Biophysical Society Conferences

Program & Abstracts

# Molecular Biophysics of Membranes

Tahoe, California | Granlibakken | June 2–7, 2024


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# Organizing Committee

Linda Columbus, University of Virginia, USA

Syma Khalid, University of Oxford, United Kingdom

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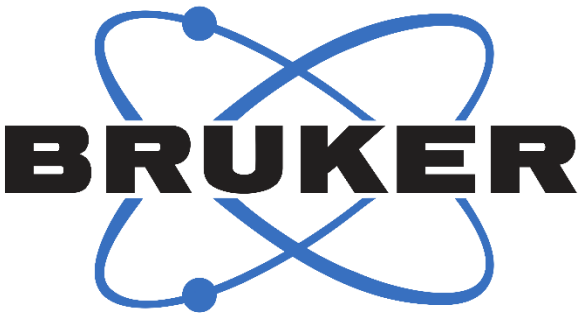


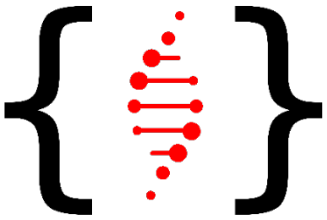

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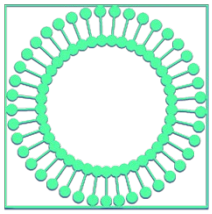

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June 2024

Dear Colleagues,

We welcome you to the Biophysical Society Conference on *Molecular Biophysics of Membranes*. This conference series is an opportunity for researchers from around the world to gather and exchange ideas within a vibrant scientific environment. 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 pursuit of our shared goals of establishing molecular understanding of membranes and membrane proteins. We have assembled an exciting program, with talks and posters on various aspects of membranes biophysics that aim to uncover the details of membrane organization, function, and their role in biological processes. The program features 47 talks and 41 posters bringing together 93 attendees from different fields, countries, and career stages promising an international and multidisciplinary environment that is as supportive as it is inspirational.

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,
Linda Columbus and Syma Khalid

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.

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# *GENERAL INFORMATION*

## *Registration/Information Location and Hours*

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

A BPS Information Desk to pick up meeting materials will be located at the Ballroom Pre-Function area at the following times:

| | |
|---|---|
| Sunday, June 2 | 4:00 PM - 6:00 PM |
| Monday, June 3 | 8:30 AM - 1:00 PM |
| Tuesday, June 4 | 8:30 AM - 1:00 PM |
| Wednesday, June 5 | 8:30 AM - 1:00 PM |
| Thursday, June 6 | 8:30 AM - 1:00 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. Monday and Tuesday posters will be available for viewing during the Monday and Tuesday poster sessions. Wednesday and Thursday posters will be available for viewing during the Wednesday and Thursday poster sessions. 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*

Breakfast, Lunch, and Dinner will be served at the Granhall. Coffee Breaks will be held at the Ballroom Pre-Function area.

## *Smoking*

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

## *Name Badges*

Name badges will be given to you when you arrive at the check-in desk to receive your room keys. 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 2-7 during registration hours.

In case of emergency, you may contact the following:

Dorothy Chaconas
Phone : 301-785-0802
Email: dchaconas@biophysics.org

Adam Vincent
Phone: 530-581-7316
Email: adamvincent@granlibakken.com

# Molecular Biophysics of Membranes

Tahoe, California, USA
June 2-7, 2024

# PROGRAM

## *Sunday, June 2, 2024*

| | | |
|---|---|---|
| 4:00 PM - 6:00 PM | **Registration/Information** | **Ballroom Pre-Function** |
| 6:00 PM - 7:00 PM | **Dinner** | **Ballroom** |
| 7:00 PM - 7:10 PM | Linda Columbus, University of Virginia, USA<br>Syma Khalid, University of Oxford, United Kingdom<br>**Opening Remarks** | |
| 7:10 PM - 8:10 PM | Barbara Baird, Cornell University, USA<br>***Keynote Address***<br>***How Does the Plasma Membrane Participate in Stimulated Cell Signaling?*** | |
| 8:15 PM - 9:45 PM | **Mixer** | |

## *Monday, June 3, 2024*

| | | |
|---|---|---|
| 7:00 AM - 8:30 AM | **Breakfast** | **Granhall** |
| 8:30 AM - 1:00 PM | **Registration/Information** | **Ballroom Pre-Function** |
| **Session I** | **Membrane Interactions and Interfaces**<br>Syma Khalid, University of Oxford, United Kingdom, Chair | |
| 9:00 AM - 9:30 AM | Alison Rodger, Macquarie University, Australia<br>***Unravelling Molecular Complexity: Small Steps with Polarised Light to Try to See*** | |
| 9:35 AM – 9:50 AM | Douglas Eaton, Emory University Medical School, USA *<br>***Electrostatic Interactions of ENaC, MLP-1, and PIP2*** | |
| 9:55 AM - 10:10 AM | Timothy Carpenter, Lawrence Livermore National Laboratory, USA *<br>***An Anisotropic Continuum Model that Captures Molecular-Level Protein-Membrane Interactions*** | |
| 10:15 AM - 10:35 AM | **Coffee Break and Group Photo** | **Ballroom Pre-Function** |
| 10:35 AM - 11:05 AM | Peter Tieleman, University of Calgary, Canada<br>***Lipid-Protein Interactions and Possible Roles in Membrane Structure*** | |
| 11:10 AM - 11:40 AM | Brian Fuglestad, Virginia Commonwealth University, USA<br>***Enhanced Tools and Strategies for Exploration of Structure, Function, and Inhibition at Protein-Membrane Interfaces*** | |
| 11:45 AM - 12:10 PM | Flash Talks I | |
| 12:10 PM - 1:00 PM | **Lunch** | **Granhall/Garden Deck** |

| | | |
|---|---|---|
| 1:00 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** | **Bacterial Membranes and Membrane Proteins**<br>Shalini Low-Nam, Purdue University, USA, Chair | |
| 7:00 PM – 7:30 PM | Markus Weingarth, Utrecht University, The Netherlands<br>***The Mechanisms of Lipid-Targeting Antibiotics*** | |
| 7:35 PM - 8:05 PM | Syma Khalid, University of Oxford, United Kingdom<br>***Computational Microbiology of the E. coli Outer Membrane: A New Picture Is Emerging*** | |
| 8:10 PM – 8:40 PM | George Ongwae, University of Virginia, USA<br>***Measurement of Accumulation of Molecules in Diderm Bacteria, and in Phagocytosed S. aureus Cells in Macrophages*** | |
| 8:45 PM – 9:15 PM | Fillipo Mancia, Columbia University Medical Center, USA<br>***Structural Basis of Lipopolysaccharide Biosynthesis and Modification*** | |
| 9:20 PM – 9:35 PM | Timothée Rivel, Masaryk University, Czech Republic *<br>***Simulating Polymyxin-Induced Divalent Ions Displacement in the Outer Membrane of Gram-Negative Bacteria*** | |

## *Tuesday, June 4, 2024*

| | | |
|---|---|---|
| 7:00 AM - 8:30 AM | **Breakfast** | **Granhall** |
| 8:30 AM - 1:00 PM | **Registration/Information** | **Ballroom Pre-Function** |
| **Session III** | **Viral and Phage Membranes and Membrane Proteins**<br>Alison Rodger, Macquarie University, Australia | |
| 9:00 AM - 9:30 AM | Mei Hong, MIT, USA<br>***Structure and Dynamics of Membrane-Bound Virus Ion Channels from Solid-State NMR*** | |
| 9:35 AM – 9:50 AM | Jinwoo Lee, University of Maryland, USA *<br>***Exploring the Vital Role of the Anionic Lipid in Initiating SARS-CoV-2 Fusion*** | |
| 9:55 AM - 10:10 AM | Elka Georgieva, Texas Tech University, USA *<br>***Viral Protein-Lipid Interactions Illustrated by the Influenza a M2 and Hepatitis C Virus Core Proteins*** | |
| 10:15 AM - 10:30 AM | Susan Fetics, Duke Human Vaccine Institute, USA *<br>***Membrane Technologies for Structural Determination of Virus Entry and Transmission*** | |
| 10:35 AM - 11:05 AM | **Coffee Break** | **Ballroom Pre-Function** |

| | | |
|---|---|---|
| 11:05 AM - 11:35 AM | Bil Clemons, California Institute of Technology, USA<br>***Mechanisms of Phage Derived Antibiotics*** | |
| 11:40 AM – 11:55 AM | Huong Kratochvil, University of North Carolina at Chapel Hill, USA *<br>***Distilling Complex Proton Channels into Simple Model Systems through Protein Design*** | |
| 12:00 PM - 1:00 PM | **Lunch** | **Granhall/Garden Deck** |
| 1:00 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 Structure and Properties**<br>Sarah Rouse, Imperial College London, United Kingdom, Chair | |
| 7:00 PM – 7:30 PM | Maria Makarova, University of Birmingham, United Kingdom<br>***Lipid Resilience: Unveiling Membrane Rescue in Oxygen-Deprived Environments*** | |
| 7:35 PM - 8:05 PM | Linda Columbus, University of Virginia, USA<br>***Structure, Organization, Packing, and Dynamics of Bicelles*** | |
| 8:10 PM – 8:25 PM | Jeriann Beiter, University of Chicago, USA *<br>***Beyond Bar Domains: Understanding Membrane Remodeling through Molecular Simulation*** | |
| 8:30 PM – 8:45 PM | Stefanie Schmieder, Boston Children's Hospital, Harvard Medical School, USA *<br>***Synthesis of a GM1 Structural Library Reveals Distinct Membrane Behavior Based on Ceramide Structure*** | |
| 8:50 PM – 9:05 PM | Shefin Sam George, Stanford University, USA *<br>***TMC Proteins Regulate Cochlear Hair Bundle Membrane Viscosity through Lipid Scramblase Activity*** | |
| 9:10 PM – 9:25 PM | Mark Arcario, Washington University in Saint Louis, USA *<br>***Flexibility of Larger Nanodiscs Allows for More Native-Like Physical Properties of Incorporated Lipids*** | |

## ***Wednesday, June 5, 2024***

| | | |
|---|---|---|
| 7:00 AM - 8:30 AM | **Breakfast** | **Granhall** |
| 8:30 AM - 1:00 PM | **Registration/Information** | **Ballroom Pre-Function** |
| **Session V** | **Membrane Protein Folding**<br>Itay Budin, University of California, San Diego, USA, Chair | |
| 9:00 AM - 9:30 AM | Heedeok Hong, Michigan State University, USA<br>***Membrane Protein Folding-What Lipids Do*** | |
| 9:35 AM - 10:05 AM | Nir Fluman, Weizmann Institute of Science, Israel<br>***Membrane Protein Sequence Features that Optimize Their Insertion and Folding*** | |

| | | |
|---|---|---|
| 10:10 AM - 10:25 AM | Katherine Clowes, Vanderbilt University, USA *<br>***A High-Throughput Screen to Identify Modifiers of KCNQ1 Trafficking*** | |
| 10:30 AM - 10:45 AM | Libin Ye, University of South Florida, USA *<br>***$^{19}$F-qNMR-Assisted Structural Elucidation of a Ligand-Free GPCR-G Protein Intermediate Complex*** | |
| 10:50 AM – 11:10 AM | **Coffee Break** | **Ballroom Pre-Function** |
| 11:10 AM - 11:25 AM | Hao Yu, Huazhong University of Science and Technology, China *<br>***Molecular Determinants of Membrane Protein Folding and Assembly Revealed by AFM-Based Force Spectroscopy*** | |
| 11:30 AM – 11:45 AM | Kelly Risch, Texas A&M University, USA *<br>***The Role of Conformational Entropy in Integral Membrane Protein Biophysics*** | |
| 11:50 AM – 12:15 PM | Flash Talks II | |
| 12:15 PM - 1:00 PM | **Lunch** | **Granhall/Garden Deck** |
| 1:00 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 Structure and Dynamics**<br>Sarah Shelby, University of Tennessee, Knoxville, USA, Chair | |
| 7:00 PM - 7:30 PM | Janice Robertson, Washington University in St. Louis, USA<br>***A Molecular Model for Ion Channel and Transporter Dimerization in Membranes*** | |
| 7:35 PM - 8:05 PM | Sarah Rouse, Imperial College London, United Kingdom - **CCPBioSim Sponsored Speaker**<br>***Modulation of Class B1 GPCRs by the Plasma Membrane Environment*** | |
| 8:10 PM – 8:40 PM | Alemayehu Gorfe, University of Texas Medical School Houston, USA<br>***Membrane Interactions and Inhibition of RAS Proteins*** | |
| 8:45 PM - 9:00 PM | Jane Allison, University of Auckland, New Zealand *<br>***PI3Ka Membrane Binding is Associated with Altered Membrane Properties*** | |
| 9:05 PM – 9:20 PM | Caroline Brown, Yale University, USA *<br>***A High-Throughput Proteome-Wide Platform for Capturing Membrane Proteins in Their Native Environment for Structural and Functional Studies*** | |
| 9:25 PM – 9:40 PM | Timothée Chauviré, Cornell University, USA *<br>***An Approach to Study Flavoproteins by in Cell Electron Spin Resonance (ESR) Spectroscopy: The Membrane Protein Aerotaxis Transducer AER*** | |

## *Thursday, June 6, 2024*

| | | |
|---|---|---|
| 7:00 AM - 8:30 AM | **Breakfast** | **Granhall** |
| 8:30 AM - 1:00 PM | **Registration/Information** | **Ballroom Pre-Function** |

| | | |
|---|---|---|
| **Session VII** | **Membrane Organization and Signaling**<br>Heedeok Hong, Michigan State University, USA, Chair | |
| 9:00 AM - 9:30 AM | Shalini Low-Nam, Purdue University, USA<br>***Shaping of CAR T Cell Activation by the Membrane Reaction Landscape*** | |
| 9:35 AM – 9:50 AM | Katherine Stefanski, Vanderbilt University, USA *<br>***Small-Molecule Modulators of Protein Lipid Raft Affinity and Lipid Raft Stability*** | |
| 9:55 AM - 10:10 AM | Nirmalya Bag, Indian Institute of Technology Kharagpur, India *<br>***Boxcar Imaging Fluorescence Correlation Spectroscopy Reveals Kinetics of Raft Stabilization in Antigen-Activated Mast Cells*** | |
| 10:15 AM - 10:30 AM | Silas Boye Nissen, Stanford University, USA *<br>***The Molecular Mechanism of the Core Planar Cell Polarity Complex Elucidated with Single-Molecule Imaging Techniques in Live Drosophila Wing Cells*** | |
| 10:35 AM – 10:55 AM | **Coffee Break** | **Ballroom Pre-Function** |
| 10:55 AM - 11:25 AM | Wade Zeno, University of Southern California, USA<br>***Functional Disorder at Biological Membranes*** | |
| 11:30 AM - 12:00 PM | Sarah Shelby, University of Tennessee, Knoxville, USA<br>***Immune Receptor Signaling Domains Arise from a Responsive Plasma Membrane*** | |
| 12:05 PM - 1:00 PM | **Lunch** | **Granhall/Garden Deck** |
| 1:00 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** | **Cholesterol, Lipids, and Phases**<br>Wade Zeno, University of Southern California, USA, Chair | |
| 7:00 PM - 7:30 PM | Itay Budin, University of California, San Diego, USA<br>***The Biophysical Tight Rope of Cell Membranes: Surprising Lessons Learned from Extreme Lipidome Adaptation in the Deep Ocean*** | |
| 7:35 PM - 8:05 PM | Kandice Levental, University of Virginia, USA<br>***Asymmetric Distribution of Phospholipids and Cholesterol Results in Unique Plasma Membrane Properties*** | |
| 8:10 PM - 8:40 PM | Francisco Barrera, The University of Tennessee Knoxville, USA<br>***Cholesterol Controls the Assembly and Activity of the EphA2 Receptor*** | |
| 8:45 PM – 9:00 PM | Niek van Hilten, University of California, San Francisco, USA *<br>***Systematic Computational Analysis of Lipid Scrambling by TMEM16 Family Members*** | |
| 9:05 PM – 9:20 PM | Malavika Varma, Carnegie Mellon University, USA *<br>***Expanding Coarse-Grained Model for Lipids to Investigate Lo/Ld Phase Coexistence*** | |

9:25 PM - 9:40 PM Jason Hafner, Rice University, USA *
***Steroid Ring Vibrations Elucidate Cholesterol's Influence on Lipid Membranes***

## *Friday, June 7, 2024*

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

**Short talks selected from among submitted abstracts*

# SPEAKER ABSTRACTS

## HOW DOES THE PLASMA MEMBRANE PARTICIPATE IN STIMULATED CELL SIGNALING?

**Barbara A Baird**[1]; David A Holowka[1];
[1]Cornell University, Chemistry & Chemical Biology, Ithaca, NY, USA

Cells are poised to respond to their physical environment and must distinguish specific stimuli from biological noise. Specific response mechanisms depend on collective molecular interactions that are regulated in time and space by the plasma membrane and its connections with the cytoskeleton and intracellular structures. Molecular stimuli engage their specific receptors to initiate a transmembrane signal, and the surrounding system efficiently rearranges to amplify this nanoscale interaction to microscale assemblies, yielding a cellular response that often reaches to longer length scales within the organism. A striking example of signal integration over multiple length scales is the allergic immune response. IgE receptors (FceRI) on mast cells are the gatekeepers of this response, and this system has proven to be a valuable model for investigating receptor-mediated cellular activation. My talk will describe our marathon efforts to measure biophysical properties associated with transmembrane signaling in live cells. We have combined quantitative fluorescence microscopy with other approaches to gain detailed insight into the poised, “resting state” of the plasma membrane and how signaling, initiated by an external stimulus, is regulated and targeted within this milieu.

## UNRAVELLING MOLECULAR COMPLEXITY: SMALL STEPS WITH POLARISED LIGHT TO TRY TO SEE HOW BIOMOLECULES WORK TOGETHER

**Alison Rodger**[1]; Soren Hoffman[2]; Nykola Jones[2];
[1]Australian National University, Research School of Chemistry, Canberra, Australia
[2]Aarhus University, Physics and Astronomy, Aarhus, Denmark

The world we live in is determined by the way molecules interact. However, it is often hard to measure what is happening. There are many techniques that we can use. In this talk I will focus on new ways of using spectroscopic measurements which are dependent on the nature of molecules and their environments so data can be interpreted to give us clues as to how molecules are behaving. The focus of this talk will be on how we can use circularly and linearly polarised light to select out respectively chiral (helical/asymmetric) interactions and oriented interactions between molecules. As well as describing what we can readily achieve with circular dichroism and linear dichroism for biomolecule characterisation in this talk I will outline new developments with combining fluorescence spectroscopy with circularly polarised light and linearly polarised light and how to use attenuated total reflectance spectroscopy to give (hopefully) reliable polarised infrared data. Applications will be to DNA, proteins, peptides and small molecules.

## ELECTROSTATIC INTERACTIONS OF ENAC, MLP-1, AND PIP2

**Douglas C. Eaton**[1]; Qiang Yue[1];
[1]Emory University Medical School, Renal Division, Atlanta, GA, USA

Using single-channel methods, we examined the interaction of a membrane-associated protein, MARCKS-like Protein-1 (MLP-1), and Epithelial Sodium Channels (ENaC), with the anionic lipid, phosphatidylinositol 4,5-bisphosphate (PIP2). PIP2 is necessary to open ENaC. However, there is a problem with a simple model of ENaC and PIP2 association by lateral diffusion in the membrane. Given the abundance of PIP2 and ENaC and the diffusion constant of PIP2 in the apical membrane of renal cells, the mean time for PIP2 to find an ENaC channel by random diffusion would be 6.3x102s or about once in 10 minutes. But, in cells expressing ENaC, the channel opens about every other second. We hypothesized that normal channel activity requires MLP-1 associated with the inner leaflet of the cell membrane. MLP-1's strongly positively charged effector domain sequesters PIP2 electrostatically. We also hypothesized that MLP-1 and functional ENaC channels are associated with specific membrane domains known as lipid rafts. PIP2 within the domains stabilizes MLP-1 and ENaC while increasing the Po of individual ENaC. ENaC in these domains can be destabilized by PIP2 degradation. To investigate MLP-1-ENaC-PIP2 interactions, we (1) examined the unusual electrostatic interaction of ENaC and MLP-1 with PIP2 in the membrane; (2) investigated ENaC stability by determining if ENaC is present in PIP2-rich lipid domain and determining if MLP-1 stabilizes ENaC in these lipid domains; (3) determined if cytoskeletal interactions maintain MLP-1 and ENaC in the PIP2-rich lipid domains by using STED FCS before and after latrunculin or cytochalasin E disruption of the cytoskeleton, and 4) examined the phenotype of renal principal cell-specific, MLP-1 knockout mice using single-channel measurements in isolated, split-open collecting ducts.

## AN ANISOTROPIC CONTINUUM MODEL THAT CAPTURES MOLECULAR-LEVEL PROTEIN-MEMBRANE INTERACTIONS

**Timothy S. Carpenter**[1]; Tomas Oppelstrup[1]; Liam Stanton[2]; Helgi Ingolfsson[1]; Tugba Ozturk[1]; Jeremy Tempkin[1];
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[2]San Jose State University, San Jose, CA, USA

Membrane proteins have important cellular roles and comprise the vast majority of all approved drug targets. These proteins interact strongly with lipids, and protein function can be affected by local lipid compositions. Furthermore, the interactions between proteins and their surrounding membranes can be distinctly anisotropic, with different patterns of lipid enrichments on opposite sides of the protein. These anisotropic patterns can have a large impact, for instance in driving protein-protein aggregation and facilitating specific interaction interfaces. The proper exploration of these relationships requires a bilayer that is not only large enough to contain relevant compositional fluctuations but is also simulated for enough time for local lipid compositions to equilibrate around the protein. For a single protein in a complex membrane mixture, the appropriate sampling time-scales are on the order of 100s of microseconds with systems containing >5,000 lipids. This extensive computational investment is required for each subsequent variable tested – rendering wide-ranging investigation prohibitively expensive in terms of time and computing resources. To that end, we extend our work on continuum membrane models from Stanton et al.[1] to allow for nontrivial protein structure, which results in a fully anisotropic protein-lipid potential interaction. We demonstrate how our model reproduces the given lipid density fields and compare the expressive complexity gained by using anisotropic potentials for two types of complex proteins on the cell membrane. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC5207NA27344.[1]Stanton, L. G., Oppelstrup, T., Carpenter, T. S., Ingólfsson, H. I., Surh, M. P., Lightstone, F. C., & Glosli, J. N. (2023). Dynamic density functional theory of multicomponent cellular membranes. Physical Review Research, 5(1), 013080.

## LIPID-PROTEIN INTERACTIONS AND POSSIBLE ROLES IN MEMBRANE STRUCTURE

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**No Abstract**

## ENHANCED TOOLS AND STRATEGIES FOR EXPLORATION OF STRUCTURE, FUNCTION, AND INHIBITION AT PROTEIN-MEMBRANE INTERFACES.

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Peripheral membrane proteins (PMPs) are water-soluble proteins that reversibly bind to membranes to perform their function. Despite a central role in a variety of biological and disease related processes, study of their functional membrane-bound forms have been hampered by technical limitations. Additionally, interest in targeting membrane proteins, including PMPs, for therapeutic intervention has grown recently. However, discovery tools for this class of protein is limited. To better understand the active, membrane bound state of PMPs, a larger toolbox must be developed. Our recently developed membrane-mimicking reverse micelles (mmRMs) are a valuable addition to the methodologies available to study PMPs using NMR and other biophysical methods. We have applied mmRMs to a variety of problems including structural study of a lipid chaperone, fatty acid binding protein 4 (FABP4), which has unveiled the structure of the elusive membrane-bound form of the protein and revealed a mechanism for lipid uptake. Fragment-based drug discovery of PMPs using biophysical methods is proving to be a promising path, demonstrated by successful screening of PX domain of $p47^{phox}$ with the goal of inhibiting of its membrane anchoring event. Conversely, proteins such as glutathione peroxidase 4 (GPx4), which are not amenable to inhibition through this strategy, present a greater challenge. Applying a fragment screening approach to the active, membrane-bound form of GPx4 housed in mmRMs has revealed small-molecule interactions within the protein-membrane interface, a challenging space for inhibitor development. Not only do these fragments represent starting points for inhibitor development, they also reveal fundamental properties about molecular interactions in the membrane-protein interface. The approaches presented here will enhance our understanding of PMPs in their functional, membrane bound state and provide avenues for building inhibitors for this challenging category of protein.

## THE MECHANISMS OF LIPID-TARGETING ANTIBIOTICS

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Antimicrobial resistance is a global health threat, calling for new antibiotics. Good candidates could be compounds that target special lipids that only exist in bacterial, but not in human cell membranes. These drugs kill pathogens without detectable resistance, which has generated considerable interest. Using solid-state NMR, microscopy, and natural product isolation techniques, our group has introduced approaches to study lipid-targeting antibiotics across different length-scales in biological membranes and intact cells[1]. Recently, we determined the killing mechanism of teixobactin[2,3]. We showed that teixobactin kills bacteria by forming supramolecular fibrils that compromises the bacterial membrane. In addition, we show the molecular mechanism of Clovibactin, a new antibiotic from 'unculturable' bacteria[4] References:[1] Medeiros-Silva, J., Jekhmane, S., Lucini Paioni, A., Gawarecka, K., Baldus, M., Swiezewska, E., Breukink, E., Weingarth, M. Nature Comm. (2018), 9, 3963, High-resolution NMR studies of antibiotics in cellular membranes[2] Shukla, S., Medeiros-Silva, J., Parmar, A., Vermeulen, B.J.A., Das, S., Paioni, L.A., Jekhmane, S., Lorent, J., Bonvin, A.M.J.J., Baldus, M., Lelli, M., Veldhuizen, E.J.A., Breukink, E., Singh, I., Weingarth, M. Nature Communications (2020), 11, 2848, Mode of action of teixobactins in cellular membranes[3] Shukla, R., Lavore, Sourav, M., F., Derks, G.N., Jones, C.R., Vermeulen, B.J.A., Melcrova, A., Morris, M.A., Becker, L.M., Wang, X., Kumar, R., Medeiros-Silva, J., van Beekveld, R., Bonvin, A.M.J.J., Lorent, J., Lelli, M., Nowick, J., MacGillavry, H., Peoples, A.J., Spoering, A.L., Ling, L.L., Hughes, Roos, W., D., Breukink, E., Lewis, K., Weingarth, M., Nature (2022) 608, 390, Teixobactin kills bacteria by a two-pronged attack on the cell envelope[4] Shukla, R., Peoples, A.J., Ludwig, K.C., Maity, S., Derks, M.G.N, de Benedetti, S., Krueger, A.M., Vermeulen, B.J.A., Lavore, F., Honorato, R.V., Grein, F., Bonvin, A.M.J.J., Kubitscheck, U., Breukink, E., Achorn, C., Nitti, A., Schwalen, C.J., Spoering, A.L., Ling, L.L., Hughes, D., Lelli, M., Roos, W.H., Lewis, K., Schneider, T., Weingarth, M., Cell (2023) A new antibiotic from an uncultured bacterium binds to an immutable target

## COMPUTATIONAL MICROBIOLOGY OF THE E. COLI OUTER MEMBRANE: A NEW PICTURE IS EMERGING

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The cell envelope that surrounds Gram-negative bacteria is composed of two membranes (the inner and outer) which are separated by an aqueous region known as the periplasm. Together these three regions provide the bacterium with a formidable defence against unwanted incoming molecules, including antibiotics. The outer membrane contains a range of beta-barrel proteins of varying sizes and functions and in terms of its lipidic composition, it has lipopolysaccharides (LPS) in the outer leaflet and phospholipids in the inner leaflet. We are using multiscale simulations in combination with the experimental work (including native mass spectrometry, cross-linking studies and AFM) of our colleagues to develop a picture of the spatial organisation of the outer membrane of E. coli. Our work has shown that outer membrane proteins (OMPs) are not uniformly distributed throughout the membrane but occupy high protein density regions or 'OMP islands'. These islands are separated by regions of high LPS (upper leaflet) and phospholipid (lower leaflet), in which there are hardly any proteins. We provide details of our current view of the organisation of OMP islands and briefly describe how this is being incorporated into our efforts to study antimicrobial penetration into the outer membrane and how this in turn, impacts the properties of the outer membrane Overall, our studies are now revealing a very different picture of the E. coli surface than the one presented in textbooks.

## MEASUREMENT OF ACCUMULATION OF MOLECULES IN DIDERM BACTERIA, AND IN PHAGOCYTOSED S. AUREUS CELLS IN MACROPHAGES

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A significant bottleneck to drug discovery and development is that few methods exist for measuring the permeation of molecules across cell membranes. Traditional methods, such as Minimum Inhibitory Concentration (MIC), have limitations in estimating drug accumulation independently from drug potency. Although mass spectrometry methodologies offer certain advantages, they also possess inherent limitations, including restricted throughput capacity and an inability to definitively ascertain cytosolic accumulation.[1,2] The ChloroAlkane Penetration Assay (CAPA) pioneered by the Kritzer Lab has become widely adopted as a method for measuring apparent accumulation; it involves the application of chloroalkane-tagged test molecules (pulse step) to cytosolic HaloTag-expressing mammalian cells.[3] Subsequent detection of chloroalkane-fluorophore signals (chase step) reveals the penetration levels. Despite the wide adoption of CAPA[5, 6], we recognized the potential confounding influence of the 15-atom long chloroalkane tag on penetration analysis in bacteria. In contrast, azides are known for their minimal size and relatively low disruptive impact as biorthogonal tags.[7] We have, therefore, introduced a robust assay, the CHloroakane Azide Membrane Permeability (CHAMP), for quantitative assessment of small molecule accumulation within Gram-negative bacteria that are engineered to express HaloTag protein. CHAMP employs biorthogonal epitopes anchored within HaloTag-expressing bacteria and measures permeation using azide-bearing test molecules through strain-promoted azide-alkyne cycloaddition(SPAAC).[8] In Mycobacterium tuberculosis (Mtb), the outer mycomembrane is hypothesized to be the principal determinant for access of antibiotics to their molecular targets. We developed a novel assay that anchors a strained alkyne on the peptidoglycan, which sits directly beneath the mycomembrane, followed by Click chemistry with test molecules, and a fluorescent labeling chase step, to measure the permeation of small molecules.[9] We have also developed an assay to measure the arrival of antibiotics within the phagosomes of infected macrophages by metabolically incorporating bioorthogonal reactive handles within the surface of S. aureus and adding Click chemistry complementary tags to antibiotics.[10] References(1) Sci Rep 2015, 5, 17968. (2) ACS Chem Biol 2014, 9 (11), 2535-2544. (3) J Am Chem Soc 2018, 140 (36), 11360-11369. (4) ACS Infect Dis 2023, 9 (1), 97-110. (5) Methods Mol Biol 2007, 356, 195-208. (6) J Am Chem Soc 2022, 144 (32), 14687-14697. (7) J Am Chem Soc 2004, 126 (46), 15046-15047. (8) ACS Chem Biol 2019, 14 (4), 725-734.(9) Angew Chem Int Ed Engl. 2023 62(20):e202217777. (10) Angew Chem Int Ed Engl. 2024 63(3): e202313870.

## STRUCTURAL BASIS OF LIPOPOLYSACCHARIDE BIOSYNTHESIS AND MODIFICATION

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The outer membrane of Gram-negative bacteria has an external leaflet that is largely composed of lipopolysaccharide (LPS), which provides a selective permeation barrier, and is also the target of select antibiotics such as the last resort polymyxins. We are interested in understanding at a molecular level how LPS is assembled and is then modified to drive antibiotic resistance, processes that are mediated by specific enzymes that reside in the bacterial inner membrane. By using a structure based integrated approach which brings together single-particle cryo-electron microscopy with genetics, biochemical experiments and molecular dynamics simulations we have come - and will present - our mechanistic understanding of the last step of LPS assembly by the O-antigen ligase WaaL, and on its subsequent modification by other glycosyltransferases that neutralize its charge to prevent binding of cationic molecules such as polymyxins.

## SIMULATING POLYMYXIN-INDUCED DIVALENT IONS DISPLACEMENT IN THE OUTER MEMBRANE OF GRAM-NEGATIVE BACTERIA

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Since their discovery over 75 years ago, polymyxins have undergone a remarkable journey in medicine. While they were recognized for their antimicrobial activity against Gram-negative bacteria, their nephro- and neurotoxicity led to their relegation as less toxic antibiotic classes went on the market. Later, the surge of multidrug-resistant bacterial strains brought them back as last-resort treatment. However, in the past decade, multiple resistance mechanisms against polymyxins were identified which set the clock to find alternative therapeutics. Polymyxins remain a rare category of drugs capable of permeabilizing the rigid and asymmetric lipopolysaccharide-containing outer membrane of Gram-negative bacteria without passing through protein channels. It is believed that polymyxins can affect the dense network of divalent ions that are known to bridge lipopolysaccharides in the outer leaflet together. However, it is still unclear how exactly that affects the outer membrane properties, and how important this is in polymyxins mode of action. In our work, we employed all-atom and coarse-grained molecular dynamics simulations to model the outer membrane of two resistant and one non-resistant strains of Salmonella enterica. We utilized enhanced sampling methods to investigate the local action of polymyxins on membrane-bound divalent cations. Additionally, we compared this local effect with global stress applied to the membrane, indicating that the action of polymyxins cannot be reduced to the local ions removal only. Our findings provide valuable insights into the role of ion displacement in outer membrane dynamics and its implications for polymyxins' permeabilization mechanism.

## STRUCTURE AND DYNAMICS OF MEMBRANE-BOUND VIRUS ION CHANNELS FROM SOLID-STATE NMR

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Enveloped viruses encode membrane-bound ion channels, also called viroporins, that are important for the lifecycle and pathogenicity of these viruses. Elucidating the structure, dynamics and mechanism of action of these viroporins is important for advancing fundamental knowledge about ion channels as well as for developing antiviral drugs. Solid-state NMR spectroscopy is well suited to studies of small viral ion channels bound to phospholipid bilayers that mimic the native membrane in which these proteins function. In this talk I will present my lab's latest structure determination of the SARS-CoV-2 envelope (E) protein, a cation-conducting channel that is associated with the inflammation response of the cell to SARS-CoV-2 infection. Using multidimensional solid-state NMR and 19F-based distance measurements, we have determined the membrane-bound E structures at neutral pH and at acidic pH in the presence of calcium. These two structures show important differences in the N-terminal and C-terminal polar segments of the helical bundle, which give insight into the activation mechanism of this viroporin. Hexamethylene amiloride (HMA) is a known inhibitor of the E channel. Measurement of protein-drug distances using 19F-enhanced solid-state NMR techniques shows that HMA surprisingly binds the protein-lipid interface instead of the channel pore. This binding mode differs from the well-known amantadine binding to the pore of the influenza M2 proton channel. We discuss this HMA binding result in terms of an aromatic belt in the middle of the E channel, the distinct hydrophobic character of E from influenza M2, and the implication of the HMA binding mode for future design of E-targeting antiviral drugs to treat COVID infections.

## EXPLORING THE VITAL ROLE OF THE ANIONIC LIPID IN INITIATING SARS-COV-2 FUSION

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Membrane fusion is a critical step in the viral lifecycle, enabling the delivery of genetic material into the host cell. For SARS-CoV-2, fusion relies on significant conformational changes within the S2 subunit of the spike glycoprotein. This process begins with a cleavage event at S2', releasing the fusion domain (FD), which then integrates into the target membrane, disrupting the lipid environment. Thus, the initial interaction between the FD and the lipid membrane of the target cell is crucial for viral fusion. Consequently, a comprehensive understanding of SARS-CoV-2 infectivity necessitates investigating the interactions between the FD and the lipid membrane of the target cell, examining both protein and lipid perspectives. Using a FRET-based in vitro fusion assay, we uncovered a clear and distinctive correlation between the fusogenicity of the fusion domain (FD) and the endosome-specific lipid BMP. Comparative analysis with other anionic lipids using various biophysical techniques revealed that BMP exerts a unique influence on lipid packing, which accounts for its specificity. To investigate further from a protein standpoint, we conducted mutagenesis on all positively charged amino acids, employing both alanine and charge-conserving mutants. Our findings indicate that certain amino acids possess distinct functional attributes tailored to anionic lipids, implying direct interactions with their negatively charged headgroups. In summary, the initiation of fusion by the SARS-CoV-2 FD is significantly enhanced in the presence of BMP due to its disruptive effect on lipid packing and the presence of multiple interactions between positively charged residues and the anionic lipid headgroup.

## VIRAL PROTEIN-LIPID INTERACTIONS ILLUSTRATED BY THE INFLUENZA A M2 AND HEPATITIS C VIRUS CORE PROTEINS

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We present our results on the interactions of influenza A M2 (IM2) and the hepatitis C virus (HCV) core proteins with lipid membranes. IM2 has single transmembrane (TM) helix and assembles in a homotetramer with proton channel activity. HCV core, critical for virus assembly and budding, has two domains binding RNA and lipid, respectively. We probed the assembly of the IM2 TM domain C-terminal region (TM helix and juxtamembrane residues) reconstituted into DOPC/DOPS liposomes and separated E. coli membranes containing the native lipids and proteins (i.e. protein crowding conditions). We mutated to cysteine and spin-labeled the residue L43C located at the end of the TM helix in the polar region and studied it by continuous wave (CW) ESR and double electron-electron resonance (DEER). We obtained similar results for DOPC/DOPS and E. coli membranes at pH 7.4. The CW ESR spectra showed the label in very slow-motional regime, indicating stable and tight assembly of the TM helix bundle at the lipid-to-solvent boundary. The DEER results analysis yielded the distance distributions with narrow peaks at 1.68 nm and 2.37 nm. The distance and amplitude ratios of 1.41±0.2 and 2:1 were as expected for four spin labels located at the corners of a square, indicative of an axially symmetric and rigid M2 tetramer. Furthermore, DEER was applied to samples of spin-labeled L43C IM2 in E. coli membranes, using protein-to-lipid molar ratios ranging from 1:230 to 1:10,400, to reveal that IM2 tetramer is likely to assemble via a dimer intermediate, well in line with our previous results based on different spin-labeling site. Finally, we present our data on recently produced, purified, and interacted with liposomes full-length HCV core protein. Our preliminary results from negative staining EM indicate that upon binding to liposome surface, the HCV core induces membrane deformation and possibly tubulation.

## MEMBRANE TECHNOLOGIES FOR STRUCTURAL DETERMINATION OF VIRUS ENTRY AND TRANSMISSION

**Susan K Fetics**[1]; Ariha Mehta[1]; Salam Sammour[1]; Jared Lindenberger[1]; Katarzyna Janowska[1]; Chan Soo Park[1]; Priyamvada Acharya[1];
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Membrane proteins, such as receptors, located on the plasma membrane of mammalian cells, account for approximately 25 % of the human proteome yet this category of proteins represents 50 – 70 % of drug targets. Therefore, these proteins are a major focus for the development of vaccines and other therapeutics. Entry of a virus into a host cell typically involves interaction of the viral surface protein with a receptor on the host cell membrane. To facilitate structural studies of viral proteins in native-like environments, we are developing a suite of tools utilizing the full-length HIV-1 Envelope (Env) protein from the JR-FL isolate as a model protein. We have expressed, purified and determined a cryo-EM structure at 6.7 Å resolution of micelle-solubilized full-length HIV-1 JR-FL Env in complex with VRC01, PGT145 and DH1317.4 antibodies. However, to establish a more native membrane environment, we are developing bicelles and nanodiscs, some of which are of novel compositions. For characterization of the membrane mimetic systems alone and in the presence of Env, we use biophysical and biochemical tools, including mass photometry, differential scanning fluorimetry (DSF), dynamic light scattering (DLS), surface plasmon resonance (SPR), and electron microscopy (EM). We have expressed and purified four membrane scaffolding proteins (MSPs) of different sizes. Combined with bicelles prepared in-house, we have used these MSPs to assemble nanodiscs that mimic various cell membrane systems. Preliminary results indicate successful exchange of Env from buffer containing micelles into nanodiscs. With our current workflow, we can continue to develop membrane mimetics for applications into other human viral systems such as coronaviruses, flaviviruses, human papillomaviruses, hepatitis, and oncogenic viruses.

## MECHANISMS OF PHAGE DERIVED ANTIBIOTICS

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A key step in the bacteriophage life cycle is the requirement to breach the peptidoglycan layer of the bacterial cell wall. While a variety of lysis mechanisms have evolved, the simplest are found in single stranded DNA or RNA bacteriophages that, constrained by the small size of their genomes, encode a single gene lysis (Sgl) protein. The first discovered and most studied example is Protein E from ΦX174 in the Microviridae family; a 91 amino acid peptide with a single transmembrane domain at its N-terminus. Protein E expression, dependent on the host chaperone SlyD, is sufficient for lysis of bacteria via inhibition of the phospho-MurNAc-pentapeptide translocase MraY, an essential enzyme in the biosynthesis of peptidoglycan. Despite the historic importance of ΦX174, the lysis mechanism remains poorly defined. Using single particle electron cryo-microscopy, we have demonstrated that Protein E forms a stable inhibitory complex with both E. coli MraY and SlyD by physically blocking access to the active site of MraY. The structure of this three-protein complex has additionally allowed us to derive new functional insight for both SlyD and MraY. Overall, the work provides exciting implications for the development of novel therapeutics.

## DISTILLING COMPLEX PROTON CHANNELS INTO SIMPLE MODEL SYSTEMS THROUGH PROTEIN DESIGN

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Proton channels and transporters move a precise and well-defined number of protons across cellular membranes to initiate bioenergetic and biocatalytic processes. Proton conduction pathways in these proteins are composed of hydrogen-bonded networks of water molecules and ionizable sidechains that enable the rapid movement of protons with minimal conformational rearrangement. Hydrophobic regions that are deceptively devoid of water also play a critical role in the selective transport of protons across the membrane. The short 8-12Å hydrophobic regions in proton channels and transporters prevent the translocation of other ions and allow for the formation of proton-conducive transient hydrogen-bonded water wires through these conduction pathways. In previous work, we used a combination of protein design, molecular dynamics (MD) simulations, crystallography, and functional liposomal assays to define proton conduction along these transient water wires in hydrophobic regions. Our designed channels, which incorporate polar Gln residues into hydrophobic pores, were proton-selective and able to move protons at rates seen in natural proton channels. We showed that the ability for these channels to form transient water wires is enough to enable proton-selective transport. Now, our lab is interested in the physicochemical nature of the sidechains in water wire formation and proton conduction rates. We have designed a new class of proton channels and are looking at how ionizable sidechains like His, Glu, and Asp affect the energetic barriers for water wire formation within these pores and how charge stabilization impacts conduction rates. Further, we also design new channels that increase the number of polar sidechains within the conduction pathway to address key questions about pore hydration and proton conduction rates. Our unique study enables us to distill complex natural channels into simple model systems, allowing us to pinpoint how different residues and hydration lengths affect proton channel selectivity and conductivity.

## LIPID RESILIENCE: UNVEILING MEMBRANE RESCUE IN OXYGEN-DEPRIVED ENVIRONMENTS

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Membrane biophysical properties are critical to cell fitness and depend on phospholipids with various acyl tail compositions. Membrane phospholipids with unsaturated acyl tails are particularly critical since they form liquid-disordered phases, with high lateral diffusion and high membrane flexibility. Unsaturated acyl tails contain double bonds that require oxygen for their production in eukaryotic organisms. This raises the question of how cells maintain bilayer properties in environments with limited oxygen. Here, I will show that this can be accomplished by the incorporation of novel asymmetric phospholipids containing one long and one medium-length fatty acid chain. I will show that the fission yeast, S. japonicus, which can grow in aerobic and anaerobic conditions, is capable of utilizing this strategy whereas its sister species, the well-known model organism S. pombe, cannot.

## STRUCTURE, ORGANIZATION, PACKING, AND DYNAMICS OF BICELLES

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Bicelles are mixtures of lipids and detergents that at certain molar ratios (q values) form a segregated lipid core with a detergent rim. The diversity of structure, organization, and properties of bicelles could be a powerful tool for investigating lipid – lipid and lipid protein interactions, as well as, identifying important membrane properties that stabilize membrane protein structure and function. Ten detergents were investigated with DMPC and a subset of these detergents were investigated with DLPC, DPPC, and POPC. Small angle X-ray scattering, laurdan fluorescence, NMR, and molecular dynamics reported on the organization and packing of these assemblies and determined which mixtures form segregated lipid cores and at what q values. The results of these investigations will be presented.

## BEYOND BAR DOMAINS: UNDERSTANDING MEMBRANE REMODELING THROUGH MOLECULAR SIMULATION

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Bin/Amphiphysin/Rvs (BAR) domains are one of the most closely studied peripheral proteins, and are involved in seemingly all cellular membrane remodeling events, particularly at the plasma membrane. Both in vitro and in vivo work have demonstrated the dramatic tubulation activity of BAR domains alone and in concert with other peripheral proteins, and the general dependence of this effect on the presence of negatively charged lipids such as phosphatidylserine (PS) and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2). Using atomistic and coarse-grained (CG) molecular dynamics simulation in tandem with in vitro and in vivo imaging, we investigate at the molecular level the interactions of BAR domains with other protein domains and with the underlying lipids in the membrane. To demonstrate the general principles we have learned in our work, we study two example BAR domain systems: the paradigmatic endophilin and the PX-BAR domain of sorting nexin 9 (SNX9). We find that BAR domains interact synergistically with other domains within the same protein, but the collective remodeling behavior of these proteins are not mediated by direct protein/protein interactions. Rather, local non-stoichiometric protein/lipid interactions act to generate long-range clustering, particularly through modulating the lipid diffusivity. The implications of this work extend broadly from considerations in structural biology of peripheral membrane proteins to understanding regulation of plasma membrane tension and cell motility.

## SYNTHESIS OF A GM1 STRUCTURAL LIBRARY REVEALS DISTINCT MEMBRANE BEHAVIOR BASED ON CERAMIDE STRUCTURE

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Each cell exhibits a diversity of ceramide acyl chain structures in their sphingolipids, which likely influence the sphingolipid's membrane behavior, endocytic trafficking, and intracellular distributions. How cells discern among the different ceramides remains unknown. To address mechanism, we synthesized a library of GM1 glycosphingolipids with naturally varied acyl chain structures and quantitatively assessed their membrane behavior. Using superresolution microscopy and single particle tracking we found a motif within the acyl chain, the C14* motif – a stretch of at least 14 saturated hydrocarbons extending from the C1 at the water-bilayer interface – that dictates incorporation into membrane nanodomains. This motif allows for lysosomal sorting by exclusion from endosome sorting tubules. Intracellular sorting by the C14* motif is cholesterol and nanodomain dependent. Perturbations of the C14* motif by unsaturation enables GM1 entry into endosomal sorting tubules of the recycling, retrograde and transcytotic pathways. These GM1 species cannot assemble into membrane nanodomains. Unsaturation occurring beyond the C14* motif (in very long unsaturated acyl chains) rescued lysosomal sorting, interaction with cholesterol and nanodomain incorporation. These results define a structural motif underlying the membrane organization and trafficking of sphingolipids and implicate cholesterol-sphingolipid nanodomain formation in endocytic sorting mechanisms.

## TMC PROTEINS REGULATE COCHLEAR HAIR BUNDLE MEMBRANE VISCOSITY THROUGH LIPID SCRAMBLASE ACTIVITY

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Lipid scramblases provide a permeation pathway to translocate phospholipids between membrane leaflets. Therefore, activation of a lipid scramblase results in the externalization of phosphatidylserine (PS), which is usually restricted to the inner leaflet, a hallmark of many relevant biological processes. Transmembrane-like Channel 1 protein (TMC1), which is an integral protein of the cochlear hair cell mechanotransduction (MET) channel, has been implicated to have membrane scramblase activity. However, there has been no direct measures of how the lipid scramblase could affect the cochlear membrane properties. As a first step towards understanding the functional relevance of TMCs in regulating membrane, we used a novel viscosity sensor BODIPY1c based on fluorescence lifetime for precise monitoring of membrane properties within live hair cells. BODIPY1c can also enter hair cells through MET channels and fluorescently label the cytoplasmic membranes, thus allowing identification of hair cells with functional MET channels. We also monitored the membrane scramblase activity using PS-specific binding protein Annexin V (AnV). We find that the membrane viscosity of mammalian cochlear hair bundles decreases during postnatal development and strongly correlates ($r^2$=0.75) with the onset of MET. We also see that the lipid scrambling increases during this time frame. This data suggests that MET machinery could reduce the membrane viscosity through lipid scrambling. To test this hypothesis, we tested how genetic deletion of TMC1 and TMC2 would affect the membrane. We find that both the TMC1 mutants and TMC1/TMC2 double mutants have significantly higher membrane viscosity along with absence of lipid scrambling compared to litter mate control. Together this data suggests that TMCs could be directly impacting the membrane through their putative scramblase activity. Membrane is potentially being locally regulated for the MET channel to be more sensitive or faster.

## FLEXIBILITY OF LARGER NANODISCS ALLOWS FOR MORE NATIVE-LIKE PHYSICAL PROPERTIES OF INCORPORATED LIPIDS

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In the current structural revolution brought about by cryo-electron microscopy (cryo-EM), nanodiscs have become an indispensable tool in understanding how the membrane environment affects membrane protein structure. Recent studies, however, have described subtle structural differences to cryo-EM structural models depending on the nanodisc scaffold size and type. While it is well-known that nanodiscs alter certain physical properties of lipid bilayers, it has been hypothesized that these alterations are due to the edge effect introduced by the nanodisc scaffold and that large enough nanodiscs would recover more native-like properties, given that proportionally less lipids would be perturbed by the rim. With the advent of nanodisc circularization technology, which makes nanodiscs up to 100 nm thermally stable, we have been able to test this hypothesis. Using nanodiscs circularized with the SpyCatcher-SpyTag modification we have tested the packing behavior of pure lipids and lipid mixtures in nanodiscs ranging from 11 nm in size to 50 nm in size using the environmentally-sensitive fluorophore, Laurdan. When compared to a 100 nm liposome of the same lipid composition, we have found that nanodiscs under 25 nm demonstrate a more ordered bilayer, have a higher melting temperature, and have significantly lower cooperativity in the phase transition. Importantly, however, increases in nanodisc size monotonically mitigate these perturbations to the lipid properties. At 50 nm, we are able to recover native-like membrane properties in a nanodisc, supporting a long-standing hypothesis in the field. To begin understanding why larger nanodiscs are more native-like, we analyzed coarse-grained molecular dynamics (CGMD) simulations of 15, 30, and 50 nm circularized nanodiscs. These simulations demonstrate that, indeed, proportionally more lipids demonstrate unperturbed physical properties compared to smaller nanodiscs, including lipid tilt and lipid diffusion. Overall, these results provide a deeper understanding of the properties of lipids contained in nanodiscs, which will help in interpretation of cryo-EM structures as well as the fundamental nature of protein-lipid interactions.

## MEMBRANE PROTEIN FOLDING-WHAT LIPIDS DO

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My talk addresses two questions regarding how the lipid bilayer mediates folding and function of membrane proteins: 1) Is the lipid bilayer a good solvent for the denatured states of membrane proteins? 2) What is the role of lipid solvation in the stability and cooperativity of membrane proteins? We have developed an array of methods to delineate thermodynamic stability, conformational features of the denatured states, and the residue interaction network of membrane proteins. The methods are based on the steric trapping strategy, which couples spontaneous denaturation of a doubly biotinylated protein to the simultaneous binding of bulky monovalent streptavidin. Using the intramembrane protease GlpG of E. coli as a model, we find that the bilayer 1) induces contraction but not collapse of the denatured state of GlpG, 2) enhances the stability of the protein by facilitating the residue burial in the protein interior, and 3) strengthens the residue-interaction network such that the whole residue-packed regions act as a single cooperative unit. These results shed light and cast shadows on the folding and function of membrane proteins. The enhanced stability and cooperativity indicate that the lipid bilayer is an adequate medium for stabilizing membrane proteins and transmitting local stimuli across the protein, which benefits function. On the other hand, the contraction of the denatured states and facilitation of residue burial point to the general lipophopic effect, which may increase the chance of nonspecific collapse of polypeptide chains in the crowded cell membranes. Furthermore, the enhanced cooperativity can render conformational integrity of membrane proteins vulnerable to local structural perturbations including missense mutations.

## MEMBRANE PROTEIN SEQUENCE FEATURES THAT OPTIMIZE THEIR INSERTION AND FOLDING

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The proper folding of multispanning membrane proteins (MPs) hinges on the accurate insertion of their transmembrane helices (TMs) into the membrane. Predominantly, TMs are inserted during protein translation, via a conserved mechanism centered around the Sectranslocon. Our study reveals that the C-terminal TMs (cTMs) of numerous MPs across various organisms bypass this cotranslational route, necessitating an alternative posttranslational insertion strategy. We demonstrate that evolution has refined the hydrophilicity and length of these proteins' C-terminal tails to optimize cTM insertion. Alterations in the C-tail sequence disrupt cTM insertion in both E. coli and human, leading to protein defects, loss of function, and genetic diseases. In E. coli, we identify YidC, a member of the widespread Oxa1 family, as the insertase facilitating cTMs insertion, with C-tail mutations disrupting the productive interaction of cTMs with YidC. Thus, MP sequences are fine-tuned for effective collaboration with the cellular biogenesis machinery, ensuring proper membrane protein folding.

## A HIGH-THROUGHPUT SCREEN TO IDENTIFY MODIFIERS OF KCNQ1 TRAFFICKING

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Long QT syndrome (LQTS) is a cardiac disorder that affects 1:2500 people and can cause syncope, arrhythmias, and cardiac arrest. Loss-of-function mutations in the potassium channel KCNQ1 cause type 1 long QT syndrome (LQT1), which accounts for ~50% of cases of LQTS. Over 250 LQT1-causing mutations in KCNQ1 have been identified, but it is unknown whether there is a common mechanism through which these mutations cause disease. Only ~20% of expressed WT KCNQ1 successfully traffics to the plasma membrane, and many LQT1-associated mutations in KCNQ1 have been found to destabilize the protein and decrease trafficking efficiency further. Protein mistrafficking has been identified as a mechanism of several diseases and has been found to be rescuable with small molecules. This led us to hypothesize that mistrafficking is a common mechanism of KCNQ1 loss-of-function in LQT1 and that fold-stabilizing small molecules can increase the trafficking efficiency of KCNQ1. To test this hypothesis, we developed an immunofluorescence-based high-throughput trafficking assay to identify compounds that alter the expression and/or trafficking of KCNQ1 in cells. Screening of ~23,000 compounds from five small molecule libraries has identified ~25 validated hits that alter the expression and/or trafficking of WT KCNQ1. Hits fall into three categories: those that increase cell surface expression and trafficking efficiency, those that increase expression but do not increase trafficking efficiency, and those that decrease both total and cell surface expression. Of the hits that decrease expression, several have been found to reduce the expression of "supertrafficking" mutant R231C back to WT-like levels. Ongoing experiments will determine the specificity and mechanism of action of hit compounds, as well as whether they bind and stabilize KCNQ1 directly. These studies will contribute to our larger hypothesis that misfolding-induced mistrafficking is a common, rescuable, mechanism of KCNQ1 dysfunction and inform potential routes for treatment of LQT1.

## $^{19}$F-QNMR-ASSISTED STRUCTURAL ELUCIDATION OF A LIGAND-FREE GPCR-G PROTEIN INTERMEDIATE COMPLEX

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Despite the cryo-EM revolution leading to the resolution of over 500 GPCR-Gαβγ complex structures, these snapshots primarily capture the fully activated end-state. Consequently, a comprehensive understanding of the conformational transitions during GPCR activation and the roles of intermediates in signal transduction remains elusive. Herein, creation of an intermediate-state-trapped mutant, guided by $^{19}$F quantitative NMR ($^{19}$F-qNMR) and Molecular Dynamics (MD) simulations[1], facilitated sampling of a homogeneous intermediate state in the adenosine $A_{2A}$ receptor ($A_{2A}R$). This approach yielded a high-resolution cryo-EM structure of intermediate ligand-free (apo) GPCR-Gαβγ complex, via the strategy of blocking conformational transition to the fully activated state. This advancement fills critical structural gap of intermediate complex in the course of GPCR signaling.

## MOLECULAR DETERMINANTS OF MEMBRANE PROTEIN FOLDING AND ASSEMBLY REVEALED BY AFM-BASED FORCE SPECTROSCOPY

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The essential forces stabilizing membrane proteins and governing their folding/unfolding are difficult to decipher. Single-molecule atomic force spectroscopy mechanically unfolds individual membrane proteins and quantifies their dynamics and energetics. However, it remains challenging to structurally assign unfolding intermediates precisely and to deduce dominant interactions between specific residues that facilitate either the localized stabilization of these intermediates or the global assembly of membrane proteins. Here, we performed force spectroscopy experiments and multi-scale molecular dynamics simulations to study the unfolding pathway of diacylglycerol kinase (DGK), a small trimeric multi-span transmembrane enzyme. The remarkable agreement between experiments and simulations allowed precise structural assignment and interaction analysis of unfolding intermediates, bypassing existing limitations on structural mapping, and provided mechanistic explanations to the formation of these states. Site-specific modifications allowed us to unfold DGK from both N- and C-terminus, which complements each other to reveal a complete interaction network that stabilizes the protein. We identified intermolecular side chain packing interactions as one of major contributions to the stability of unfolding intermediates. Mutagenesis creating packing defects induced dramatic decrease to the mechano-stability of corresponding intermediates and also to the thermo-stability of DGK trimer, in good agreement with predictions from simulations. Hence, the molecular determinants of the mechano- and thermo-stability of a membrane protein can be identified at residue resolution. The accurate structural assignment established and microscopic mechanism revealed in this work may substantially expand the scope of single molecule studies of membrane proteins.

## THE ROLE OF CONFORMATIONAL ENTROPY IN INTEGRAL MEMBRANE PROTEIN BIOPHYSICS

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Understanding the motions of amino acid side chains is crucial for a complete picture of the thermodynamics governing protein stability, structure, and function. Here we focus on the conformational entropy manifested in sub-nanosecond motion. Using an NMR relaxation-based approach, we have shown that changes in conformational entropy in soluble proteins can be a pivotal contribution to the thermodynamics of protein structure-function relationships, either favorably or unfavorably.[1,2] We have overcome several technical barriers that have historically hindered similar investigation of integral membrane proteins (IMPs). In the first examples, we found that photo sensory rhodopsin II and outer membrane protein W are more dynamic that any soluble protein studied in this way.[3] This degree of side chain motion corresponds to an extraordinary level of conformational entropy and helps explain the stability of the folded state in the absence of the hydrophobic effect.[4] The role of this "excess" entropy in the thermodynamics of molecular recognition by IMPs remains a mystery. We are continuing to investigate the nature of internal motion and conformational entropy of IMPs and their sensitivity to the host membrane mimetic such as micelles, bicelles and nanodiscs. Results from similar studies of human VDAC-1 in nanodiscs also show the dynamic signature of the previous study. Supported by Texas A&M University, the NIH and the Mathers Foundation.Caro et al (2017) Proc. Nat. Acad. Sci. USA 114, 6563.Wand & Sharp (2018) Annu. Rev. Biophys. 47, 4. O'Brien et al (2020) Angewandte Chemi Intl. Ed. 59, 11108Corin & Bowie, J.U. (2022) EMBO Rep. 23, e53025

## A MOLECULAR MODEL FOR ION CHANNEL AND TRANSPORTER DIMERIZATION IN MEMBRANES

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Ion channels and transporters are the molecular gatekeepers of biology, governing the passage of atoms and molecules in and out of cells. They provide essential nutrients for metabolism, eliminate waste, enable cell-to-cell communication, and store the potential energy that fuels life. Yet, despite their overwhelming importance, we lack a physical and molecular understanding of why membrane transport proteins form stable structures that enable their specific functions within the oil-filled environment of the lipid bilayer. Our lack of understanding here is well rationalized by the inherent challenge of measuring equilibrium reactions of membrane protein assembly in membranes, compounded by the complexity of the reaction solvent as a structured lipid bilayer. However, through a combination of novel experimental single-molecule microscopy approaches coupled with coarse-grained and all-atom computational modeling, we can now measure equilibrium constants of protein association in membranes and dissect out the important molecular contributions from the protein and surrounding membrane. Here, I present our results on two systems where reversible binding within membranes can be quantitatively assessed - the homodimeric CLC-ec1 chloride/proton antiporter and the dual-topology homodimeric Fluc fluoride ion channel. By combining experimental and computational studies, we identify a driving force for protein association that arises from state-dependent perturbation of the membrane structure, and a mechanism for tuning dimerization stability by altering solvation energetics through preferential solvation. These results present a generalizable driving force for membrane protein assembly in membranes that is expected to apply to all ion channels and transporters.

## MODULATION OF CLASS B1 GPCRS BY THE PLASMA MEMBRANE ENVIRONMENT

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Class B1 GPCRs are key targets for prevalent diseases, but existing peptide drugs targeting these receptors have limitations, requiring the development of novel small molecules, particularly of allosteric modulators with greater potential to fine-tune receptor outputs. GPCR-lipid binding sites represent a large, untapped opportunity for allosteric drug development, but our understanding of lipid modulation of Class B1 GPCRs remains limited. We present molecular dynamics simulations of all 15 Class B1 family members in model plasma membranes, allowing us to determine patterns of specific lipid interactions across this subfamily. We observe that GM3 plays a modulatory role in the dynamics of the extracellular domain (ECD), in a GPCR state-dependent manner. We further assess in vitro the impact of GM3 on GLP1R ECD conformational dynamics using a TR-FRET GM3 inhibitor Eliglustat assay.

## MEMBRANE INTERACTIONS AND INHIBITION OF RAS PROTEINS

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Compared to protein-based membrane targeting motifs, much less is known about the structural and biophysical basis of membrane targeting by the intrinsically disordered lipid-based membrane targeting motifs of small GTPases. In this presentation, I will discuss new insights from molecular simulations and experiments into how the flexible lipid anchor of KRAS recognizes membrane lipids in a conformational ensemble-dependent manner, and its role in the dynamics of the full-length protein on membrane surfaces. I will then briefly discuss our efforts toward the development of small-molecule non-covalent allosteric KRAS inhibitors.

## PI3KA MEMBRANE BINDING IS ASSOCIATED WITH ALTERED MEMBRANE PROPERTIES

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The class IA phosphatidyl-inositide-3'-kinase PI3Kα is frequently mutated in cancers and tumours, so understanding its function and malfunction is important for development of new therapeutics. PI3Kα is a lipid kinase that phosphorylates the inositol ring at the 3'-OH position in phosphatidyl-inositides (PIs), with the most significant reaction being the phosphorylation of $PI(4,5)P_2$ ($PIP_2$), converting it to $PI(3,4,5)P_3$ ($PIP_3$). $PIP_3$ is a secondary lipid messenger that acts as a recruitment site for effector proteins that contain a pleckstrin homology domain, which further leads to the downstream signalling of the PI3K/Akt/mTOR signalling pathway that governs crucial cellular functions such as cell growth, metabolism, motility and survival. To access the lipid substrate $PIP_2$ and carry out its catalysis, PI3Kα must bind to the plasma membrane in its activated state. While there have been several molecular dynamics (MD) studies of PI3Kα, none have included the plasma membrane. Here, we present the results of MD simulations of PI3Kα and its cellular partner HRas in the presence of a model plasma membrane. We show that the presence of membrane-anchored HRas is crucial for PI3Kα to bind to the membrane in a catalytically-competent orientation, and that regardless of the presence of HRas, PI3Kα membrane binding is associated with altered membrane properties such as thickness, curvature and $PIP_2$ spatial distribution.

## A HIGH-THROUGHPUT PROTEOME-WIDE PLATFORM FOR CAPTURING MEMBRANE PROTEINS IN THEIR NATIVE ENVIRONMENT FOR STRUCTURAL AND FUNCTIONAL STUDIES

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The native membrane's local molecular environment significantly shapes membrane protein (MP) biology. However, the prevalent method, use of detergent-like molecules for MP study, removes this crucial local context, hindering our quantitative understanding of how the local bilayer governs MP structure, function, and biogenesis. Addressing this, we generated a library of membrane active polymers (MAPs) that enable nanoscale spatially-resolved extraction of MPs, directly from endogenous membranes, into native nanodiscs while maintaining the local nanoscale membrane environment. We then developed a label-free quantitative proteomics workflow to assess protein-specific extraction efficiency directly into native MAPdiscs. Leveraging this, we created a proteome-wide quantitative screening platform, reporting extraction efficiency of individual MAPs from our library in a protein-specific manner. Applying this platform to mammalian cells, using 15 different MAPs, we built a quantitative guide that reports the most optimized extraction condition of ~ 2500 unique MPs, directly from their physiological membranes. We've packaged this data into an open-access database, facilitating gene-name-specific searches yielding optimal MAP-extraction conditions for efficient extraction of target MPs or even multi-protein complexes into native nanodiscs. Using our database, we demonstrated the applicability of this platform by extracting and purifying structurally and functionally diverse MPs, directly from various organellar membranes, such as plasma membrane, endoplasmic reticulum, mitochondria, lysosome, Golgi, and even transient organelles such as autophagosome. Together, this provides a broadly applicable, rapid, and efficient avenue to capture target MPs, within local membrane 'nano-domains', directly from endogenous organellar membranes, maintaining physiological expression levels. We envision interfacing this platform with diverse bioanalytical approaches like MS-based OMICS, single-molecule microscopy, EM-imaging, and biochemical assays generating a transformative tool for membrane biology by offering a quantitative molecular view of the local membrane context and its regulatory impact on MPs.

## AN APPROACH TO STUDY FLAVOPROTEINS BY IN CELL ELECTRON SPIN RESONANCE (ESR) SPECTROSCOPY: THE MEMBRANE PROTEIN AEROTAXIS TRANSDUCER AER.

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Most mobile bacteria move towards favorable environments by exhibiting a behavior known as chemotaxis [1]. In Escherichia coli (E. coli), the transmembrane receptor Aerotaxis (Aer) is a primary energy sensor for mobility. Aer responses are determined by the redox state of the flavin adenine dinucleotide (FAD) cofactor, which sends signals from the electron-transport chain to the chemotaxis histidine kinase CheA [2]. In its oxidized state (quinone), Aer activates CheA autophosphorylation, which causes the cell to tumble. When the cell encounters terminal electron acceptors, the FAD cofactor reduces to the neutral semiquinone state (neutral (NSQ•) or anionic semiquinone state (ASQ•-)), which deactivates the CheA autophosphorylation and enables, via a change in flagellar rotation, the "smooth swimming" of the cell. Until now, Aer has been primarily studied by electron spin resonance (ESR) in reconstituted systems by mimicking the cell structure with membranes or nanodiscs [3]. In this study, we aim to investigate Aer in whole-cell by ESR spectroscopy and obtain insight by pulse dipolar spectroscopy on the organization of Aer in chemosensory arrays.In cell ESR spectroscopy of E. coli identify the type of radical created and characterize distances (via pulse dipolar spectroscopy) between the semiquinone radical located in the FAD-containing Per-Arnt-Sim (PAS) domains of associated Aer subunits. Whole cell isotopic labeling (2H and 15N) allows effective characterization of the spin system of the FAD radical by a combination of continuous-wave ESR and advanced pulsed ESR techniques (3P-ESEEM: Electron Spin Echo Envelope Modulation, ENDOR: Electron Nuclear Double Resonance). These live-cell studies have the potential to reveal the flavin redox couples involved in signaling and structural features of the receptor key to its function.
References:[1] Taylor B.L., Zhulin I.G., Johnson M.S.; Annu. Rev. Microbiol., 53, 103–128 (1999)[2] Maschmann Z.A., Chua T.K., Chandrasekaran S., Ibáñez H., Crane B.R.; J. Biol. Chem., 298, 102598-25814 (2022)[3] Samanta D.; Widom J.; Borbat P.P.; Freed J.H.; Crane B.R.; J. Biol. Chem., 291, 25809-25814 (2016)

## SHAPING OF CAR T CELL ACTIVATION BY THE MEMBRANE REACTION LANDSCAPE

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T cells interpret physiochemical cues within the contact zone with another cell and make fate decisions that control adaptive immune responses. For example, normal T cells detect even low densities of weak agonist ligands to activate and either relay cues to recruit other immune cells or directly kill targets. Remarkably, these global outcomes can result from the integration of signals from small numbers of binding events, collected stochastically. Recent engineering efforts have generated T cells expressing chimeric antigen receptors (CARs) that consolidate key components of T cell triggering machinery into a single transmembrane receptor capable of scanning for specific tumor antigens. There has been limited success in the application of CAR T cells in cancer immunotherapy and patients often exhibit toxicity from hyperactivation of the immune response. The mechanistic determinants for threshold setting in CAR T cells are still poorly understood. Signaling onset, at the cell surface, may be tuned by spatiotemporal, topographic, mechanical, and chemical parameters. Additionally, the local composition of the membrane itself may be consequential. We map CAR T cell inputs to cellular activation and cytotoxic responses using single molecule, single cell in vitro reconstitution assays. We find that, surprisingly, CAR T cells can mobilize cytotoxic responses to a small number of antigenic binding events, suggesting a different molecular threshold than previously appreciated. Thus, rare cells that respond to low input levels may be a source of therapeutic failures. A mechanistic understanding of CAR T cell activation setpoints and how this tuning is altered during tumor evolution will create actionable insights to optimize these therapies.

## SMALL-MOLECULE MODULATORS OF PROTEIN LIPID RAFT AFFINITY AND LIPID RAFT STABILITY

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Lipid rafts remain an active area of membrane biophysics research but due to their nanoscale size and theorized short lifetime their exact cellular functions have yet to be clarified. Giant plasma membrane vesicles (GPMVs), which spontaneously separate into ordered (raft) and disordered (non-raft) phases, are a practical a tool for studying rafts and raft-resident proteins. The lack of tools to manipulate raft-affinity of proteins has stymied our ability to study the functions of lipid rafts and raft proteins in cells. One such protein, PMP22 shows a high affinity for ordered phases (lipid rafts) in GPMVs. Genetic defects in PMP22 cause Charcot-Marie-Tooth disease, the most common inherited peripheral neuropathy. Several disease-causing mutations in PMP22 exhibit decreased raft partitioning which correlates with disease severity, but the functional consequences of PMP22 raft affinity are not understood. Using a high-throughput screening pipeline, we screened 20,000+ small molecules in search of compounds that alter the raft affinity of PMP22. Hits were counter-screened against another raft partitioning protein, MAL. Here, we describe two classes of compounds discovered in this screen. First, we discovered a novel class of compounds which modulate both PMP22 raft-partitioning and raft stability. Interestingly, these compounds, which we call global modulators, are not protein specific but are protein-dependent suggesting a cooperativity between lipids and proteins that form rafts. In contrast, we have also characterized small-molecule modulators of raft stability that act independently of protein content. We also show these compounds have differential effects on subcellular trafficking and aggregation of PMP22. The global modulators of protein raft affinity and raft modulators described here deepen our understanding of raft biophysics and present a new set of tools for probing the functional importance of lipid rafts and their constituent proteins.

## BOXCAR IMAGING FLUORESCENCE CORRELATION SPECTROSCOPY REVEALS KINETICS OF RAFT STABILIZATION IN ANTIGEN-ACTIVATED MAST CELLS

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Antigen (Ag) crosslinking of immunoglobulin E (IgE) receptor, FcεRI, leads to the clustering of the receptors followed by their phosphorylation by Lyn kinase at the plasma membrane (PM). Recently, we exploited unprecedented statistical robustness of camera-based imaging fluorescence correlation spectroscopy (ImFCS) to show reorganization of lipidic interactions in the inner leaflet of the PM corresponding to stabilization of ordered regions (colloquially known as rafts) around Ag-clustered IgE- FcεRI in live RBL mast cells (Bag et al, PNAS, 2021). We have now developed a time-resolved diffusion analysis in which the raw image stack is divided in overlapped segments (boxcar), and ImFCS is performed on each segment. In this manner, we obtained diffusion coefficients of membrane components at every 35 sec from live cells. Our data reveals membrane organization, as sensed by order- and disorder-preferring lipid probes, occurring within 500 sec of Ag-stimulation corresponding to about 50 FcεRI per cluster. These results represent one of the first direct demonstrations of raft stabilization in live cells in real time. We anticipate widespread application of this method in future studies of membrane dynamics.

## THE MOLECULAR MECHANISM OF THE CORE PLANAR CELL POLARITY COMPLEX ELUCIDATED WITH SINGLE-MOLECULE IMAGING TECHNIQUES IN LIVE DROSOPHILA WING CELLS

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The planar cell polarity (PCP) signaling pathway polarizes epithelial cells along an axis parallel to the epithelial sheet to generate front-back asymmetry at the tissue level. Failures in PCP signaling underlie developmental defects and diseases such as neural tube and heart defects, deafness, and contribute to cancer. Six core PCP proteins assemble into large, asymmetric complexes at cell-cell junctions, with Van Gogh and Prickle recruited to the proximal side of the cell-cell junction while Frizzled, Diego, and Dishevelled are recruited to the distal side. Flamingo forms asymmetric inter-cellular bridges connecting polarity between adjacent cell. Neither the potential requirement for clusters, nor their detailed organization are understood. To explore these questions, we used total internal reflection fluorescence microscopy to image GFP-tagged PCP proteins in the Drosophila pupal wing disc. Using this approach, we find that the molecular size distribution of PCP complexes follows an exponential function, suggestive of a single underlying growth mechanism. Mutations that block Dishevelled oligomerization decrease average cluster sizes, and also result in PCP phenotypic defects, demonstrating a requirement for large clusters in polarization. In conclusion, our findings provide a quantitative framework for understanding how cluster formation is coupled to the induction of tissue-level asymmetry during early embryonic development.

## FUNCTIONAL DISORDER AT BIOLOGICAL MEMBRANES

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The prevailing structure-function paradigm posits that protein function only arises from specific structural features. However, our recent work has revealed that proteins lacking secondary structure – Intrinsically Disordered Proteins (IDPs) – can be potent sensors and generators of membrane curvature. The mechanisms for this functional behavior arise from the polyampholytic nature of these unfolded, amino acid chains. This characteristic imbues IDPs with unique biophysical properties when they come into proximity of biological membranes. Interestingly, IDPs comprise ~40% of the human proteome but only a handful of IDPs have been examined in the context of protein-lipid interactions. Therefore, this paradoxical finding represents a significant, yet unexplored frontier in the field of membrane remodeling. Our most recent findings highlight an example of this disordered functionality in α-Synuclein, which is a neuronal protein that interacts with synaptic vesicles and is heavily implicated with the onset of Parkinson's Disease. We explore the complex interplay between membrane composition and protein sequence in protein-lipid interactions. Specifically, we find that the C-terminal domain of α-Synuclein contributes to electrostatic repulsion with bilayer surfaces, ultimately regulating its ability to sense and generate membrane curvature. These interactions are highly sensitive to membrane composition. We also investigate the dynamics of these protein-lipid interactions as a function of membrane physiochemical properties. Our work provides fundamental mechanistic insight into the biophysical function of IDPs, which are an underappreciated class of proteins in membrane remodeling processes.

## IMMUNE RECEPTOR SIGNALING DOMAINS ARISE FROM A RESPONSIVE PLASMA MEMBRANE

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Long-standing evidence supports a role for plasma membrane heterogeneity in modulating interactions of immune receptors such as the B cell receptor with its signaling partners, suggesting that membrane domains participate in the regulation of B cell receptor activation. Our recent work uses super-resolution microscopy to directly visualize membrane domains that arise upon B cell receptor clustering on the surface of intact, live cells. This approach enables a quantitative comparison of protein partitioning in cellular domains and phase-separated domains in isolated model membrane vesicles. Our results point to membrane phase separation as a physical driver of membrane domain formation in cells, with implications for receptor signaling output. We propose a model in which membrane organization is responsive, where domains are easily induced upon assembly of receptor complexes because the thermodynamic state of the membrane is poised near a miscibility phase transition. Our ongoing work is applying these concepts to predict and manipulate signaling in engineered immunoreceptor systems.

## THE BIOPHYSICAL TIGHT ROPE OF CELL MEMBRANES: SURPRISING LESSONS LEARNED FROM EXTREME LIPIDOME ADAPTATION IN THE DEEP OCEAN

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[1]University of California, San Dieg, La Jolla, CA, USA

Lipid bilayers are the universal structure of all cell membranes and depend on the propensity of specific phospholipid classes to form fluid lamellar phases. However, no biological membranes are composed entirely of bilayer forming lipids, instead balancing species with low and high curvatures. I will describe how this dynamic plays out in organisms that have adapted to hydrostatic pressure, which increases by 1 atmosphere every 10m in the water column. Small angle x-ray scattering of lipid suspensions from deep sea comb jellies (ctenophores) collected at depths up to 4km revealed a remarkable ability to access non-lamellar lipid phases, which are inhibited by pressure. Lipidomic analysis across a wide range of species identified phospholipids with large, negative spontaneous curvature as a depth-specific adaptation across the phylum. High pressure molecular dynamics of lipidome-derived bilayers supports this adaptation. Based on these results, we propose a homeocurvature adaptation model for cell membranes, in which the effect of pressure on lipid shape can contribute to both fitness and specialization in the deep oceans. The model predicts pressure tolerances of lipid engineered bacterial cells. I will discuss implications of this model for the regulation of phospholipid composition in all cell membranes.

## ASYMMETRIC DISTRIBUTION OF PHOSPHOLIPIDS AND CHOLESTEROL RESULTS IN UNIQUE PLASMA MEMBRANE PROPERTIES

**Kandice R. Levental**[1]; Milka Doktorova[1]; Fred Heberle[2]; Ed Lyman[3]; Ilya Levental[1];
[1]University of Virginia, Molecular Physiology and Biophysics, Charlottesville, VA, USA
[2]University of Tennessee, Chemistry, Knoxville, TN, USA
[3]University of Delaware, Physics and Astronomy, Newark, DE, USA

The plasma membrane is the interface between a cell and its environment and is therefore responsible for a myriad of parallel processing tasks that must be tightly regulated to avoid aberrant signaling. To achieve this functional complexity, mammalian cells produce hundreds of lipid species, nearly all of which are asymmetrically distributed between the two membrane leaflets. Using quantitative lipidomics we determined the asymmetric distribution of all phospholipids in human erythrocyte plasma membranes. In addition to defining the asymmetric lipidomes of the two PM leaflets, we discovered that the cytoplasmic leaflet contains 50% more phospholipids than the exoplasmic leaflet. This finding invalidates the long-standing assumption that the two leaflets of a lipid bilayer should contain equal numbers of phospholipids. We show that this imbalance of phospholipids in the plasma membrane is enabled by the large abundance of cholesterol (~40%) in the plasma membrane, which can rapidly flip between leaflets to buffer stresses. Through computational and experimental approaches, we find that the combination of the preference of cholesterol for the more saturated exoplasmic lipids and the overabundance of lipids in the cytoplasmic leaflet yields a dramatic enrichment of cholesterol in the exoplasmic leaflet. Furthermore, we show that this asymmetric distribution of phospholipids and cholesterol results in unique plasma membrane properties, namely low permeability, fast cholesterol diffusion, and more cytoplasmic hydrophobic defects which enables lipidated protein interactions with the cytoplasmic leaflet. Our observations of these previously overlooked aspects of membrane asymmetry represents an evolution of existing models of plasma membrane structure and physiology.

## CHOLESTEROL CONTROLS THE ASSEMBLY AND ACTIVITY OF THE EPHA2 RECEPTOR

**Francisco N. Barrera**[1];
[1]University of Tennessee, Knoxville, TN, USA

The receptor tyrosine kinase EphA2 plays a central role during embryogenic development of the neural system. EphA2 has low expression in adult tissues, with the exception of tumors, where it promotes malignancy in different cancer types, with a salient role in metastasis. At the molecular level, EphA2 can be found in different self-assembly states: as a monomer, dimer and oligomer. However, we have a poor understanding of which specific EphA2 state is responsible for oncogenic signaling, which is characterized by phosphorylation of cytoplasmic serine residues. To address this question, we have developed SiMPull-POP, a new single-molecule method for accurate quantification of the self-assembly of membrane proteins. SiMPull-POP uses the amphipathic DIBMA co-polymer to isolate native-like nanodiscs from human cells. The oligomeric state of membrane proteins in the resulting DIBMALPs is determined by single-molecule photobleaching. We applied SiMPull-POP to GFP-tagged EphA2. Our experiments revealed that a reduction of plasma membrane cholesterol had a strong effect on EphA2 self-assembly. Indeed, low cholesterol caused a similar effect than an EphA2 ligand. These results indicate that cholesterol inhibits EphA2 assembly. Phosphorylation studies in different cells lines revealed that low cholesterol increased phospho-serine levels, the signature of oncogenic signaling. Investigation of the mechanism that cholesterol uses to inhibit the assembly and activity of EphA2 failed to reveal binding to the transmembrane domain of EphA2. Instead, our data indicate an in trans effect, where EphA2 serines are phosphorylated by protein kinase A downstream of beta-adrenergic receptor activity, which cholesterol also inhibits. Our investigation not only enriches our understanding on how EphA2 works, but it also indicates that plasma membrane cholesterol acts as a molecular safety mechanism that prevents uncontrolled self-assembly and oncogenic activation of EphA2.

## SYSTEMATIC COMPUTATIONAL ANALYSIS OF LIPID SCRAMBLING BY TMEM16 FAMILY MEMBERS

**Niek van Hilten**[1]; Yisheng Zheng[1]; Christina A Stephens[1]; Michael Grabe[1];
[1]University of California, San Francisco, San Francisco, CA, USA

Calcium-activated TMEM16 proteins play important roles in eukaryotes through their passive ion transport and lipid scrambling activity. In fact the latter has been associated with blood coagulation, viral entry, and cancer. Experimental and simulation studies have demonstrated that both mammalian and fungal TMEM16 members scramble lipids by inducing a strong membrane deformation near a hydrophilic groove that facilitates lipid translocation in a "credit-card" like mechanism. However, given the variety of identified calcium-bound conformations, individual members may scramble in different ways. Here, we employ systematic coarse-grained molecular dynamics simulations on every available TMEM16 structure to address important open questions about open/closed states, scrambling pathways, and lipid specificity. This study highlights the similarities and differences between the different family members and will help us better understand the different physiological roles of these proteins in both health and disease.

## EXPANDING COARSE-GRAINED MODEL FOR LIPIDS TO INVESTIGATE LO/LD PHASE COEXISTENCE

**Malavika Varma**[1]; Markus Deserno[1];
[1]Carnegie Mellon University, Dept. of Physics, Pittsburgh, PA, USA

Lipid rafts are nanoscopic assemblies of sphingolipids, cholesterol, and specific membrane proteins that are widely believed to underlie the experimentally well-established lateral heterogeneity of eukaryotic plasma membranes. Membrane rafts function as signaling platforms in diverse cellular processes, such as immune regulation, cell cycle control, membrane trafficking and fusion events. The coexistence of distinct fluid phases, particularly the liquid-ordered (Lo) and liquid-disordered (Ld) phases, is considered to be a highly plausible model for raft formation. Additionally, cholesterol significantly influences this process by modulating membrane fluidity, permeability, lipid packing, and protein mobility. We have expanded the Cooke model for lipids, a highly coarse-grained model extensively utilized for exploring membrane mechanics, so that it can explore the physics of Lo/Ld phase coexistence, thereby introducing the thermodynamics of mixtures into the model. Our model simplifies a complex system and captures its large-scale physics, helping us to explore the interplay of membrane mechanics and phase behavior. In particular, we outline the phase diagram roughly and estimate the location of the critical point, shedding light on the system's thermodynamic state. In addition, we measure observables such as membrane area expansion modulus (KA) and lipid diffusion constants, uncovering trends like anti-registered domains and increased KA with reduced phase separation. Because Lo/Ld phase behavior relies on leaflet cholesterol fraction, which in turn is sensitive to differential stress [Varma & Deserno, BiophysJ 2022], adjusting lipid packing in the inner leaflet enables us to control raft formation in the outer leaflet. We leverage the capabilities of our simulation model to apply differential stress to membranes exhibiting Lo/Ld phase coexistence, thus obtaining valuable insight into the interplay between various types of asymmetries and membrane phase behavior.

## STEROID RING VIBRATIONS ELUCIDATE CHOLESTEROL'S INFLUENCE ON LIPID MEMBRANES

**Jason H. Hafner**[1]; Kyra R Birkenfeld[1]; Mathieu L Simeral[1];
[1]Rice University, Physics & Astronomy, Houston, TX, USA

Cholesterol is a major component of the plasma membrane of animal cells that impacts membrane fluidity, permeability, and lateral organization. Despite its importance, the molecular mechanisms of cholesterol's many functions are not clearly understood. Our objective is to use cholesterol's low-frequency molecular vibrations to determine its conformational structure in different lipid environments. This will reveal the molecular mechanism behind cholesterol's effects on lipid membrane structure and order. The methodology employed is based on our recent discovery that a steroid ring vibration band from 300 – 650 $cm^{-1}$ is sensitive to the conformational structure of cholesterol's iso-octyl chain. The chain structures fall into ten types that depend on the pattern of trans/gauche dihedral angles around four bonds in the chain. Each structure type is a set of conformers with the chain set at a similar angle relative to the plane of the steroid rings. Through a comparison of measured and DFT-calculated Raman spectra, we recently showed that each structure type has a unique Raman spectrum in the 300 – 650 $cm^{-1}$ band. The corresponding spectral types are sufficiently distinct that they can be used to fit experimentally measured Raman spectra of cholesterol to directly measure the distribution of conformers in a sample. This process was carried out for cholesterol in phospholipid vesicles with different degrees of acyl chain saturation: DPPC, POPC, and DOPC. The resulting cholesterol conformer distributions are distinct for each phospholipid species. In DPPC cholesterol has the largest fraction of straight chains, consistent with the order and saturation of that phospholipid. In DOPC, which is unsaturated and has the lowest order, cholesterol has a higher fraction of bent chain conformers. We conclude that the cholesterol chain structure responds to phospholipid saturation, with straighter cholesterol chains increasing the membrane thickness to influence lipid membrane properties.

# POSTER ABSTRACTS

## Monday, June 3
## POSTER SESSION I
## 4:00 PM – 6:00 PM
## Ballroom

Monday and Tuesday posters are available for viewing during the Monday and Tuesday poster sessions. However, below are the formal presentations for Monday. 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

| | | |
|---|---|---|
| **Bradley Baker** | **1-POS** | **Board 1** |
| **Mandira Dutta** | **5-POS** | **Board 2** |
| **Sonya Hanson** | **9-POS** | **Board 3** |
| **Ehsaneh Khodadadi** | **13-POS** | **Board 4** |
| **Korbinian Liebl** | **17-POS** | **Board 5** |
| **Daniel Milshteyn** | **21-POS** | **Board 6** |
| **Reza Razeghifard** | **25-POS** | **Board 7** |
| **Ryan Schuck** | **29-POS** | **Board 8** |
| **Ming-Feng Tsai** | **33-POS** | **Board 9** |
| **Mouzhe Xie** | **37-POS** | **Board 10** |

Posters should be set up on the morning of Monday, June 3 and removed by 11:00 PM on Tuesday, June 4. All uncollected posters will be discarded.

**1-POS Board 1**

## OPTICAL DEMONSTRATION THAT THE LIPID ENVIRONMENT IN THE PLASMA MEMBRANE INFLUENCES THE ACTIVITY OF A VOLTAGE SENSING DOMAIN

Haeun Lee[1]; Nazarii Frankiv[1]; Eugene Park[1]; **Bradley J. Baker**[1];
1Korea Institute of Science and Technology, Seoul, South Korea

Genetically encoded voltage indicators (GEVIs) convert membrane potential transients into an optical signal. The ArcLight family of GEVIs consists of a classic voltage sensing domain with four transmembrane segments (S1-S4) from the voltage sensing phosphatase gene family fused to a fluorescent protein. Positively charged arginines positioned every third amino acid in S4 cause a conformational change when the membrane potential is altered. The consensus charge distribution in S4 of the phosphatase gene family is R1-X-X-R2-X-X-I3-X-X-R4 (isoleucine occupies the R3 position). To investigate the role counter charges in S1-S3 play in determining the voltage response, we performed a lysine scan of the arginines in S4. The hypothesis was that the reduced hydrogen bond capability of lysine with only one nitrogen in its sidechain could act as a stop signal for further movement of S4. Lysines at the 2nd, 3rd, or 4th positions yielded uniform optical responses in the plasma membrane when expressed in HEK cells and subjected to whole cell voltage clamp. In contrast, the K1-X-X-R2-X-X-I3-X-X-R4 mutant despite uniform membrane expression, displayed a discontinuous voltage-dependent optical response in the plasma membrane suggesting that in some regions of the cell S4 could not respond to voltage. Voltage-dependent optical signals from the entire plasma membrane could be observed from the K1 mutant with the additional mutation of arginine at the I3 position (K1-X-X-R2-X-X-R3-X-X-R4), however, different optical patterns were still detected in different regions of the plasma membrane. These results indicate that the varied lipid environment of the plasma membrane influences the conformation of the voltage sensing domain resulting in diverse functions of the protein. Other GEVI mutants exhibit similar behaviors indicating that GEVI activity may provide new insights into the effects membrane chemistry has on cell physiology.

**5-POS Board 2**

## INVESTIGATION OF THE ASSEMBLY AND BUDDING MECHANISMS OF SARS COV-2 M PROTEIN

**Mandira Dutta**[1]; Gregory A Voth[1];
[1]The University of Chicago, Department of Chemistry, Chicago, IL, USA

The profound repercussions on both public health and the global economy resulting from the COVID-19 pandemic, instigated by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have spurred unparalleled research efforts worldwide aimed at eradicating this virus. The SARS-CoV-2 virion is composed of four major structural proteins: Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N). The M protein is the most abundant structural protein in the virion surface. Its pivotal role lies in facilitating the assembly and budding processes of SARS-CoV-2. Our study investigates the intricate protein-protein and protein-lipid interactions of M protein within biologically relevant complex membrane models. We have performed atomistic molecular dynamics simulations based on recent experimental structures of M proteins; we have explored crucial lipid binding properties inherent to M proteins. These properties, we hypothesize, are critical for the assembly and budding processes of SARS-CoV-2. Additionally, we have scrutinized the conformational dynamics of distinct conformations of the M protein, elucidating how it orchestrates the curvature of the membrane through interactions with lipids. Our findings illuminate the mechanisms underpinning M protein-driven assembly and budding, thereby advancing our understanding of viral pathogenesis. Furthermore, this understanding aids in the advancement of novel antiviral therapeutics targeting viral budding processes.

**9-POS Board 3**

## NARROWING DOWN THE TEMPERATURE SENSOR IN VOLTAGE-GATED SODIUM CHANNELS OF MARINE BACTERIA

**Sonya M. Hanson**[1]; Gerhard Hummer[2]; Carlos Gonzalez-Leon[3]; Andres Jara-Oseguera[3];
[1]Flatiron Institute, New York, NY, USA
[2]Max Planck Institute of Biophysics, Frankfurt, Germany
[3]University of Texas at Austin, Austin, TX, USA

Life depends sensitively on temperature, yet how temperature is sensed remains poorly understood. Here we focus on the temperature sensitivity of the voltage-gated sodium channel channel of Silicibacter pomeroyi (NavSp1), for which previous studies have found the helical C-terminal domain to be critical to temperature sensing. On the basis of bioinformatics analysis, Alphafold2-multimer models, molecular dynamics simulations, and electrophysiology experiments, we found that the coiled coil domain plays a critical role in determining temperature sensing - not just acting as a stable anchor. Through building NavSp1 chimeras, leaving the neck region intact, and swapping in the sequences of the coiled coil of other species of sodium channel or even designed coiled coils we are able to further elucidate what determines the temperature sensitivity of these channels.

**13-POS Board 4**

## MODULATORY EFFECTS OF LIPID COMPOSITION ON THE CONFORMATIONAL DYNAMICS OF MGLUR2 IN VARIOUS FUNCTIONAL STATES: INSIGHTS FROM ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS

**Ehsaneh Khodadadi**[1]; Shadi Badiee[1]; Mortaza Derakhshani-Molayousefi[1]; Ehsan khodadadi[1]; Mahmoud Moradi[1];
[1]university of Arkansas, Department of Chemistry and Biochemistry, Fayetteville, AR, USA

Metabotropic glutamate receptor 2 (mGluR2) is a G protein-coupled receptor (GPCR) integral to regulating neurological functions and can be significantly affected by its surrounding lipid membrane environments. This study utilizes all-atom molecular dynamics simulations to explore the conformational dynamics of mGluR2 in both active and inactive states, contextualized by lipid interactions. Here, we embedded mGluR2 protein in various micelles using BLMNG detergent and lipid bilayers composed of palmitoyl-oleoyl phosphatidylcholine (POPC) and Cholesteryl hemisuccinate (CHS) and investigated conformational dynamics of mGluR2 across different CHS concentrations (0%, 10%, 25%) to understand the lipid's modulatory effects on receptor conformation. Our simulations have brought to light distinct conformational variations between the inactive and active mGluR2. In the inactive state, the receptor exhibited enhanced conformational flexibility, particularly within the transmembrane helices, which contrasts with the more stable conformation of its active state. CHS plays a significant role in these dynamics, especially at 10%, facilitating notable conformational changes in transmembrane domains. This result underscores the critical role of lipid composition in GPCR activity. We have uncovered substantial conformational rearrangements, particularly in the TM7 transmembrane domains, which play a significant role in the behavior of mGluR2. Furthermore, we have identified key electrostatic interactions that have significant contributions on the conformations of the inactive and active mGluR2, specifically influenced by TM7. These insights not only underscore the importance of the lipid environment in modulating GPCR structure and function but also provide a deeper understanding of the dynamic behavior of mGluR2. This understanding can be harnessed to develop targeted therapeutics for mGluRs and potentially other GPCRs by exploiting their lipid-dependent conformational states. This study significantly advances our understanding of receptor behavior in diverse lipid contexts, paving the way for innovative drug discovery and therapeutic optimizations targeting neurological and synaptic disorders.

**17-POS Board 5**

## MULTISCALE-SIMULATIONS OF MEMBRANE-REMODELING BY CAVEOLIN-1

**Korbinian Liebl**; Gregory A Voth[1];
[1]University of Chicago, Chicago, IL, USA

Caveolae are ~50-100 nm large invaginations in the plasma membrane, initiating the formation of endocytic vesicles. Mature caveolae are built from 8S complexes that consist of Caveolin-1 (CAV1) protomers oligomerized into disk-like structures with a central beta-barrel. Intriguingly, the CAV1-8S complexes accumulate cholesterol and are embedded in only one lipid layer, leaving the beta-barrel exposed to solvent. However, how this molecular architecture orchestrates the intricate molecular functions that are central to caveolae biogenesis is not understood. In addition, also the role of posttranslational modifications (palmitoylation) of the CAV1 monomers has remained elusive. To gain better understanding of the molecular functioning of the CAV1-8S complexes, we have performed extensive atomistic Molecular Dynamics (MD) simulations. Unbiased microsecond-long simulations of a CAV1-8S complex indicate only minor membrane-bending due to the protein complex. To overcome the modest lipid reorganization on this timescale, we have performed comparative MetaDynamics simulations that greatly enhance the sampling of lipid conformations. In this way, we have been able to monitor the concentration of cholesterol within the beta barrel of the CAV1-8S complex. Furthermore, the obtained free energy profiles reveal that posttranslational modifications reinforce accumulation of cholesterol. Nevertheless, the atomistic simulations are not sufficient to explain how CAV1-8S complexes organize a flat-to-curved transition of the lipid bilayer to form endocytic vesicles. Thus, we have built a rigorous bottom-up coarse-grained model that enables accurate simulations of multiple membrane embedded CAV1-8S complexes. Based on these cutting-edge simulations, we give new insight into caveolae formation by showing how CAV1-8S complexes cooperatively leverage membrane bending. We also discuss the impact of electrostatic repulsion between distinct complexes and bring to light the importance of cholesterol concentration. Finally, we emphasize that our approach extends the state-of the art of protein-lipid coarse-graining and paves the way for accurate coarse-grained models on length-scales up to ~1 μm.

## 21-POS Board 6
## HIGH PRESSURE BIOPHYSICS FOR DISSECTING ROLES OF NON-BILAYER LIPIDS IN BIOLOGICAL MEMBRANES

**Daniel Milshteyn**[1]; Jacob R Winnikoff[2]; Itay Budin[1];
[1]University of California, San Diego, Chemistry and Biochemistry, La Jolla, CA, USA
[2]Harvard University, Organismic and Evolutionary Biology, Cambridge, MA, USA

While lamellar bilayers are the basis of cell membranes, many abundant biological lipids form nonlamellar structures, like the inverse hexagonal ($H_{II}$) phase, that could facilitate high membrane curvature and fusion/fission processes in cells. To better understand the regulation of global membrane curvature and cellular access to nonlamellar phases, we have employed the use of hydrostatic pressure incubations of model microorganisms as an assay to challenge and test biophysical roles of non-bilayer lipids. Hydrostatic pressure compresses the acyl chains of conical lipids, like phosphatidylethanolamine (PE), into cylindrical geometries with less negative curvature resembling that of phosphatidylcholine (PC). To assess the roles of non-bilayer lipid abundance in preserving membrane dynamics under pressure, we screened the fitness of yeast strains engineered with varied PE/PC ratios under high pressure incubation. Polar lipid extracts of these strains were then analyzed by high pressure small angle x-ray scattering (HP-SAXS) to evaluate accessibility of lamellar to $H_{II}$ phases. In parallel, we have tested the dependence of membrane fusion on negatively-curved lipids with synthetic vesicles featuring varied PE/PC ratios using a FRET-based high pressure stopped-flow assay. In combination, these methodologies allowed us to investigate the role of PE, and other non-bilayer lipids, in preserving access to nonlamellar topologies and membrane dynamics under biophysically constrained conditions. We demonstrate the applicability of high pressure experimental biology to uncover subtle roles of lipids and biochemical pathways involved in fundamental membrane biophysics that may be difficult to detect in ambient environments.

**25-POS Board 7**

## INCREASING THE ENZYME ACTIVITY AND STABILITY BY CONTROLLING THE MEMBRANE COMPOSITION.

**Reza Razeghifard**[1]; Rachel Gruboy [1]; Kena Patel[1];
[1]Nova Southeastern University, Chemistry and Physics, Fort Lauderdale, FL, USA

The purpose of this study is to make membrane assemblies capable of increasing and maintaining the catalytic activity of enzymes. Cytochrome P450 (CYP) and Carbonic anhydrase are the two enzymes studied here. CYP enzymes are responsible for the metabolism of a variety of drugs. This enzyme versatility in the choice of substrates can be beneficial since it can show if the new membrane assembly can indeed increase the enzyme activity/stability in the presence of different substrates. The enzyme activity is tested by detecting and quantifying the product concentrations using LCMS and GCMS depending on the product volatility. Butylated Hydroxytoluene (BHT) was used as the control substrate since it has a high affinity for the CYP enzyme. The most common products are hydroxylated substrates. However, the enzyme also produces hydrogen peroxide when the reaction is uncoupled. The coupling can be about 5% meaning that 95% of reduced NADP is wasted by the CYP as hydrogen peroxide. The CYP enzyme can then gradually lose its activity over time even under mild reaction conditions. Carbonic anhydrase is responsible for converting carbon dioxide into bicarbonate. The enzyme rapidly performs the reaction but the enzyme stability plays a key role in employing this enzyme for CO2 storage and capture since the reaction needs to be carried out at elevated temperatures. The enzyme activity can be measured spectrophotometrically by detecting a color compound produced by the esterase reaction.

**29-POS Board 8**

## CHOLESTEROL BLOCKS ONCOGENIC ACTIVITY AND SELF-ASSEMBLY OF THE EPHA2 RECEPTOR

**Ryan Schuck**[1]; Alyssa E Ward[1]; Amita R Sahoo[5]; Jennifer A Rybak[3]; Robert J Pyron[3]; Thomas N Trybala[2]; Timothy B Simmons[1]; Joshua A Baccile[2]; Ioannis Sgouralis[4]; Matthias Buck [5]; Rajan Lamichhane[1]; Francisco N Barrera [1];
[1]University of Tennessee , BCMB, Knoxville , TN, USA
[2]University of Tennessee , Chemistry , Knoxville , TN, USA
[3]University of Tennessee , GST, Knoxville , TN, USA
[4]University of Tennessee , Mathematics, Knoxville , TN, USA
[5]Case Western Reserve University, Physiology and Biophysics, Cleveland, OH, USA

The EphA2 receptor exerts a profound influence on cancer malignancy. EphA2 has two modes of activation that have opposite effects on cancer outcomes. EphA2 acts as a tumor suppressor when it is phosphorylated at cytoplasmic tyrosines after binding of Ephrin ligands, which triggers receptor monomers to self-assemble. On the other hand, EphA2 can be activated in the absence of ligand when kinases phosphorylate key serine residues. Under these conditions, EphA2 triggers oncogenic signaling that drives an invasive phenotype that leads to metastasis. We hypothesized that specific cellular mechanisms must exist to keep EphA2 in check by maintaining its optimal self-assembly levels and preventing its oncogenic activation by kinases. However, improved methods to quantify protein oligomers are needed to rigorously evaluate this idea. Here, we developed a single-molecule method, SiMPull-POP, which achieves high sensitivity to quantify oligomeric populations of membrane proteins. Our approach captures the native environment of the eukaryotic cell membrane in DIBMA-derived nanodiscs (DIBMALPs). SiMPull-POP delivers single-molecule sensitivity to quantify protein interactions via photobleaching analysis. We used SiMPull-POP to discover that cholesterol (Chol) is a potent inhibitor of EphA2 assembly. Additionally, we observed that Chol regulates EphA2 activity by preventing oncogenic phosphorylation of key residue Ser897. We performed cell biology experiments that suggest that Chol controls EphA2 serine phosphorylation by regulating protein kinase A activity via changes in the levels of the second messenger cAMP. Based on the data presented, we propose that the presence of cholesterol in the plasma membrane acts as a safety mechanism that prevents uncontrolled self-assembly and oncogenic activation of EphA2.

**33-POS Board 9**

## HUMAN MRS2 IS A CALCIUM-REGULATED NON-SELECTIVE CATION CHANNEL IN MITOCHONDRIA

**Ming-Feng Tsai**[1,2]; Yung-Chi Tu[2]
[1]University of Colorado Anschutz, Physiology and Biophysics, Aurora, CO, USA
[2]University of Virginia, Molecular Physiology and Biological Physics, Charlottesville, VA, USA

The human Mitochondrial RNA Splicing 2 protein (MRS2) has been implicated in $Mg^{2+}$ transport across mitochondrial inner membranes, thus playing an important role in $Mg^{2+}$ homeostasis critical for mitochondrial integrity and function. However, the molecular mechanisms underlying its fundamental channel properties such as ion selectivity and regulation remain unclear. Here, we present structural and functional investigation of MRS2. Cryo-electron microscopy structures in various ionic conditions reveal a pentameric channel architecture and the molecular basis of ion permeation and potential regulation mechanisms. Electrophysiological analyses demonstrate that MRS2 is a $Ca^{2+}$-regulated, non-selective channel permeable to $Mg^{2+}$, $Ca^{2+}$, $Na^{+}$ and $K^{+}$, which contrasts with its prokaryotic ortholog, CorA, operating as a $Mg^{2+}$-gated $Mg^{2+}$ channel. Moreover, a conserved arginine ring within the pore of MRS2 functions to restrict cation movements, thus preventing the channel from collapsing the proton motive force that drives mitochondrial ATP synthesis. Together, our results provide a molecular framework for further understanding MRS2 in mitochondrial function and disease.

**37-POS Board 10**

## DIAMOND QUANTUM SENSOR HOLDS POTENTIAL TO REVOLUTIONIZE PHOSPHOLIPID AND MEMBRANE BIOPHYSICS RESEARCH

**Mouzhe Xie**[1];
[1]Arizona State University, School of Molecular Sciences, Tempe, AZ, USA

Quantum sensing technologies enable some of the most precise measurements that human beings have ever achieved. In recent years, optically addressable nitrogen-vacancy (NV) color center hosted by diamond crystal has been widely used as a quantum bit (qubit), which has exquisitely sensitive response to magnetic fields. This novel magnetometer therefore enables micro-/nano-scale NMR experiments with unprecedented sensitivity and spatial resolution (i.e. single-molecule regime) to provide insights about dynamics and interactions of biological systems at molecular and cellular level. Examples include the nanoscale 31P NMR detection and characterization of a single layer self-assembled 12-pentafluorophenoxydodecylphosphonic acid (PFPDPA) phospholipid molecules [1]. Interfacing a diamond quantum sensor with physiologically relevant biological samples in a controlled manner is key to the successful application of this new technology to tackle important research questions in biophysics and biomedicine. Our recent development of the biocompatible diamond surface functionalization toolkits provides general means to enrich biomolecules of interest, including phospholipids, to the near-surface sensing volume for improved sensitivity [2]. Furthermore, we fabricated diamond membrane platforms with tunable quantum properties and integrated with optical, electrical, and fluidic controls as a hybrid sensing device [3]. Parallel to experimental advances, we performed theoretical calculations and simulations to guide the design of sensing protocols for surface-supported phospholipids. These comprehensive efforts promise a new way to study phospholipid and membrane biophysics regarding their compositional rigidity, structural dynamics, and the intricate connection to functionality. [1] K. Liu et al. PNAS. 2022, 119, e2111607119.[2] M. Xie & X. Yu et al. PNAS. 2022, 119, e2114186119.[3] X. Guo et al. 2022. arxiv 2306.04408.

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

Monday and Tuesday posters are available for viewing during the Monday and Tuesday poster sessions. However, below are the formal presentations for Tuesday. 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

| | | |
|---|---|---|
| **Rahul Benani** | **2-POS** | **Board 11** |
| **Seamus Gallagher** | **6-POS** | **Board 12** |
| **Nolan Jacob** | **10-POS** | **Board 13** |
| **Katelyn Kraichely** | **14-POS** | **Board 14** |
| **Tsung Yun Liu** | **18-POS** | **Board 15** |
| **Collin Nisler** | **22-POS** | **Board 16** |
| **Rituparna Samanta** | **26-POS** | **Board 17** |
| **Mohamed Seghiri** | **30-POS** | **Board 18** |
| **Yung-Chi Tu** | **34-POS** | **Board 19** |
| **Yue Xu** | **38-POS** | **Board 20** |

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**2-POS Board 11**

## EXPLORING TWO-DIMENSIONAL PROTEIN-PROTEIN INTERACTIONS IN SUPPORTED LIPID BILAYERS IN PRESENCE OF SMALL ORGANIC MOLECULES

**Rahul Benani**[1]; Paul Cremer[1,2];
[1]The Pennsylvania State University, Department of Chemistry, University Park, PA, USA
[2]The Pennsylvania State University, Department of Biochemistry and Molecular Biology, University Park, PA, USA

Membrane-associated proteins are a major component of plasma membranes and other cellular organelles. Herein, we used biotinylated binding sites to anchor avidin, a positively charged protein, and streptavidin, a negatively charged protein, to the surface of two-dimensionally fluid phospholipid membranes with phosphatidylcholine as the major component. Both the protein and lipid molecules could be labeled with dye molecules and studied inside polydimethylsiloxane (PDMS) on glass microfluidic devices. Studies were performed as a function of pH, salt concentration, as well as in the presence of small molecules, like osmolytes and denaturants, such as urea. The results showed a surprisingly variety of protein-protein interaction (PPI) behaviors. Fluorescence recovery after photobleaching (FRAP) was employed to monitor both protein and lipid diffusion as local population densities. The results revealed that protein diffusion behavior can change in a complex fashion and strongly depends not only on the exact buffer conditions and surface protein densities, but also on the presence of sugars, amino acids and osmolytes in the solution that can drastically alter PPI under what would otherwise appear to be identical conditions. These results are explained in terms of disrupting sites that enhance or impede PPI.

**6-POS Board 12**

## FUSION OF COARSE GRAINED, IMPLICIT-SOLVENT LIPID MEMBRANES WITH VARIABLE GAUSSIAN CURVATURE MODULI

**Seamus Gallagher**[1]; Markus Deserno[1];
[1]Carnegie Mellon Univ, Dept Physics, Pittsburgh, PA, USA

The Gauss-Bonnet theorem states that the total Gaussian curvature over a closed membrane is a topological invariant. This suggests the Gaussian curvature modulus is important for topology changing events such as fusion. In support of this point, recent experiments show lipid nanoparticles (LNPs) prepared with lipids that demonstrate a preference towards negative Gaussian curvature phases more readily fuse with the endosome, escaping a well known bottleneck in the LNP drug delivery pathway. To complement these experimental results in simulation, we used a coarse grained lipid model in which we succeeded to prepare a set of lipids with systematically varying Gaussian curvature moduli independently of other elastic parameters and subsequently studied the fusion propensity of such membranes. While our model is well equipped to characterize elastic determinants of fusion, limitations of the implicit solvent used in our model indicate caution is required when hydration dynamics are important.

**10-POS Board 13**

## IDENTIFYING THE EFFECTS OF PROTON CHANNEL HYDRATION ON SELECTIVE CONDUCTION ACROSS A MEMBRANE

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Membrane channels are important for performing basic biological mechanisms such as regulating intracellular pH and creating ion gradients, both of which are necessary for proper cellular function and signaling. However, the size, complexity, and stability of these channels make them difficult to study in vitro. De novo protein design allows us to distill these proteins into simple model systems which we can use to test basic mechanisms of channel and transporter function. Specifically, previous research determined the role of water wires in the transport of protons within a designed pentameric scaffold channel by replacing nonpolar pore-facing residues with polar side chains to introduce water into the channel and achieve selective proton conduction across the membrane. My project sought to extend water wire formation throughout the channel by adding additional polar residues to address questions of pore hydration's effect on proton conductivity. We used computational molecular dynamics simulations, X-ray crystallography, and functional assays to define the roles of water wires in proton movement. From these experiments, we show that the relative placement of polar residues in the pore not only affects their channel assembly, but also alters their ability to explore different rotameric states, which ultimately impacts water accessibility into the pore.

**14-POS Board 14**

## THE TWO SNARE MOTIFS OF SNAP25 ARE STRUCTURALLY AND FUNCTIONALLY DISTINCT

**Katelyn Kraichely**[1]; Connor Sandall[1]; Volker Kiessling[1]; Binyong Liang[1]; Lukas K Tamm[1]; [1]University of Virginia Health System, Department of Molecular Physiology and Biological Physics, Charlottesville, VA, USA

Eukaryotic cells sustain protein and membrane homeostasis through protein-regulated fission and fusion of lipid membranes. Many intracellular membrane fusion events are catalyzed by members of the SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) protein family. SNARE proteins form heterotrimers or tetramers that bridge separate membranes and assemble a coiled helical bundle of four 60-70 residue "SNARE motif" domains to induce fusion. The particularly highly regulated fusion events between neuronal synaptic vesicles and the plasma membrane are catalyzed by a SNARE complex composed of syntaxin-1a and SNAP25 (target membrane or "tSNAREs") on the plasma membrane and synaptobrevin-2 on the vesicle membrane ("vSNARE"). Unlike its synaptic SNARE partners and most other cellular SNAREs, SNAP25 contains two SNARE motif domains connected by a flexible linker and does not contain a transmembrane domain; it is instead associated to the plasma membrane through posttranslational lipidation of its linker domain. These distinctions led us to hypothesize that the unusual arrangement of dual SNARE motifs in SNAP25 may serve a functional role in supporting tightly regulated synaptic exocytosis. We have generated both soluble and membrane-associated single SNARE domain constructs of SNAP25 that have allowed us to dissect the structural and functional contributions of each domain. Magnetic resonance experiments revealed distinct structural preferences of the two domains. FRET-based measurements in model membranes showed a correlation between these structural preferences and ability of the individual domains to interact with partner SNAREs. Lastly, a total internal reflection fluorescence microscopy-based fusion assay demonstrated that each domain forms a fusion-capable SNARE complex with its cognate SNAREs, though with different efficiency and kinetics. Overall, these data support a model in which the two SNARE domains of SNAP25 serve complementary roles in optimizing fast and regulated membrane fusion at the synapse.

**18-POS Board 15**

## NEW EVIDENCES SUPPORTING THE OCCLUSION MECHANISM UNDERLYING MICU1 REGULATION OF THE MITOCHONDRIAL CALCIUM UNIPORTER

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The mitochondrial Ca2+ uniporter which is a Ca2+ channel that transports Ca2+ into mitochondria across the inner mitochondrial membrane (IMM), is a key player regulating intracellular Ca2+ signaling. It is known that the uniporter is regulated by a MICU1 subunit, but the exact mechanism is under debate. There are two opposite regulatory mechanisms. The occlusion model proposes that MICU1 blocks the uniporter's calcium pathway when calcium concentration is low. When calcium concentration increases, MICU1 would then separate from the calcium pathway to open the channel. By contrast, the potentiation model argues that MICU1 never blocks, but potentiates the uniporter when calcium increases. To test these two models, we utilized a sodium-dependent IMM depolarization assay in WT versus MICU1-knockout (KO) HEK cells. After chelating divalent cations with EDTA, MICU1-KO cells exhibit significantly faster IMM depolarization. This result suggests that MICU1 blocks the uniporter when calcium concentration is low, thus providing support for the occlusion mechanism. We then investigated the effects of MICU1 depletion on uniporter activity in mouse embryonic fibroblast (MEF) cells. MICU1-KO led to reduced rate of mitochondrial calcium uptake, but we found that this is caused by decreased EMRE expression. When we fused EMRE to MCU to enforce an obligatory 1:1 MCU-EMRE stoichiometry, the uniporter exhibits similar activity in WT and MICU1-KO cells. These results suggest that MICU1 does not potentiate the uniporter. Altogether, our experiments advance our knowledge of uniporter regulation by providing new evidences supporting the occlusion model and refuting the potentiation mechanism.

**22-POS Board 16**

## SIMULATIONS AND EXPERIMENTS REVEAL HOW A HETEROGENEOUS LIPID COMPOSITION IN MODEL PROTOCELL MEMBRANES MODULATES STABILITY AND PERMEABILITY

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The transformation from a non-living collection of interacting molecules to a self-replicating system capable of Darwinian evolution marks the origin of life on Earth. While a plethora of chemical and physical processes are necessary for the origin and evolution of complex life, encapsulation is widely recognized as a fundamental requirement for the concentration and sequestering of early self-replicating molecules. Due to the lack of complex molecules required to tightly regulate traffic in and out of cells as seen in modern biology, primitive cells would have had to rely on inherent membrane properties to modulate the selective permeation of feedstock and byproduct molecules, while retaining longer functional molecules and remaining stable in response to various external selection pressures. Here, we present a combination of experiments as well as coarse-grain and all-atom molecular simulations to characterize the permeability, stability, and structure of membranes and vesicles composed of different mixtures of prebiotically plausible lipids. We find that a mixture of short-chain and long-chain fatty acids significantly enhances the stability relative to membranes formed by either lipid alone. Simulations suggest a tradeoff between membrane thickness and dynamics tunes the stability and permeability of fatty-acid membranes in a manner that depends on the size of the permeant and, in the case of RNA, the sequence. Our results provide fundamental insight into the function of potentially prebiotic protocells and will guide future efforts to design a self-sustaining protocell system.

**26-POS Board 17**

## FLEXIBLE BACKBONE MEMBRANE-ASSOCIATED PROTEIN DOCKING IMPROVES ITS PERFORMANCE ON EXPANDED TRANSMEMBRANE-PROTEIN COMPLEX DATASETS.

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The oligomerization of protein macromolecules on cell surfaces plays a fundamental role in regulating cellular function, including signal transduction and the immune response. Despite their importance, membrane proteins (MPs) represent only 2% of all protein structures in the protein data bank (PDB), and their complexes are even scarcer. Computational modeling provides a promising alternative to model MP interfaces and predict protein complex structures. Here, we present RosettaMPDock, a flexible transmembrane protein docking protocol that captures binding induced conformational changes. To generate diversity in backbone conformations for the RosettaMPDock, we used three conformer generation methods: perturbation of the backbones along the normal modes by 1 Å, refinement using the Relax protocol, backbone flexing using the Rosetta Backrub protocol. RosettaMPDock samples large conformational ensembles of flexible monomers and docks together protein targets within an implicit membrane environment. To improve the scoring efficiency, we have used a combination of low-resolution Motif Dock Score and membrane based high-resolution score Franklin2023. RosettaMPDock is benchmarked on 30 transmembrane-protein complexes of variable flexibility dataset. Our results show RosettaMPDock successfully predicts the correct interface (success defined as achieving 3 near-native structures in the 5 top-ranked ones) for 67% of moderately flexible targets (unbound-bound backbone motion within 1.5-2.5Å) and 60% of the highly flexible targets (unbound-bound backbone motion greater than 2.5Å), a substantial improvement from the existing membrane protein docking methods. We have also developed a hybrid protocol that refines AlphaFold-multimer structures with RosettaMPDock and further improves prediction success rates from 64% to 73%.

**30-POS Board 18**

## PIE-FCCS MODEL FOR COMPLEX MEMBRANE HETEROMERS STOICHIOMETRY

**Mohamed Seghiri**[1]; Adam W Smith[1];
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Pulsed-interleaved excitation fluorescence cross-correlation spectroscopy (PIE-FCCS) is an advanced fluorescence method that can quantitatively measure biomolecular interactions in living cells. The correlation of fluorescence fluctuations in PIE-FCSS provides information about the density, diffusion, and oligomerization of two-color labeled species. Oligomerization is determined by the amplitude of the correlation functions, the accuracy of which is affected by several factors, including non-overlapping observation volumes, immature and dark states in fluorescent protein labels, photobleaching, and incomplete dimerization. We have previously developed a mathematical model for membrane protein homo-oligomers and compared it with experimental data. However, to account for membrane protein hetero-oligomers, we present a mathematical model describing the different combinations of heteromerization levels and include corrections for non-fluorescent proteins. To validate the model, we used FKBP (FK506-binding protein) and FRB (FKBP rapamycin-binding domain) constructs and induced their heterodimerization with rapamycin. We compared the mathematical model with the experimental data of FRB-FKBP-induced hetero-oligomerization and FKBP-induced homo-oligomerization, showing the relative change in cross-correlation values ($f_c$). We also assessed the predictive capacity of the mathematical model with respect to the structural organization and binding affinity of these controls.

**34-POS Board 19**

## MECHANISM OF DUAL MODULATORY EFFECTS OF SPERMINE ON THE MITOCHONDRIAL CALCIUM UNIPORTER COMPLEX

**Yung-Chi Tu**[1]; Ming-Feng Tsai[1];
[1]University of Virginia, Molecular Physiology and Biological Physics, Charlottesville, VA, USA

The mitochondria calcium uniporter is a multi-subunit calcium channel complex, which mediates mitochondrial calcium uptake. It is crucial for regulating mitochondria metabolism and intracellular calcium signaling. The uniporter is composed of the pore-forming subunit MCU, an EMRE subunit, and the regulatory subunits MICU1 and MICU2, which can form a MICU1-2 heterodimer. We have shown before that MICU1-2 regulates the uniporter by occluding the MCU pore under resting cellular [$Ca^{2+}$]. When [$Ca^{2+}$] increases, MICU1-2 would separate from MCU to open the uniporter. For decades, it has been known that spermine, a polyamine ubiquitously present in animal cells, can potentiate the uniporter, but the underlying molecular mechanism remains unclear. Here, we demonstrate that spermine exhibits dual modulatory effects on the uniporter. Under physiological concentrations of spermine, it enhances the uniporter's activity by attaching to the bilayer surface and disrupting MICU1-2 block of MCU, allowing the uniporter to take up more $Ca^{2+}$ in low $Ca^{2+}$ conditions. This potentiation effect does not require MICU2 or the EF-hand $Ca^{2+}$-binding motif in MICU1. Lastly, we show that when spermine concentration rises to millimolar levels, it can inhibit the uniporter by blocking the pore in a MICU1-independent manner. Together, our findings solved the decade-old puzzle about how spermine regulates the mitochondria calcium uptake process. Furthermore, our work nicely explains why cardiac mitochondria show no response to spermine — the uniporter in cardiac mitochondria is MICU1-derugalated.

**38-POS Board 20**

## TREHALOSE SUGAR PROTECTS LIPID MEMBRANE AGAINST AMYLOID-BETA TOXICITY IN ALZHEIMER'S DISEASE

**Yue Xu**[1]; Danielle M McRae[1]; Carina T Filice[2,3]; Zoya Leonenko[1,2,3];
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The amyloid-beta peptide (Aβ1-42) is one of the main pathogenic factors in Alzheimer's disease and is known to induce damage to the lipid membrane (Drolle et al. 2017). Trehalose, a naturally existing disaccharide, has been shown to protect plant cellular membranes in extreme conditions and has been attracting attention in neurodegeneration research due to its ability to reduce Aβ misfolding (Khan and Kumar, 2017). We hypothesize that trehalose can also protect the neuronal membrane from amyloid toxicity. In this work, we aimed to explore the potential protective effect of trehalose against Aβ-induced damage in model lipid membranes (DPPC/POPC/Cholesterol in mass ratio of 4:4:2), used to mimic neuronal membranes. We used atomic force microscopy (AFM), Kelvin Probe Force Microscopy (KPFM), Black lipid membrane (BLM) and Localized Surface Plasmon Resonance (LSPR) techniques. Our AFM and KPFM results demonstrated that trehalose modifies the properties of model lipid membranes and monolayers (both topography and electrical surface potential), especially in combination with NaCl. Our BLM data show that Aβ induced damage to membranes and led to ionic current leakage across membranes due to the formation of various defects and pores. The presence of trehalose reduced the ion current caused by Aβ peptides' damage to membranes. Our LSPR results revealed that trehalose potentially reduces the binding of Aβ to lipid membranes, indicating the protective effect through suppression of Aβ-membrane interaction. These findings suggest that trehalose sugar can be useful in protecting neuronal cellular membranes against amyloid toxicity, and thus, this study may contribute to the development of membrane-targeted preventive approaches to overcome AD.

**Wednesday, June 5**
**POSTER SESSION III**
**4:00 PM – 6:00 PM**
**Ballroom**

Wednesday and Thursday posters are available for viewing during the Wednesday and Thursday poster sessions. However, below are the formal presentations for Wednesday. 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

| | | |
|---|---|---|
| **Tanner Blocker** | **3-POS** | **Board 1** |
| **Beatrix Goggin** | **7-POS** | **Board 2** |
| **Andres Jimenez Salinas** | **11-POS** | **Board 3** |
| **I-Chi Lee** | **15-POS** | **Board 4** |
| **Connor McDermott** | **19-POS** | **Board 5** |
| **Ece Özdemir** | **23-POS** | **Board 6** |
| **Jonathan Schlebach** | **27-POS** | **Board 7** |
| **Alexandra Stuer** | **31-POS** | **Board 8** |
| **Ruan van Deventer** | **35-POS** | **Board 9** |
| **Matthew Necelis** | **39-POS** | **Board 10** |

Posters should be set up on the morning of Wednesday, June 5, and removed by 11:00 PM on Thursday, June 6. All uncollected posters will be discarded.

**3-POS Board 1**

## CHANGES IN ENVIRONMENTAL CONDITIONS DIFFERENTIALLY AFFECT SNAP-25 ISOFORMS

**Tanner M Blocker**[1]; Thomas D Weed[1]; Jason T Carlson[1]; Joseph H Jackson[1]; Jarom S Sumsion[1]; Samuel W Shumway[1]; Hunter S Malquist[1]; Dixon J Woodbury[1];
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In the brain, neurons communicate via releasing and detecting neurotransmitters. Release occurs through exocytosis following fusion of synaptic vesicles to neuronal cell membranes. This process is driven by formation of a dynamic quaternary protein structure known as the SNARE complex. SNAP-25 contributes two (of four) alpha helical domains to the SNARE complex. Neurons express SNAP-25 in two isoforms, SNAP-25A (25A) and SNAP-25B (25B) which appear to function similarly. These two isoforms vary by just 9 amino acids and are expressed differently depending on brain region and the developmental stage of the neuron (Bark et al. PNAS, USA. 92:1510). The amino acid sequences of 25A and 25B and their effect on SNARE complex stability have been studied (Nagy et al, 2005, Molecular Biology of the Cell, Vol. 16:5675). However, the difference in secondary structures of 25A and 25B remains unclear. Using circular dichroism (CD) spectroscopy, we show that SNAP- 25A and 25B are differentially altered by temperature, redox state, and alcohol. We hypothesize these differences provide neurons alternative responses depending on environmental stresses encountered during stages of development and in different regions of the brain.

**7-POS Board 2**

## DO ANTIDEPRESSANTS MODULATE BIOLOGICAL MEMBRANES?

**Beatrix L Goggin**[1,2]; Jane R Allison[1]; Oleg O Glebov[3,4];
[1]University of Auckland, School of Biological Sciences, Auckland, New Zealand
[2]University of Auckland, School of Chemical Sciences, Auckland, New Zealand
[3]Qingdao University, Institute of Neuroregeneration and Neurorehabilitation, Qingdao, China
[4]King's College London, Department of Old Age Psychiatry, London, United Kingdom

SARS-CoV-2 has been a pervasive threat to global health since its emergence in December 2019. The unprecedented need for rapid and readily available treatments and prophylactics coupled to the initial lack of information on the virus itself lead many researchers to turn to drug repurposing in favour of de novo drug development. Initial results identified 11 existing drugs that may block endocytic pathways facilitating SARS-CoV-2 entry into cells. Further work revealed that the antidepressant (AD) Fluvoxamine may cause rapid and significant modulation of endocytosis at even sub-therapeutic concentrations. Given the ubiquitous prescription and assumed safety of ADs, they prove a highly attractive candidate as a prophylactic for SARS-CoV-2 or similar viruses that may emerge in the future. The widespread use of ADs also makes the discovery of unexpected off-target effects of significant interest for further investigation. Though these drugs are highly prescribed, relatively little is known about their exact mechanism of action or cause behind side effect profiles. Molecular dynamics simulations allow for investigation of interactions of ADs with membranes of varying compositions as models for different body tissues. The preliminary work reported here was carried out with a selection of ADs, including Fluvoxamine, and a model blood-brain barrier membrane. As AD action is generally considered in a central nervous system context, the blood-brain barrier proves a cogent choice as the first membrane type with which to investigate AD interaction. These results will inform direction of future in vitro and computational research, including expanding the range of drugs tested and construction of additional membrane models (such as the gut epithelium). This research is hoped to aid elucidation of the mechanism of potential prophylactic effect of ADs against SARS-CoV-2, as well as improve insight into the mechanism of action of ADs for their intended use.

**11-POS Board 3**

## REGULATION OF RAF KINASE THROUGH MEMBRANE RECRUITMENT AND MULTIVALENT PROTEIN-LIPID INTERACTIONS

**Andres Jimenez Salinas**[1]; Youngkwang Lee[1];
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Multivalent membrane-protein interactions dictate and regulate signaling pathways. The MAPK pathway controls various cellular outcomes including differentiation, proliferation and apoptosis. An important node of the MAPK pathway consists of Raf Kinase and its regulatory domains of Raf, the Ras binding domain (RBD) and the cysteine rich domain (CRD), drives membrane recruitment as an initial step for its activation. Its activation mechanism is not fully understood due to its complexity of molecular events and multivalent interactions and its challenge of investigating molecular processes on a relevant membrane environment. To better understand the activation mechanism of Raf, we quantitively characterized membrane binding of Raf on supported lipid bilayers. Total internal reflectance fluorescent microscopy measurements were preformed to obtain kinetic and thermodynamic parameters. Our data demonstrate the functional coupling between RBD and CRD domain in which binding of RBD to active Ras promotes lipid engagement of CRD. Detailed kinetic analysis revealed that Raf integrates protein and membrane interactions through kinetically distinct binding modes. The RBD and CRD determine membrane association and dissociation rates, respectively. The functional coupling between RBD and CRD is critical to achieve specific and efficient membrane translocation in response to Ras activation. Our studies provide a novel insight into mechanisms of Raf activation, which requires the extraction of CRD from the autoinhibited complex upon binding of RBD to Ras.

**15-POS Board 4**

## INVESTIGATING THE ROLE OF THE MCUR1 PROTEIN IN THE MITOCHONDRIAL CALCIUM UNIPORTER COMPLEX

**I-Chi Lee**[1]; Ming-Feng Tsai[1];
[1]University of Virginia, Molecular Physiology and Biological Physics, Charlottesville, VA, USA

The Mitochondrial Calcium Uniporter, which is a calcium channel complex that mediates mitochondrial calcium uptake, plays critical roles in maintaining mitochondrial calcium homeostasis, regulating energy production, cell survival, and metabolic balance. MCUR1 was identified as a subunit of the uniporter complex, but its function is currently under debate — whether it is a uniporter regulator, a regulator of the mitochondrial permeability transition pore, or an assembly factor of the cytochrome c oxidase. To investigate MCUR1 function, we generated an MCUR1 knockout (KO) cell line using CRISPR-Cas9. Measuring mitochondrial calcium uptake shows that MCUR1 KO only reduces uniporter function by 15%. Moreover, we did not observe changes in the mitochondrial permeability transition in these cells. While MCUR1 KO causes a modest 15% reduction in the expression of COX1, a protein in the cytochrome c oxidase complex, we found the effect is indirectly caused by altered mitochondrial calcium homeostasis. We have now conducted extensive studies to elucidate MCUR1's molecular properties and interactions within the uniporter complex. Our data show that MCUR1 binds to MCU, MCU paralogue MCUb, and MCUR1, but not EMRE, MICU1, or MICU2. Furthermore, the interaction between MCUR1 and MCU occurs through the transmembrane helix and is highly conserved. Based on our findings, we propose that MCUR1 functions to connect MCU together to enhance the uniporter's ability to generate larger calcium microdomains. These results shed light on the regulatory mechanisms governing mitochondrial calcium transport and underscore the importance of MCUR1 in modulating MCU complex activity.

**19-POS Board 5**

## INVESTIGATING THE ROLE OF LIPOPOLYSACCHARIDE IN OPACITY-ASSOCIATED PROTEIN-HOST RECEPTOR SPECIFICITY AND STRUCTURE

**Connor M McDermott**[1]; Meagan Belcher Dufrisne[1]; Linda Columbus[1];
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Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) are a group of 12 cell surface receptors within the immunoglobulin family. Of the 12 members, four CEACAMs are pathogen binding: CEACAM1, CEACAM3, CEACAM5, and CEACAM6, and of those four, three CEACAMs: CEACAM1, CEACAM5, and CEACAM6, are well-known biomarkers for a variety of cancers. Almost all, CEACAM-ligand interactions occur at the conserved N-terminal domain including CEACAM-pathogen interactions. Neisseria gonorrhoeae (Ng) and Neisseria meningitidis (Nm) are the bacterial species which cause the sexual transmitted infection known as gonorrhea and bacterial meningitidis, respectively, and are both bacterial species that interact with human CEACAMs. These pathogenic Neisseria species are unique amongst CEACAM-targeting pathogens because, unlike other pathogens that bind to CEACAM, Ng and Nm are able to exploit CEACAM binding to trigger uptake into non-phagocytic cells. This interaction is mediated by opacity-associated (Opa) proteins, which are eight-stranded outer membrane (OM) β-barrel proteins with four highly dynamic extracellular loops. Two of these loops contain regions of high amino acid sequence variability known as the hypervariable regions 1 (HV1) and 2 (HV2). Previous research has demonstrated that Opa-CEACAM specificity is dictated by the combination of specific HV sequences, however, the high sequence variability in the HV regions has prevented the identification of a CEACAM binding motif. Previous attempts to study specific Opa proteins in vitro have been unable to replicate the observed in vivo Opa-CEACAM specificity. We hypothesize the inability to recapitulate the in vivo specificity is because previous in vitro binding assays lacked a critical component of the Neisserial OM: lipooligosaccharide (LOS), which is a type of lipopolysaccharide (LPS) expressed by both pathogenic Neisseria species that lacks an O-antigen. Preliminary results show that Opa in the presence of LPS forms an SDS-resistant complex. Furthermore, using a microscale thermophoresis (MST) binding assay with Opa expressed to and subsequently extracted and purified from E. coli OM, we found that the monovalent binding affinity was weak (~ 2 μM). These results will not only help provide a greater understanding of the Neisseria pathogenesis, but provide an understanding of a mechanism that induces phagocytosis in non-phagocytic cells that may be of interest for receptor-mediated cellular uptake of foreign material such as therapeutics.

**23-POS Board 6**

## QUANTIFYING EGF-EGFR BINDING AFFINITIES IN THE PRESENCE OF EGFR HETEROPARTNERS

**Ece Özdemir**[1,2]; Kalina Hristova[1,2];
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Epidermal Growth Factor Receptor (EGFR) is a member of the RTK family which is critically important for the development of the epithelium and has been implicated in many cancers. EGFR is known to have multiple interaction partners in the plasma membrane and some of these heterointeractions have been studied in the literature. Understanding the complexities in EGFR signaling will help us develop better therapeutics in the future that are more specific and targeted. In this project, we investigate how the binding characteristics of EGFR are modulated by interaction partners, such as Fibroblast Growth Factor Receptor1 (FGFR1) and Ecadherin, in the plasma membrane. We quantify the binding of EGF to the EGFR, with and without the partner proteins, using a recently developed model membrane system which allows high-throughput measurements by using confocal microscope. The results reveal a novel way in which interaction partners can modulate EGFR activation and signaling, by altering EGF-EGFR binding.

**27-POS Board 7**

## IDENTIFICATION OF CONFORMATIONALLY-BIASED PHARMACOLOGICAL CHAPERONES FOR SLC6A8 CREATINE TRANSPORTER DEFICIENCY SYNDROME

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Creatine uptake by the SLC6A8 creatine transporter protein (CT1) is critical for energy homeostasis in the central nervous system. Over one hundred loss-of-function (LOF) mutations in the SLC6A8 gene are known to cause creatine transporter deficiency syndrome (CTD)- a spectrum of X-linked neurological disorders associated with intellectual disability, autism, and epilepsy. These incurable disorders fundamentally arise from a loss of CT1 expression and/ or activity, which results in insufficient creatine uptake within the brain. Most CTD mutations appear to promote CT1 misfolding. The misfolding of related SLC6 transporters can be corrected by atypical inhibitors that selectively bind to the inward-facing conformation of the transporter protein. To identify small molecules that correct this primary defect in CTD, we constructed structural homology models of CT1 in each of its conformational states and carried out a virtual screen for conformationally-biased ligands selectively stabilize its inward-facing state. An experimental characterization of our top hits has identified several compounds that enhance the plasma membrane expression CT1. Our most potent pharmacochaperones appear to bind within the substrate binding site and to make extensive contact within creatine's intracellular permeation pathway. These compounds represent promising candidates for the development of pharmacological chaperones for CTD.

**31-POS Board 8**

## DYNAMICS WITHIN THE LONG PERIODICITY PHASE (LPP) OF THE STRATUM CORNEUM

**Alexandra Stuer**[1]; Peter D Olmsted[1];
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The Human Stratum Corneum (SC) is the protective outermost layer of the epidermis. This primary barrier for permeation comprises corneocytes embedded in a complex lipid matrix with many different lipids in a gel-like state. In dermatology or cosmetics, the performance of a particular active ingredient, "active", requires understanding how and whether the active penetrates the lipid matrix. The complexity (composition and structure) and slow dynamics of the lipid matrix create significant challenges that hinder computational studies to investigate molecule permeation across the skin with atomistic resolution. Here we investigate this phenomenon at atomistic resolution using MD simulations to understand the dynamics during permeation in the lipid region. More specifically, we focus on the mobility of the LPP's lipids as well as of the actives. We try to understand their lateral movement within the layers of the LPP as well as between different layers, a phenomenon described in literature as 'flip-flop' and their re-organisation during long production runs. In addition, we examine diffusion of the active inside the LPP both laterally and along the z direction. We conclude that the heterogeneity of the LPP is strongly affected by the mobility of cholesterol and the free fatty acids between the inner bilayers and the slab. The cholesterol depleted and rigid Ceramide-EOS abundant outer leaflet of the bilayers is relatively immobile both along the xy-plane as well as along the bilayer normal. We propose that cholesterol rich regions affect the mobility of small permeants.

**35-POS Board 9**

## INTERACTION OF AMYLOID-BETA PEPTIDES WITH PHOSPHOLIPID BILAYER.

**Ruan van Deventer**[1]; Yuri Lyubchenko[1];
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The amyloid-β peptide, Aβ42, is capable of self-assembly into aggregates of various sizes, and this aggregation process depends on several conditions. Membrane interaction is one of the critical factors involved in the aggregation of Aβ42 at physiological conditions. There is evidence that Aβ42 can change the membrane structure, damaging the membrane through membrane thinning. However, these effects were observed at Aβ42 concentrations significantly higher than physiological, leading to contradictory evidence regarding their relevance. Previously, we have shown that Aβ42, at physiologically relevant low nanomolar concentration, assembles into aggregates but does not damage the bilayer. Here, we investigated the role of the phospholipids' mechanical properties on the interaction with Aβ42 aggregates. We used POPC: POPS-supported lipid bilayers (SLB) with different concentrations and employed time-lapse atomic force microscopy (AFM) to directly visualize the effect of Aβ42 on the bilayer morphology. Equimolar POPC: POPS SLBs were assembled at 0.1 mg/ml and 0.25 mg/ml concentrations, and 50 nM of Aβ42 was subsequently incubated on top of the bilayers. In the case of 0.1 mg/ml SLBs, we revealed assembly of oligomers within hours, followed by the formation of pore-like defects, the number of which grew over time. No such effect was observed for 0.25 mg/ml SLBs. The mechanical properties of the bilayers were characterized, where the Young's modulus of 0.1 mg/ml SLBs were half that of 0.25 mg/ml SLBs. These observations suggest that the Aβ42-induced membrane damage depends on the membrane stiffness, so local mechanical properties of the cell membrane can be a factor resulting in their damage with Aβ42. Membrane damage is considered as one of the factors contributing to the development of Alzheimer's disease, and local mechanical properties of the membrane should be considered as potential factors for disease development.

**39-POS Board 10**

## INVESTIGATING LIPID-DETERGENT SEGREGATION IN BICELLES

**Matthew Necelis**[1]; Christopher Baryiames[1]; Nicole Swope[1]; Linda Columbus[1];
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Bicelles have long been used in biophysical studies of membrane proteins due to the more bilayer like environment they provide on a similar size scale as micelles. A bicelle's inert behavior of self-segregation into its two components has been an area of particular interest for the Columbus Lab. Historically, the composition of bicelles has been mainly focused on DMPC and DHPC (6:0) combinations. Here we present the diversity of bicelle combinations and the multiple ways to observe bicelle segregation. Using small angle x-ray scattering (SAXS) for morphology, and $^{31}$P NMR and Laurdan fluorescence for lipid behavior, we've characterized several lipid/detergent combinations. Short Lyso PC detergents, being in a less stable mixed micelle, exhibited a distinct shift in the observed segregation behavior when compared to the two tailed DH6PC. This is indicative that the overall geometry of the detergent monomers has a significant impact on the packing and stabilization of DMPC bicelles.

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

Wednesday and Thursday posters are available for viewing during the Wednesday and Thursday poster sessions. However, below are the formal presentations for Thursday. 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

| | | |
|---|---|---|
| **Alex Compton** | **4-POS** | **Board 11** |
| **Yu-Lun Huang** | **8-POS** | **Board 12** |
| **Sachin Katti** | **12-POS** | **Board 13** |
| **Ming-Tao Lee** | **16-POS** | **Board 14** |
| **Nanqin Mei** | **20-POS** | **Board 15** |
| **Tugba Ozturk** | **24-POS** | **Board 16** |
| **Thorsten Schmidt** | **28-POS** | **Board 17** |
| **Harry Thorne** | **32-POS** | **Board 18** |
| **Liying Wu** | **36-POS** | **Board 19** |
| **Sangbae Lee** | **40-POS** | **Board 20** |
| **Sandor Volkan-Kacso** | **41-POS** | **Board 21** |

Posters should be set up on the morning of Wednesday, June 5, and removed by 11:00 PM on Thursday, June 6. All uncollected posters will be discarded.

**4-POS Board 11**

## SNARE MIMICRY BY CD225 PROTEINS, INCLUDING IFITM3, UNDERLIES MEMBRANE TRAFFICKING REGULATION

Kazi Rahman[1]; Isaiah Wilt[1]; Siddhartha Datta[1]; **Alex A. Compton**[1];
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The CD225 superfamily consists of integral membrane proteins that regulate vesicular transport and membrane fusion events driving critical cellular functions, including glucose transport, neurotransmission, and antiviral immunity. However, how the conserved CD225 domain contributes to the diverse roles played by CD225 proteins during membrane trafficking was unknown. Here, we reveal that the CD225 domain contains a SNARE-like motif that enables interaction with cellular SNARE fusogens and modulation of SNARE complex formation. CD225 member interferon-induced transmembrane protein 3 (IFITM3) exhibits broad-spectrum antiviral activity and has been shown to accelerate the trafficking of incoming virions to lysosomes for degradation. We demonstrate that IFITM3 interacts with and regulates endosomal SNARE proteins responsible for homotypic late endosome fusion. Specifically, IFITM3 binds to Q-SNARE syntaxin 7 in cells and in vitro, and this interaction requires key residues residing within a SNARE-like motif in IFITM3. Mutations in IFITM3 that abrogate syntaxin 7 binding exhibited a loss of antiviral activity against Influenza A virus. IFITM3 deficiency in human cells resulted in a greater extent of co-immunoprecipitation between Q-SNARE syntaxin 7/8 and R-SNARE VAMP8, while IFITM3 overexpression inhibited this interaction. Structural modeling showed that IFITM3 adopts a VAMP-like fold that may permit displacement of VAMP8 from the SNARE complex controlling homotypic late endosome fusion. Our results suggest that IFITM3 selectively inhibits homotypic fusion of late endosomes, a pathway ensuring persistence and recycling of endocytic cargo (including viruses). Therefore, our findings invoke a mechanistic model whereby IFITM3 diverts incoming virus towards lysosomes by preventing homotypic late endosome fusion via a SNARE-like motif in its CD225 domain. Importantly, SNARE-binding activity and its determinants were shared among other members of the CD225 superfamily, including PRRT2 and TUSC5, suggesting that SNARE modulation plays a previously unrecognized central role in the diverse membrane trafficking functions performed by these proteins.

**8-POS Board 12**

## PROBING CONFORMATIONAL CHANGES OF THE MITOCHONDRIAL CALCIUM UNIPORTER USING TRANSITION METAL ION FRET

**Yu-Lun Huang**[1]; Ming-Feng Tsai[1];
[1]Physiology and Biophysics, Aurora, CO, USA

The mitochondrial calcium uniporter is a $Ca^{2+}$ channel that mediates $Ca^{2+}$ influx into the mitochondrial matrix. The uniporter plays a crucial role in regulating ATP synthesis, apoptosis, and intracellular calcium signaling. The uniporter contains the pore-forming MCU subunit, the auxiliary EMRE protein, and the regulatory MICU1/MICU2 subunits. It's known that EMRE binds to MCU to open the pore, and that MICU1 gates MCU by blocking/unblocking the pore via a "ball-and-chain" mechanism. However, the mechanism by which EMRE induces conformational changes to open MCU remains unclear. Moreover, the kinetics of MICU separation from MCU is unknown. Transition metal ion FRET (tmFRET) is a powerful tool to elucidate protein conformational dynamics. I introduced a tmFRET acceptor/donor pair into the transmembrane helix 1 and coiled-coil 2 (CC2) in MCU to test the hypothesis that EMRE binding to MCU causes the movement of CC2 away the center of the pore to open the channel. I am also in the process of using stopped-flow tmFRET to determine the rate constants governing $Ca^{2+}$-induced MICU separation from MCU. These studies represent an exciting direction to gain new insights into the molecular mechanisms underlying the uniporter's function.

**12-POS Board 13**

## STRUCTURAL BASIS OF BRYOSTATIN-1 INTERACTIONS WITH PERIPHERAL MEMBRANE BINDING C1 DOMAINS OF PROTEIN KINASE C

**Sachin S Katti**[1]; Tien Nguyen[1]; Lokendra Poudel; Savana M Green[1]; Vytas A Bankaitis[1]; Tatyana I Igumenova[1,2];
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Several isoforms of Protein Kinase C (PKC) family are lipid-activated Ser/Thr kinases that translate GPCR-mediated external stimuli into intracellular signaling events. Pharmacological modulation of PKC activity at membranes holds significant promise in the treatment of various cancers and neurodegenerative disorders. To that end, several classes of lipophilic compounds have been identified that target the diacylglycerol (DAG)-dependent membrane-anchoring modules of PKC regulatory region, the conserved homology 1 domains (C1 domains). Among the DAG-mimicking macrocyclic lactones, Bryostatin-1 has gained considerable traction due to its antineoplastic, neuroprotective, and anti-retroviral activities observed in clinical studies. However, the mechanism of Bryostatin-1 driven PKC activation at the membrane remains poorly characterized. In this work, we integrate solution NMR spectroscopy, atomistic molecular dynamics simulations, and cellular membrane translocation assays to describe the interactions of Bryostatin-1 with the DAG-sensitive C1 domains of PKC$\alpha$ and ε isoforms. NMR of the Bryostatin-1-complexed C1 domain in lipid bicelles identified the protein residues that undergo membrane insertion upon complex formation. Additional information on the geometry of protein-membrane interactions and the isoform-specific dynamics of protein and Bryostatin-1 was obtained from atomistic MD simulations. Consistent with those findings, in-cell imaging experiments show clear isoform-specific differences in Bryostatin-1-induced membrane translocation properties. Taken together, this integrative approach offers new insights into future design of effective Bryostatin analogs.

**16-POS Board 14**

## MEMBRANES MEDIATED INHIBITION ON THE CHAPERONE ACTIVITY OF ALPHA-CRYSTALLINS

Jui-Kai Chen[1]; Jheng-Hao Lin[1]; Yu-Ting Liu[1,2]; **Ming-Tao Lee**[1,2];
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alphaA-crystallin and alphaB-crystallin, the water soluble proteins, are able to carry out their chaperone activtiy to maintain the transparency of eye lens. The cataract, induced by light scattering from eye lens clouding, is the major cause of the blindness for the older people. Previous studies show denature of alpha-crystallins cause abnormal aggregation of proteins and consequently lead to eye lens clouding. The mechanism is still a puzzle. Besides protein structure, the interaction between alpha-crystallins and membranes have been considered as possible mechanism of the function loss. In this study, the different thicknesses of lipid membranes composed of Di18:1PC (DOPC) and Di20:1PC are used to be model membranes and interact with alpha-crystallins. The lamellar X-ray diffraction (LXD) was used to probe the structural change of lipid membranes to investigate the effect of hydrophobic match between alpha-crystallins and lipid membranes. ADH and insulin assays were used to monitor the chaperone activity of alpha-crystallins in the presence and absence of lipid membranes. Our result indicates the insertion of alpha-crystallins into lipid membranes decreases their chaperone activity. Furthermore, hydrophobic mismatch between alpha-crystallins and thinner membranes may recover their chaperone activity via suppressing their insertion into membranes. We proposed the inhibition on chaperone activity of alpha-crystallins was mediated by membranes.

**20-POS Board 15**

## PROTECTIVE EFFECT OF MELATONIN AGAINST AMYLOID-BETA-INDUCED DAMAGE IN MODEL LIPID MEMBRANES.

**Nanqin Mei**[1,2]; Carina T Filice[3]; Julia I Lumini[3]; Jingwen Liang[1]; Danielle McRae[1]; Weilin Wei[4]; Ayumi Sumino[4]; Takeshi Fukuma[4]; Zoya Leonenko[1,2,3];
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[4]Kanazawa University, Nano Life Science Institute, Kanazawa, Japan

In Alzheimer's disease (AD), amyloid toxicity to neuronal membranes results in cell damage and dysfunction, with the nature of toxicity changing with age and disease states. Our prior work showed that the structure and composition of model lipid membranes are crucial factors in this interaction. This study explores the potential protective effect of melatonin against amyloid toxicity by altering various membrane properties. We exposed model lipid membranes, representing both healthy and diseased states, to melatonin and examined the resulting effects on membrane properties as well as the resulting changes in amyloid interaction. Using Localized Surface Plasmon Resonance (LSPR), we measured amyloid accumulation on membranes with/without melatonin's presence. The Black Lipid Membrane (BLM) technique was employed to study changes in permeability of membranes under amyloid attack, measuring melatonin's impact on this change. Additionally, we used Atomic Force Microscopy (AFM) and High-Speed AFM (HS-AFM) to image and record realtime videos of amyloid-membrane interactions. Our findings demonstrate that the protective effect of melatonin against amyloid toxicity varies with the disease state of the membrane and is more effective in membranes mimicking early disease stages. These findings may aid the development of novel preventative strategies towards AD.

**24-POS Board 16**

## CHARACTERIZATION OF FRANCISELLA TULARENSIS LIPIDS

**Tugba N. Ozturk**[1]; W. F. Drew Bennett[1]; Timothy S Carpenter[1]; Helgi I Ingolfsson[1]; [1]Lawrence Livermore National Laboratory, Physical and Life Sciences Directorate, Livermore, CA, USA

Francisella tularensis (Ft) is a highly pathogenic species of gram-negative bacteria, capable of effectively invading host cells and suppressing the host immune system to facilitate infection. Ft lipids exhibit a unique tail-length asymmetry; the most abundant Ft phospholipids have acyl chain lengths of C24:0-C10:0, which is significantly more asymmetric compared to, for example, phosphatidylcholine lipids in budding yeast membranes, which have acyl chain lengths of C16:1-C16:1 or C18:1-C16:1. Characterizing Ft's unique tail-length-asymmetric lipids is essential to understand Ft pathogenesis. We hypothesize that when invading mammalian membranes, unique Ft lipids significantly alter the host membrane properties. In this study, we will carry out coarse-grained and all-atom molecular dynamics simulations to test this hypothesis by contrasting and comparing structural properties of Ft, Ecoli and mammalian membranes and studying how the asymmetric incorporation of Ft lipids into mammalian membranes alters their properties. By investigating the molecular mechanism of Ft's unique tail-length-asymmetric lipids, we hope to identify novel Ft countermeasures that achieve specificity by targeting Ft's unique tail-length-asymmetric lipids.

**28-POS Board 17**

## DNA-BASED LIPID BILAYER MIMETICS FOR CRYO EM OF MEMBRANE PROTEINS

**Thorsten Schmidt**;
[1]Kent State University, Physics, Kent, OH, USA

DNA is a unique polymer. It is the information storage molecule of all known life forms but can also be used to form nanoscale structures that are not found in nature. Our group is leveraging this programmability to engineer nanoscale architectures and tools for applications in Biophysics and Structural Biology. One goal of our lab is to establish DNA-lipid nanodiscs as a new customizable nanoscale lipid bilayer mimetic for single-particle cryo-EM of membrane proteins. In our first approach (Iric et al. Nanoscale 2018, 18463–18467.), we functionalized the inside of a DNA minicircle with hydrophobic side chains to hold a lipid bilayer. Alternatively, we are employing amphiphilic peptides and polymers to modulate interactions with lipids while retaining good water solubility and preventing aggregation. Our DNA nanotechnology-based approach will overcome some existing limitations of established bilayer mimetics and offer unprecedented control over structural, chemical, and physical design parameters. We expect DNA-based systems to enable qualitatively new types of experiments in structural biology and single-molecule biophysics.

**32-POS Board 18**

## INVESTIGATING HOW HOPANOIDS MAINTAIN FUNCTIONAL EUKARYOTIC MEMBRANE PROPERTIES DURING HYPOXIA.

**Harry Thorne**[1]; Maria Makarova[1];
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Sterols are highly important membrane constituents that are critical for maintaining the integrity and functional biophysical properties of eukaryotic membranes. Through lipid-lipid interactions, sterols play pivotal roles in modulating the fluidity, permeability, lipid organisation and phase behaviour of membranes. However, sterol biosynthesis can only occur in the presence of molecular oxygen. Therefore, this poses the question of how, in the absence of oxygen, can functional eukaryotic membrane properties be maintained. Here, we investigate how the biosynthesis of hopanoids, a class of cyclic triterpenoids with structural similarities to eukaryotic sterols, facilitates the sterol-independent anaerobic growth of the fission yeast Schizosaccharomyces japonicus. This study aims to determine whether diploptene and diplopterol can functionally compensate for the absence of ergosterol through imparting similar membrane properties. We will employ advanced microscopy and polarity sensitive probes to quantitatively characterise the level by which hopanoids, and ergosterol, induce order within eukaryotic model membranes, both in vitro and in vivo. Using genetic tools and antifungal drugs, membrane lipid compositions of live cells can be carefully manipulated, allowing for differential measurements of lipid order, induced by hopanoids and/or ergosterol, to be linked to cell fitness. The expected significance from this study will aid our understanding of how novel, non-canonical eukaryotic lipids can be leveraged for environmental adaptation to oxygen availability and antifungal drugs.

**36-POS Board 19**

## NANOSCALE ORGANIZATION AND DYNAMIC OF SUN1 LINC COMPLEX AS MECHANOTRANSDUCING COORDINATOR ACROSS NUCLEAR MEMBRANES

**Liying Wu**[1]; Fabien Pinaud[1,2];
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The nucleus is a central coordinator of cellular adaption to environmental forces, and its defective mechanical responses can lead to severe diseases, such as muscular dystrophies or premature aging. At the nuclear membrane, mechanical signals are transduced via the LINC complex (SUN1, SUN2, emerin, nesprin), which bridges the space between the outer nuclear envelope (ONE) and the inner nuclear envelope (INE) to transmit bidirectional forces between the cytoskeleton and the nucleoskeleton. While considerable progress was made to understand the structure and composition of LINC complexes, how they integrate and convey mechanical signals across the two membranes of the NE remains unclear. To assess how LINC complexes might organize as a function of force, we studied the nanoscale spatial distribution and the mobility of SUN1 using single molecule tracking and 3D super-resolution microscopy, while modulating nuclear mechanics in cells. We found that SUN1 protomers rapidly assemble as trimers after ER membrane insertion and accumulate as slow diffusing homo-trimers (33%) and near-immobile nanoclusters (61%) at the INE. Mechanical challenges to the nucleus induce an increase mobility of SUN1 and a local de-clustering of SUN1 homotrimers in a SUN1/nesprin interaction-dependent manner. We also identified multiple amino acid residues in SUN1 that participate in two distinct force transmission steps across the NE: (i) force transfer initiation at the ONE via SUN1/nesprin contacts and (ii) force transfer regulation across the NE by the SUN1 coiled-coil backbone, via (de)clustering of SUN1. Those residues and the clustering state of SUN1 homotrimers directly impact the stability and the shape of the NE. Together, our research provides an initial physical model of how LINC complexes function as mechanotransducing hubs across the NE to modulate nuclear mechanics in cells.

**40-POS Board 20**

## IN-SILICO VALIDATION OF BINDING AFFINITIES FOR BIOLOGICAL GPCRS WITH BAR ALGORITHM

**Sangbae Lee**; Minkyu Kim[1]; Jian Jeong[1]; Donghwan Kim[1]; Sangbae Lee[1]; Art E Cho[1]; [1]inCerebro, Seoul, South Korea

Binding affinity prediction of ligand-receptor complex structure using computational approach often falls short in providing satisfactory validation of experimental results due to frequent insufficient sampling. As a solution to these challenges, re-cent emphasis has been placed on the re-sampling of new trajectories, and in this study, we propose a simulation protocol that achieves efficient sampling. We have re-engineered the widely used Bennett acceptance ratio (BAR) method as a representative approach and tested its efficacy across various membrane protein targets to verify its efficient applicability to the targets. We chose GPCR (G-protein coupled receptor) targets with diverse structural landscapes and experimentally validated binding affinities. Subsequently, using BAR-based binding free energy, we confirmed correlations to demonstrate the validity and performance of the computational approach.

**41-POS Board 21**

## EXTRACTING SHORT-LIVED STATES IN SINGLE F-ATPASE MOLECULAR MOTORS

**Sandor Volkan-Kacso**[1,2];
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Recently, we proposed a method to analyze fast rotation trajectories in F1-ATPase using the distribution of angular velocity. The analysis involves the transitions during the stepping between pauses. A theoretical-computational approach is used to model the fluctuation of the imaging probe as the molecular motor undergoes stepping rotation. A key quantity in this method is the angular velocity vs. rotation angle extracted from both experimental data and computer simulations. When applying the method on Thermophilic Bacillus F1-ATPase rotation data, we detected the presence of a short-lived substep previously not detectable in the histograms. The comparison between the experimental and theory reveals that an $80^{O}$ substep of the "concerted" ATP binding and ADP release involves an intermediate state reminiscent of a 3-occupancy structure. Its lifetime (~10 μs) is about six orders of magnitude smaller than the lifetime for spontaneous ADP release. By detecting this short-lived state the method provides "temporal super-resolution". Most recently, this method was applied to single-molecule imaging data from Paracoccus Denitrificans F1-ATPase and it yielded a similar hidden state in the transitions between dwells. Our recent findings indicate a common mechanism for the acceleration of ADP release in the F1-ATPase motor of the two species.