in the E. coli BAM complex according to our simulations. Finally, we compare the results of simulations of both BAM complexes to identify general mechanisms across different species, and the results will be discussed.

20-POS Board 5

CONCERTED DIFFERENTIAL CHANGES OF HELICAL DYNAMICS AND PACKING INDUCED BY LIGAND OCCUPANCY IN A BACTERIAL CHEMORECEPTOR

Jesse B Gordon[1]; **Mikaila C Hoffman**[1]; Julianne M Troiano[1]; Mingshan Li[2]; Gerald L Hazelbauer[2]; Gabriela S Schlau-Cohen[1];
[1]Massachusetts Institute of Technology, Chemistry, Cambridge, MA, USA
[2]University of Missouri, Biochemistry, Columbia, MO, USA

Bacterial infections can be initiated by detection of and motion towards favorable chemical environments in a process known as chemotaxis. Transmembrane chemoreceptors initiate chemotaxis upon ligand binding and generate conformational signals that regulate a histidine kinase. Ligand-induced signaling through the periplasmic and transmembrane domains of the receptor involves a piston-like helical displacement, but the nature of this signaling through the four-helix coiled coil of the cytoplasmic domain had not yet been identified. We performed single-molecule Förster Resonance Energy Transfer measurements on Escherichia coli aspartate receptor homodimers inserted into Nanodiscs. Upon ligand binding, changes in conformation and dynamics of the four-helix cytoplasmic coiled-coil domain were observed, and these ligand-induced effects were asymmetric across helical pairs. We suggest this reflects a conformational change in which the differential alterations to the packing and dynamics of the helices are coupled. These coupled changes could represent a previously unappreciated mode of conformational signaling that may well occur in other coiled-coil signaling proteins.

32-POS Board 8

ELECTROSTATICS OF THE MSCS CYTOPLASMIC CAGE DOMAIN OPTIMIZES FAST ELECTRONEUTRAL OSMOLYTE RELEASE FROM BACTERIAL CELLS

Elissa Moller[1,3]; Madolyn Britt[1,3]; Praveen Kumar[2]; Maureen A Kane[2]; Andriy Anishkin[1]; Jeffery B Klauda[4]; Sergei Sukharev[1,5];
[1]University of Maryland, Department of Biology, College Park, MD, USA
[2]University of Maryland, School of Pharmacy, Baltimore, MD, USA
[3]University of Maryland, Biophysics Graduate Program, College Park, MD, USA
[4]University of Maryland, Department of Chemical and Biomolecular Engineering, College Park, MD, USA
[5]University of Maryland, Institute for Physical Science and Technology, College Park, MD, USA

Free-living microorganisms have little control of their environment and are often subjected to changes in osmolarity. Therefore, bacteria have evolved robust osmoadaptation, evading lysis by efficiently ejecting metabolites through mechanosensitive channels from the MscL and MscS families. The larger MscL is non-selective, but the smaller MscS is slightly anionic ($2K^+$: $3Cl^-$). Substitution of chloride for acetate, a more prevalent intracellular anion, renders MscS non-selective, implying that it is designed to pass carboxylic substances together with counterions. We reason that these channels should select for dispensable intracellular osmolytes to offset the metabolic cost of release. Further, a drastic ionic preference would generate a Donnan potential, which would preclude further transport and be detrimental to efflux. Electrostatic analysis of MscS structures prompted investigation of the positive 'trim' around the windows in the cytoplasmic cage domain as the basis of selectivity. Charge reversing and neutralizing mutations were introduced at residues R156, K161, R184, R185, R224 and R238 and substitution for glutamines at all positions (6xQ) produced a decisive change in selectivity ($3K^+$: $1Cl^-$). 6xQ still expresses comparably to WT and has similar unitary conductance; however, it provides no rescuing function in osmotic viability assays. Additionally, the mutant shows a slightly higher activation midpoint, faster kinetics and prolonged inactivation indicating an allosteric effect of the selectivity filter on gating. Molecular dynamics simulations corroborated the experimental data and provided further insight into ion flux and channel permeability. Stopped-flow light scattering experiments of 6xQ show slower osmolyte release rates and a higher fraction of permeable osmolytes. Based on metabolomic analysis of shock fluids, 6xQ mediates decreased release of carboxylic compounds and increased release of basic amino acids compared to WT. Clearly, the cytoplasmic domain of MscS acts as a release filter, with electrostatics finely tuned to the repertoire of intracellular osmolytes allowing for electroneutral transport.

36-POS Board 9

BIOPHYSICAL INVESTIGATION OF THE MEMBRANE BINDING AND FUSION MECHANISMS OF SENDAI VIRUS WITH MODEL LIPID MEMBRANES

Amy Lam[1]; Abraham Park[1]; **Robert J Rawle**[1];
[1]Williams College, Chemistry, Williamstown, MA, USA

For all membrane-enveloped viruses, the first steps in infection are 1) binding of the virus to a receptor in the host cell membrane, and 2) virus-catalyzed fusion between the viral and host membranes. Both of these steps are mediated by viral glycoproteins, associated with or embedded in the viral membrane, but the role of the host cell membrane(s) involved in these steps are also important determinants of whether or not productive infection will occur. Here, we discuss mechanistic investigations into membrane binding and fusion of Sendai virus (SeV), the prototypical paramyxovirus, focusing on the influence of the target membrane and membrane receptor. To do this, we ask biophysical questions by observing interactions between individual SeV particles and model lipid membranes by fluorescence microscopy. We investigate the influence of the chemical structure of the ganglioside receptor on SeV membrane binding, and present support for a cooperative binding mechanism. We study the influence of cholesterol-mediated nano-cluster receptor formation on viral binding, and perform a comparative analysis with influenza A virus, which indicates that viral size may modulate the role that receptor nano-clusters can play in the viral binding step. We also present preliminary data on single virus fusion measurements of SeV, and discuss the biophysical implications of those results.

40-POS Board 10

TOWARDS AN IMPLICIT MODEL TO CAPTURE ELECTROSTATIC FEATURES OF MEMBRANE ENVIRONMENT

Rituparna Samanta[1]; Jeffrey J Gray[1,2];
[1]Johns Hopkins University, Chemical and Biomolecular Engineering, Baltimore, MD, USA
[2]Johns Hopkins University, Program in Molecular Biophysics, Baltimore, MD, USA

Membrane proteins are challenging to predict structure or design due to lipid layers. Implicit models accelerate this complex biomolecular problem by representing the solvent as a continuous medium. However, such models often do not consider the effect of pH, lipid head group, or dielectric constant of membrane environment. In this work, we are developing an implicit approach that captures the crucial electrostatic interactions due to the membrane, such as the effect of lipid head groups the influence of pH and dielectric variations inside the membrane layer. Our energy function franklin2022 is built upon franklin2019, an existing energy function based on experimentally derived hydrophobicity scales that could capture the anisotropic structure, the shape of water-filled pores, and nanoscale dimensions of membranes with different lipid compositions.Our method uses a constant-pH algorithm to sample the protonated and de-protonated states of protein residues. Further, it captures the effect of the lipid head group using a mean field-based approach and uses a depth-dependent dielectric constant to characterize the membrane environment. Relative to franklin2019, this model improved the calculation of ddG_{pH} of low pH insertion peptides (pHLIP) in extracellular acid environments, important biomarkers of cancer cells. We will further benchmark the performance of franklin2021 on predicting the stability, structure, and ability to design membrane proteins. The speed of implicit models will help access biophysical phenomena at different time and length scales to accelerate the design pipeline for membrane proteins.

44-POS Board 11

WHY WE SHOULD STUDY PROTEIN BINDING TO BICONTINUOUS INVERTED CUBIC PHASES, TO BETTER UNDERSTAND MEMBRANE TRAFFICKING.

David P Siegel[1];
[1]Givaudan Inc., 1199 Edison Drive, Cincinnati, OH, USA

The membrane binding constants of proteins are sensitive to membrane curvature. This is true of some adaptors/effectors in the vesicle budding and fusion stages of membrane trafficking. Recently, others showed that some proteins bind preferentially to membranes with Gaussian curvature: they bind much more strongly to spherical vesicles (with positive Gaussian curvature) than to tubules with the same mean curvature but zero local Gaussian curvature. Fusion and fission pores in budding & fusion have special curvature properties. Here I show that the Gaussian curvature, range of mean curvature, and mean curvature gradient on the surfaces of such pores are different than in the membrane systems conventionally used to assess protein curvature sensing and induction (vesicles and the insides of tubules pulled from GUVs or cells). This is true for both symmetric membranes and asymmetric biomembranes. However, the curvature properties of bicontinuous inverted cubic (Q_{II}) phases are quite similar to those of pores. Q_{II} phases and pores both have negative Gaussian curvature, true of neither vesicles nor tubules; have more negative mean curvature than many tubules; and gradients in mean curvature that are either absent or smaller in vesicles and tubules. It's been shown that proteins with molecular weights of several tens of kDa can enter the water channel networks in Q_{II} phases, and that the phases can be equilibrated with peptides through the aqueous phase. This suggests that we might find new adaptors/effectors of trafficking by studying binding of peptides to Q_{II} phases, compared to flat membranes of similar composition. Some features of Q_{II} phases make such experiments awkward. Only certain ranges of lipid compositions form Q_{II} phases, and, in phospholipids, temperature-cycling techniques or particular electrolyte compositions may be needed. Appropriate protocols will be proposed.

48-POS Board 12

COUPLING BETWEEN PROTEIN CONDENSATES AND ORDERED MEMBRANE DOMAINS FACILITATES T CELL ACTIVATION

Hongyin Wang[1]; Kandice R Levental[1]; Simli Dey[1]; Ivan Castello-Serrano[1]; Jonathon A Ditlev[2]; Michael K Rosen[3]; Ilya Levental[1];
[1]University of Virginia, Molecular Physiology and Biological Physics, Charlottesville, VA, USA
[2]University of Toronto, Department of Biochemistry, Toronto, ON, Canada
[3]University of Texas Southwestern Medical Center, Department of Biophysics, Dallas, TX, USA

Liquid-liquid phase separation of proteins have been broadly observed in many cellular machineries, but the biological roles of many of these protein condensates remains ambiguous. Condensates of the membrane protein LAT with Grb2+Sos1 have been implicated in T cell activation, possibly by excluding inhibitory phosphatase CD45 through electrostatic repulsion and/or increasing the dwell time of effector protein Sos1 near the plasma membrane (PM). Intriguingly, LAT has been widely shown to reside in ordered PM domains known as lipid rafts. Here, we provide evidence that LAT condensates can induce and stabilize raft domains in the PM, and in turn that raft domains can nucleate and stabilize cytoplasmic protein condensates. Coupling between condensates and ordered domains were studied in both lipid model membranes and live T cells. First, reconstituted LAT condensates induced phase separation in biomimetic giant unilamellar vesicle membranes, with liquid ordered (Lo) domains being recruited to condensates. Lo domain recruitment by condensates were also observed in supported lipid bilayers. Importantly, phase separated membranes could concentrate LAT molecules in Lo regions, with this effect facilitating condensate formation. Concordantly, in activated Jurkat T cells, LAT condensates induced microscopic cholesterol-rich raft domains, evidenced by enrichment of raft markers, including endogenous GPI-anchored protein CD90, and exclusion of non-raft constructs, including transmembrane domain of CD45. Moreover, stabilizing raft domains by clustering the GPI-anchored protein CD90 not only potentiated the formation of LAT condensates, but also increased their size, reinforcing the effect of condensates for cell activation. The coupling of raft domains with the condensates also explains the long-lingering mysterious observation that crosslinking raft components can activate T cells. Altogether, we conclude that protein condensates are thermodynamically and mechanistically coupled to ordered membrane domains to regulate the functional organization underlying immune cell signal transduction.

52-POS Board 13

ATAXIA-LINKED SLC1A3 MUTATIONS ALTER EAAT1 CHLORIDE CHANNEL ACTIVITY AND GLIAL REGULATION OF CNS FUNCTION

Qianyi Wu[1]; Azman Akhter[2]; Shashank Pant[3]; Eunjoo Cho[4]; Jin Xin Zhu[4]; Alastair R Garner [5]; Tomoko Ohyama[5]; Emad Tajkhorshid[3]; Donald J van Meyel [2,4,5]; Renae M Ryan[1];
[1]University of Sydney, School of Medical Sciences, Faculty of Medicine and Health, New South Wales, Australia
[2]McGill University, Department of Neurology and Neurosurgery and Integrated Program in Neuroscience, Montreal, QC, Canada
[3]University of Illinois at Urbana-Champaign, Urbana, IL, USA
[4]Research Institute of the McGill University Health Centre, Montreal, QC, Canada
[5]McGill University, Department of Biology, Montreal, QC, Canada

Glutamate is the predominant excitatory neurotransmitter in the mammalian central nervous system (CNS). Excitatory Amino Acid Transporters (EAATs) regulate extracellular glutamate by transporting it into cells, mostly glia, to terminate neurotransmission and to avoid neurotoxicity. EAATs are also chloride (Cl-) channels, but the physiological role of Cl- conductance through EAATs is poorly understood. Mutations of human EAAT1 (hEAAT1) have been identified in patients with episodic ataxia type 6 (EA6). One mutation showed increased Cl- channel activity and decreased glutamate transport, but the relative contributions of each function of hEAAT1 to mechanisms underlying the pathology of EA6 remain unclear. Here we investigated the effects of five additional EA6-related mutations on hEAAT1 function in Xenopus laevis oocytes, and on CNS function in a Drosophila melanogaster model of locomotor behaviour. Our results indicate that mutations with decreased hEAAT1 Cl- channel activity and functional glutamate transport can also contribute to the pathology of EA6, highlighting the importance of Cl- homeostasis in glial cells for proper CNS function. We also identified a novel mechanism involving an ectopic sodium (Na+) leak conductance in glial cells, due to protein-lipid interactions between the EA6-related mutant and lipids in the membrane bilayer. Together, these results reveal how these mutant transporters contributes to the pathology of EA6 and strongly support the idea that EA6 is primarily an ion channelopathy of CNS glia.