Beyond Simple Models: The Consequences of Membrane Complexity in Living Systems

Copenhagen, Denmark | July 7-10, 2025

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July 2025

Dear Colleagues,

We welcome you to the Biophysical Society Thematic Meeting entitled, ***Beyond simple models: the consequences of membrane complexity in living systems***. This meeting will broadly explore the emergent properties and functional roles arising from the lipid and protein complexity of living membranes. Presentations and discussions will span a wide range of synthetic and living membrane models, and methodologies ranging from computational modeling to state-of-the-art microscopy and spectroscopy. Finally, this meeting is designed as a celebration of the life and science of Luis Bagatolli, a champion of membrane biophysics across the decades and around the world.

The conference is organized into 12 distinct sessions, each with 3 invited talks, a talk chosen from submitted abstract, and a 15-minute round-table discussion moderated by the Session Chair. We hope these discussions will resolve controversies, identify new research directions, and promote collaborations and connections. These discussions must be conducted with civility, respect, and good-faith argumentation; organizers and moderators will actively manage any departures from these principles.

We invite you to actively take part in these discussions, the vibrant poster sessions, and the informal exchanges during the coffee breaks and meals. We also hope that you will also enjoy the beautiful city of Copenhagen!

Finally, we would like to thank our generous sponsors: University of Copenhagen-Department of Chemistry, Lipotype GmbH, The Company of Biologists (Journal of Cell Science), American Chemical Society (ACS) Publications, the FEBS Journal, and the Biophysical Journal.

The Organizing Committee:
Ilya Levental
Ed Lyman
Dimitrios Stamou
Juan Vanegas
Sarah Veatch

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

The meeting will take place at the University of Copenhagen, Department of Chemistry, located at Ole Maaløes Vej 5, 2100 København, Denmark. The BPS Registration Desk, to pick up your badge and meeting materials, will be located in the Auditorium Foyer during the following times:

Monday, July 7	7:45 – 18:30
Tuesday, July 8	8:30 – 17:00
Wednesday, July 9	8:30 – 12:30
Thursday, July 10	8:30 – 15:00

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in the Lundbeckfond Auditorium. 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 Auditorium Foyer.

2) A display board measuring 90 cm by 190 cm (portrait orientation) will be provided for each poster. Poster should be printed in A0 format 84.1 cm wide x 118.9 cm high (portrait orientation). Poster boards are numbered according to the same numbering scheme as listed in the E-book.

3) There will be formal poster presentations on Monday and Tuesday from 15:00 – 17:00. Please refer to the daily schedule for your formal presentation date and time. Poster should be set up the morning of your formal presentation date and removed by 18:00 by the end of your presentation date. Two hours (120 minutes) have been allotted for poster presentations each day. Presenting authors with odd-numbered poster boards should present during the first 60 minutes, and those with even-numbered poster boards should present during the last 60 minutes.

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

5) All posters left uncollected at the end of the meeting will be discarded.

Note Pads/Pens
Society pens will be provided, however please bring your own note pad.

Meals, Coffee Breaks, and Socials
There will be a Welcome Reception on Monday evening from 18:40 – 20:00 in the Canteen.

Coffee Breaks (Monday, Tuesday, Wednesday, and Thursday) will be served in the Canteen.

Lunches (Monday, Tuesday, and Thursday) will be served in the Canteen.

Smoking
Please be advised that the University of Copenhagen is a non-smoking facility.

Name Badges
Name badges will be given to you when you check-in at the Registration Desk in the Auditorium Foyer. Badges are required to enter all scientific sessions, poster sessions, and social functions. Please wear your badge throughout the conference.

Internet
Wi-Fi will be provided at the venue. Information will be available at the registration desk.

On-Site Contact Information
If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from July 7-10 during registration hours.

In case of emergency, you may contact the following:

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

Umi Zhou
Phone: 703-507-0523
Email: uzhou@biophysics.org

Beyond Simple Models:
The Consequences of Membrane Complexity in Living Systems

Copenhagen, Denmark
July 7-10, 2025

All scientific sessions will be held in the Lundbeckfond Auditorium unless otherwise noted.

PROGRAM

Monday, July 7, 2025

7:45 – 18:30	Registration/Information	**Auditorium Foyer**
8:45 – 9:00	***Welcome and Opening Remarks***	
Session I	**Lipid Sensing and Homeostasis** Chair: Ilya Levental, University of Virginia, USA	
9:00 – 9:20	Itay Budin, University of California, San Diego, USA ***Visualizing Nanoscale Heterogeneity in Cell Membranes Using Fluorogenic Probes***	
9:20 – 9:40	Robert Ernst, University of Saarland, Germany ***Membrane Property Sensors for Membrane Homeostasis and Adaptation***	
9:40 – 10:00	Maria Laura Fanani, Universidad Nacional de Córdoba, Argentina ***Bioactive Amphiphiles and Lipid Membranes: Unraveling the Biophysical Foundations of Functional Interactions***	
10:00 – 10:15	Carolyn Shurer, University of Virginia, USA * ***Probing Biophysical Paralipidomes: A Novel Toolbox for Studying Lipid Nano-Environments in Live Cells***	
10:15 – 10:30	Round Table Discussion	
10:30 – 11:00	**Coffee Break**	**Canteen**
Session II	**Consequences of Lipid Asymmetry** Chair: Ilya Levental, University of Virginia, USA	
11:00 – 11:20	Maria Makarova, University of Birmingham, United Kingdom ***Membrane Remodeling Strategies for Low Oxygen Survival in Eukaryotes***	
11:20 – 11:40	Erwin London, Stony Brook University, USA ***Constitutive Plasma Membrane Raft Formation in Both Exponential and Stationary Growth Phase Saccharomyces Cerevisiae and Other Fungi: Relationship to Vacuolar Rafts***	
11:40 – 12:00	Milka Doktorova, University of Stockholm, Sweden	

	Phospholipid Number Imbalance: An Emerging New Angle of Plasma Membrane Organization
12:00 – 12:15	Rana Ashkar, Virginia Tech, USA * ***Unified Laws of Membrane Elasticity: From Fundamental Principles to Practical Applications***
12:15 – 12:30	Round Table Discussion
12:30 – 13:30	**Lunch** **Canteen**
Session III	**Sterols and Hopanoids as Membrane Masters** Chair: James Saenz, Technische Universität Dresden, Germany
13:30 – 13:50	Arun Radhakrishnan, UT Southwestern Medical Center, USA ***Chemical Activity of Cholesterol in Membranes***
13:50 – 14:10	Sarah Keller, University of Washington, USA ***Micron-Scale, Liquid-Liquid Phase Separation in Vacuole Membranes in Living Yeast***
14:10 – 14:30	Alex Sodt, NIH, USA ***Membrane Mechanical Effect of Small Changes in Lipid and Sterol Chemical Structure***
14:30 – 14:45	Ankur Gupta, University of Copenhagen, Denmark * ***Exploiting Plasma Membrane Heterogeneity to Delineate Molecular Mechanisms of GPCR Potency and Efficacy***
14:45 – 15:00	Round Table Discussion
15:00 – 17:00	**Poster Session I** **Auditorium Foyer**
Session IV	**Special Symposium on the Science of Luis Bagatolli** Chair: John Ipsen, Copenhagen University, Denmark
17:00 – 17:15	Adam Cohen Simonsen, University of Southern Denmark * ***Synergy Between Membrane Curvature and Area Expansion in A Mechanism of Plasma Membrane Repair***
17:15 – 17:30	Yannick Azhri Din Omar, Massachusetts Institute of Technology, USA * ***Beyond Saffman–Delbrück Theory: Diffusion of Membrane Protein Aggregates***
17:30 – 17:50	Leonel Malacrida, Institut Pasteur de Montevideo, Uruguay ***The Phasor Approach for Studying Cellular Complexity with Dan Probes in Membranes and Cellular Crowding: Persuading Luis Constituted a Remarkable Journey Spanning Over a Decade of Friendship***
17:50 – 18:10	Natalia Wilke, Universidad Nacional de Córdoba, Argentina **– *Lipotype Sponsored Speaker*** ***The Selectivity of the Antimicrobial Peptide Mp1 Is Controlled by Multiple Membrane Factors***
18:10 – 18:25	Johannes Thoma, University of Gothenburg, Sweden * ***In-Situ Structure of the Bacterial Outer Membrane Protein A***

18:25 – 18:40	Round Table Discussion	
18:40	**Reception**	**Canteen**

Tuesday, July 8, 2025

8:30 – 17:00	Registration/Information	**Auditorium Foyer**
Session V	**Advances in Modeling Biomembranes** Chair: Edward Lyman, University of Delaware, USA	
9:00 – 9:10	Kandice Levental, University of Virginia * ***Plasma Membrane Cholesterol Asymmetry Regulates Cellular Cholesterol Homeostasis***	
9:10 – 9:20	Benjamin Machta, Yale University, USA * ***Condensate Prone Bulk Mixtures Can Prewet Membranes***	
9:20 – 9:40	Naomi Oppenheimer, Tel Aviv University, Israel ***Flow, Friction, and Elasticity: Theoretical Insights into Membrane Mechanics***	
9:40 – 10:00	Mark Uline, University of South Carolina, USA ***Using Molecular Field Theories to Study Plasma Membrane Complexity***	
10:00 – 10:15	Yuka Sakuma, Tohoku University, USA * ***Long-Range Membrane Viscosity of Living Cell***	
10:15 – 10:30	Round Table Discussion	
10:30 – 11:00	**Coffee Break**	**Canteen**
Session VI	**Engineering Lipid Metabolism in Microbiology** Chair: Itay Budin, University of California, San Diego, USA	
11:00 – 11:20	Chad Leidy, Universidad de los Andes, Colombia ***The Membrane of Staphylococcus Aureus: Modulation of Membrane Biophysical Properties and the Inhibition of Antimicrobial Peptide Activity***	
11:20 – 11:35	Raya Sorkin, Tel Aviv University, Isreal * ***Pressure-Dependent Tension Propagation in Crumpled Cell Membranes***	
11:35 – 11:55	Diego de Mendoza, IBR-CONICET, Argentina ***The Role of Lipid Environment in Membrane Protein Signaling and Catalysis***	
11:55 – 12:10	Camilo Aponte-Santamaria, Max Planck Institute for Polymer Research, Germany * ***Staphyloxanthin Modifies the Structure of Bacterial-Model Phosphoglycerol Membranes***	
12:10 – 12:25	Anand Srivastava, Indian Institute of Science, Bangalore, India * ***Soft Dynamic Channels in Liquid Ordered Lipid Membrane***	
12:25 – 12:40	Round Table Discussion	
12:40 – 13:30	**Lunch**	**Canteen**

Session VII	**Lipidomic Complexity and Engineering** Chair: Sarah Keller, University of Washington, USA	
13:30 – 13:50	Andre Nadler, Max Planck Institute of Molecular Cell Biology and Genetics, Germany ***Tracking Lipids Through Physical and Chemical Space***	
13:50 – 14:10	Takeshi Harayama, CNRS-IPMC, France ***Regulation of Plasma Membrane Organization by Lipid Tail Lengths***	
14:10 – 14:30	Isabella Graf, European Molecular Biology Laboratory, Germany ***Critical Behavior in Multicomponent Mixtures with Structured Interactions: From Tuning to Function***	
14:30 – 14:45	Sergei Sukharev, University of Maryland, USA * ***On the Lipid Dependence of Bacterial Mechanosensitive Channels Gating***	
14:45 – 15:00	Round Table Discussion	
15:00 – 17:00	**Poster Session II**	**Auditorium Foyer**

Wednesday, July 9, 2025

8:30 – 12:30	Registration/Information	**Auditorium Foyer**
Session VIII	**Protein Regulation by Lipids** Chair: Sarah Veatch, University of Michigan, USA	
9:00 – 9:20	Karen Fleming, Johns Hopkins University, USA ***The Lipid Bilayer Modulates Membrane Protein Energetics***	
9:20 – 9:40	Matt Eddy, University of Florida, USA ***Mechanisms of Lipid-Driven GPCR Activation***	
9:40 – 10:00	Anna Duncan, Aarhus University, Denmark ***Protein-Lipid Interactions in Complex Crowded Membranes***	
10:00 – 10:15	Sami Tuomivaara, University of Helsinki, Finland * ***A Cell-Based Scrambling Assay Reveals Phospholipid Headgroup Preference of Tmem16f on the Plasma Membrane***	
10:15 – 10:30	Round Table Discussion	
10:30 – 11:00	**Coffee Break**	**Canteen**
Session IX	**Membrane Mechanobiology** Chair: Juan Vanegas, Oregon State University, USA	
11:00 – 11:20	Satyajit Mayor, University of Warwick, United Kingdom ***Active Stresses Functionally Shape the Local Composition of the Living Cell Membrane***	
11:20 – 11:40	John Ipsen, Copenhagen University, Denmark	

	To See or Not to See: Lateral Organization of Biological Membranes
11:40 – 12:00	Margarita Staykova, Durham University, United Kingdom ***Interstitial Hydrodynamic Instabilities Sculpt Cell Adhesions***
12:00 – 12:15	Peter Kasson, Georgia Institute of Technology, USA * ***Membrane Complexity in Enveloped Viral Entry: Multifactorial Effects and Hints of Emergent Phenomena***
12:15 – 12:30	Round Table Discussion
12:30 – 17:00	**Free Time**

Thursday, July 10, 2025

8:30 – 15:00	Registration/Information	**Auditorium Foyer**
Session X	**Advances in Imaging and Spectroscopy** Chair: Sarah Veatch, University of Michigan, USA	
9:00 – 9:20	Jay Groves, University of California, Berkeley, USA ***Stochastic Signaling Reactions on the Membrane Surface***	
9:20 – 9:40	Ana Garcia-Saez, Max Planck Institute for Biophysics, Germany ***Shedding New Light on Mitochondrial Permeabilization in Apoptosis***	
9:40 – 10:00	Kenichi Suzuki, Gifu University, Japan ***Regulation Mechanisms of Receptor and Signaling Molecule Activities in Lipid Domains as Revealed by Single-Molecule Imaging and Super-Resolution Microscopy***	
10:00 – 10:15	Robert Vacha, Masaryk University, Czech Republic * ***Peptide Translocation Across Asymmetric Phospholipid Membranes***	
10:15 – 10:30	Round Table Discussion	
10:30 – 11:00	**Coffee Break**	**Canteen**
Session XI	**Coupling Between Cytoplasmic Condensates and Membrane Structure** Chair: Benjamin Machta, Yale University, USA	
11:00 – 11:20	Jeanne Stachowiak, University of Texas at Austin, USA ***Intrinsic Disorder as an Organizing Principle for Biological Membranes Biology***	
11:20 – 11:40	Rumiana Dimova, Max Planck Institute of Colloids and Interfaces, Germany ***Shaping Membranes with(out) Proteins: How Simple Physicochemical Factors Drive Curvature and Remodeling***	
11:40 – 12:00	Siddharth Deshpande, Wageningen University, The Netherlands ***Flowers, Petals & Buds: Membrane Remodeling Via Associative and Segregative Phase Separation***	
12:00 – 12:15	Sasiri Vargas Urbano, University of Delaware, USA * ***Pressure Dependent Elastic Constants of Membranes***	

12:15 – 12:30	Round Table Discussion
12:30 – 13:30	**Lunch** **Canteen**
Session VII	**Membrane Organization in vivo** Chair: Dimitrios Stamou, University of Copenhagen, Denmark
13:30 – 13:50	James Saenz, Technosphere Universität Dresden, Germany ***Hopanoids and the Evolution of Lipid Ordering***
13:50 – 14:10	Erdinc Sezgin, Karolinska Institute, Sweden ***Biophysical Properties of Cells and Nanoscale Bioparticles as New Bi-Omarkers in Health and Disease***
14:10 – 14:30	Anne Kenworthy, University of Virginia, USA ***Biophysical Basis for Caveolae Formation and Function***
14:30 – 14:45	Stefanie Schmieder, Boston Children's Hospital, USA* ***A Functionalized GM1 Structural Library Reveals Distinct Membrane Organization***
14:45 – 15:00	Round Table Discussion
15:00 – 15:30	**Closing Remarks and *Biophysical Journal* Poster Awards**

**Contributed talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

VISUALIZING NANOSCALE HETEROGENEITY IN CELL MEMBRANES USING FLUOROGENIC PROBES

Itay Budin
University of California, San Diego, USA

No Abstract

MEMBRANE PROPERTY SENSORS FOR MEMBRANE HOMEOSTASIS AND ADAPTATION

Jona Causemann[1,2]; Barbara Schmidt[2,4]; Thorsten Mosler[3]; Heiko Rieger[2,4]; Ivan Dikic[3]; **Robert Ernst**[1,2];
[1]Saarland University, Medical Biochemistry and Molecular Biology, Saarbrücken, Germany
[2]Saarland University, Center for Biophysics, Saarland, Germany
[3]Goethe University Frankfurt, Biochemistry II, Frankfurt, Germany
[4]Saarland University, Theoretical Physics, Saarbrücken, Germany

Biophysical membrane homeostasis and adaptation is crucial for maintaining biomembrane functions in response to stress and metabolic perturbation. All living organisms use sophisticated sensory mechanisms to regulate key membrane properties such as membrane compressibility or membrane fluidity. One of the key challenges of membrane property sensing is the necessity to integrate and amplify small, fluctuating signals from the lipid bialyer, in order to make robust decisions with wide impact on lipid metabolism, membrane biogenesis, and cell fate. Using the lipid saturation sensor Mga2 as a paradigm, we show that signal amplification can be provided by an energy-consuming reaction, which involves a covalent modification for the sensor protein with ubiquitin molecules. Ultimately, ubiquitylation of Mga2 provides a means to proteolytically process Mga2 and upregulate fatty acid desaturation. Using biochemical reconstitution and physical modeling, our work uncovers a sophisticated mechanism of signaling amplification for biophysical membrane homeostasis and adaptation.

BIOACTIVE AMPHIPHILES AND LIPID MEMBRANES: UNRAVELING THE BIOPHYSICAL FOUNDATIONS OF FUNCTIONAL INTERACTIONS

Maria L Fanani[1,2];
[1]Universidad Nacional de Cordoba, Biological Chemistry Ranwel Caputto, School of Chemical Science, CORDOBA, Argentina
[2]National Scientific and Technical Research Council - Argentina (CONICET), Center for Research in Biological Chemistry of Córdoba (CIQUIBIC), CORDOBA, Argentina

Amphiphilic bioactive molecules are vital for health, disease prevention, and biological regulation. Their interaction with cell membranes and lipid structures defines their bioactive properties. Unlike bioactive compounds targeting specific protein receptors, those interacting with membranes primarily engage through numerous weak interactions and entropic factors, determining their function. Thus, amphiphile selectivity is heavily influenced by the rheological properties of target membranes. Amphiphilic compounds also effectively modulate membrane rheology, which can affect the insertion of other membrane-associated components.In contrast to lipids, which have low solubility in aqueous environments, amphiphilic bioactive compounds generally exhibit a critical micelle concentration in the micromolar range, facilitating dynamic exchanges of monomeric amphiphiles between micelles and the target membrane. Introducing a new component to a membrane can alter surface properties through various physical mechanisms. The new component may exhibit varying partitioning in different lipid hemilayers and phases. This transient imbalance in compositional and physical membrane properties can create tension and catalyze membrane-related processes.We've analyzed the effects of asymmetric amphiphilic drug adsorption on the in-plane and three-dimensional structure of membranes in model lipid membranes over the past decades. In this talk, I will present examples of bioactive amphiphiles interacting with lipid membranes, emphasizing how modulation of membrane properties enhances bioactive functionality. This positions them as promising candidates for healthcare applications, both as drugs and additives, as well as for functions related to ambient and plant health, attracting interest from new biotechnology sectors.

PROBING BIOPHYSICAL PARALIPIDOMES: A NOVEL TOOLBOX FOR STUDYING LIPID NANO-ENVIRONMENTS IN LIVE CELLS

Carolyn Shurer[1]; Kandice Levental[1]; Ilya Levental[1];
[1]University of Virginia, Charlottesville, VA, USA

Membrane proteins constitute ~30% of the mammalian proteome and 60% of all drug targets, localizing a major fraction of cellular bioactivity at membrane interfaces. Mammalian membrane proteins are solvated by hundreds of distinct lipid species that are actively turned over and sorted. Membrane protein function is influenced by the biophysical phenotypes arising from lipid collective behaviors such as membrane tension, packing, and fluidity. Further, proteins can selectively recruit local lipid environments, called paralipidomes, that are distinct from the bulk membrane, with varying preferences for lipid headgroups, saturation, and sterols. Experimental studies of cell membrane biophysics rely largely on synthetic fluorescent reporters whose photophysical properties are sensitive to their local environment, which have almost exclusively been applied for measuring bulk membrane properties. Thus, the biophysical properties of local protein paralipidomes remain elusive. To address this technology gap, we have deployed HaloTags to covalently modify membrane proteins with membrane-sensitive probes to measure the biophysical properties of a protein's local, native paralipidome in living cells. We show the utility of this technology to characterize the differences in local lipid packing between the inner and outer plasma membrane leaflets. Next, we identify differences in local lipid packing between established raft and non-raft transmembrane proteins, representing direct measurements of nanoscopic raft domains in the living plasma membrane. Future work will further utilize these probes to track lipid packing through the protein secretory pathway, during immune signaling events, and during functional plasma membrane scrambling.

MEMBRANE REMODELING STRATEGIES FOR LOW OXYGEN SURVIVAL IN EUKARYOTES

Maria Makarova;
[1]University of Birmingham, Birmingham, United Kingdom

Cell membranes are essential to cell function, acting as physical barriers and dynamic platforms that regulate key biological processes. Membrane biophysical properties—such as fluidity, thickness, and phase behaviour—are fundamental to their biological function, influencing processes like signalling, transport, and membrane protein activity. They are largely determined by lipid composition—particularly sterols and phosphoglycerolipids. In eukaryotes, however, the synthesis of both sterols and unsaturated phospholipid acyl tails is oxygen-dependent. This raises a fundamental question: how do eukaryotic organisms maintain membrane integrity and function under oxygen-limited conditions? In this study, we investigate how the unicellular eukaryote Schizosaccharomyces japonicus has adapted to survive in hypoxic environments through distinct lipid remodeling strategies. First, it replaces unsaturated acyl tails of phospholipids with those containing asymmetric saturated acyl tails. Second, it substitutes sterols with diplopterol—a sterol-like molecule. These two components co-evolve to preserve membrane biophysics in the absence of oxygen. Additionally, we identify metabolic adaptations that further support eukaryotic survival in anoxic niches, shedding light on the flexibility and resilience of membrane lipid architecture under extreme conditions.

CONSTITUTIVE PLASMA MEMBRANE RAFT FORMATION IN BOTH EXPONENTIAL AND STATIONARY GROWTH PHASE SACCHAROMYCES CEREVISIAE AND OTHER FUNGI: RELATIONSHIP TO VACUOLAR RAFTS

Betty Du[1]; **Erwin London**[1];
[1]Stony Brook University, Stony Brook , NY, USA

Liquid ordered state membrane domains (lipid rafts) that coexist with disordered domains, and which are abolished at higher temperatures, have been characterized in the vacuoles of stationary phase Saccharomyces cerevisiae. In contrast, membrane lipid domain formation and properties in the yeast plasma membrane are complex and poorly understood. Using a FRET assay that has previously identified rafts in artificial lipid vesicles and mammalian plasma membranes, and fluorescence anisotropy, the formation and thermal stability of rafts in the plasma membrane of S. cerevisiae was characterized. Ordered lipid domain formation was detected both in the plasma membrane of whole yeast and in isolated yeast plasma membranes. These domains have properties similar to those of domains in vacuoles. In intact cells, a first thermal transition involving "melting" of rafts occurred with a midpoint of 45 ± 1oC, similar to stationary phase vacuoles. Like vacuolar rafts they were slightly less thermally stable when the yeast were grown at 20oC than when grown at 30oC. However, unlike vacuoles, rafts were detected in both exponential and stationary growth phase. The properties and thermal stability of ordered domains in isolated plasma membranes and isolated vacuoles were also similar. Yeast plasma membrane rafts were stable over a wider temperature range than in mammalian plasma membranes, and unlike mammalian plasma membrane rafts could not be induced by reconstitution under conditions that degrade lipid asymmetry. S. cerevisiae strains with mutations in sphingolipid or ergosterol synthesis showed minimal effects on raft formation. Interestingly, FRET also detected raft formation with thermal stability similar to that in S. cerevisiae in the fungi Candida albicans and Cryptococcus neoformans. The constitutive presence of ordered lipid domains over a wide range of conditions may be a general feature of fungal plasma membranes.

PHOSPHOLIPID NUMBER IMBALANCE: AN EMERGING NEW ANGLE OF PLASMA MEMBRANE ORGANIZATION

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The functions of cell membranes are determined in part by properties conferred by their constituent lipids. Advanced lipidomics has revealed a vast diversity of lipids in biological membranes, highlighting the importance of lipid composition. At a molecular level, lipids participate in biochemical processes as substrates for specific proteins, but regulation of cellular activity also emerges at the level of collective lipid behaviors. As a well-known example, lateral heterogeneity arising from nonideal lipid interactions can compartmentalize membrane proteins, bringing together interaction partners and facilitating signaling cascades. In contrast to lateral lipid organization, far less is known about the regulatory roles of transverse (i.e., across leaflet) lipid organization, yet these interactions are also a defining feature of living systems. Through comprehensive lipidomics analysis of red blood cell plasma membranes (PMs), we have confirmed the ability of biological membranes to sustain not only compositional asymmetry, but also large imbalances in the overall phospholipid abundances between their two leaflets. These two types of asymmetries—in both lipid composition and number—result in internal elastic stresses that stretch one leaflet while compressing the other. We found that cholesterol plays an important role in shaping this biophysical profile, with its abundance directly related to the membrane's tolerance for (and utilization of) phospholipid asymmetries. These observations prompt a novel, experimentally validated molecular model of a mammalian PM. Computational analysis of this model, corroborated by in vitro experiments, reveals a large cholesterol concentration gradient between the two leaflets that dynamically regulates their properties and elastic stresses, as well as the membrane's response to changes in phospholipid abundance asymmetry. Our findings demonstrate the multifaceted nature of interleaflet lipid organization and highlight the need for a new conceptual framework for investigating the biological roles of membrane asymmetry.

UNIFIED LAWS OF MEMBRANE ELASTICITY: FROM FUNDAMENTAL PRINCIPLES TO PRACTICAL APPLICATIONS

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Cell membranes are a remarkable class of molecular assemblies that have evolved to perform and regulate life functions with unparalleled efficiency and precision – features that scientists and engineers strive to emulate. Their superb functionality is enabled through adaptive compositional remodeling that accommodates dietary or environmental changes by maintaining an optimal biophysical medium for protein activity. Recent lipidomic studies have demonstrated that such adaptive remodeling is largely regulated through a balance between lipid unsaturation and cholesterol content, which feature opposite effects on the lipid packing density. For decades, these competing factors have resulted in contradictory observations of membrane elasticity across different measurement scales. To explain incompatible structural and elastic effects of cholesterol in membranes with varying degrees of chain unsaturation, chemical specificity is typically invoked. In contrast, our recent studies demonstrate that – on the mesoscale – lipid membranes exhibit a unified elastic behavior irrespective of cholesterol content, lipid unsaturation, or temperature. Our findings imply that compositional complexity can be simplified into unified structure-property relations that directly map local membrane elasticity to the molecular packing density. What is more, the obtained relations closely align with theoretical laws based on conformational chain entropy, demonstrating the molecular mechanisms responsible for emergent membrane elasticity. These new observations provide unique insights into the membrane design rules optimized by nature and unlock predictive capabilities for guiding the functional performance of lipid-based materials in synthetic biology, liposomal gene delivery, and biosensing platforms.

CHEMICAL ACTIVITY OF CHOLESTEROL IN MEMBRANES

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Biomembranes are complex two-dimensional liquids composed of hundreds of lipid species that interact in a myriad of ways. One such interaction, that between sphingomyelin (SM) and cholesterol in plasma membranes of animal cells, provides many functional benefits, including protection from microbial infection, prevention of unrestrained cell growth, and proper maintenance of cellular lipid composition. However, owing to the liquid nature of membranes, determination of the structure of this functionally critical lipid-lipid complex, or any other functionally critical lipid-lipid interaction, has remained elusive. Here, we overcome this challenge using a fungal toxin called Ostreolysin A (OlyA), that specifically binds to SM/cholesterol complexes in membranes. We used OlyA to stabilize SM/cholesterol complexes much in the same way as antibodies are used to stabilize pre-existing protein complexes. Extensive cryo-electron microscopy analysis of OlyA bound to SM/cholesterol membranes reveals a tightly bound complex of the two lipids, where the steroid nucleus of cholesterol packs against the acyl chains of SM and a hydrogen bond forms between the nitrogen on SM's ceramide base and the oxygen on cholesterol's hydroxyl group. Mutational analysis confirms the importance of hydrogen bonding in stabilizing SM/cholesterol complexes. These complexes control varied cellular signaling pathways by dampening the chemical activity of membrane cholesterol and its interaction with sensor proteins. The strategies described herein promise to be a prototype for understanding interactions between other pairs of lipids and their protein sensors.

MICRON-SCALE, LIQUID-LIQUID PHASE SEPARATION IN VACUOLE MEMBRANES IN LIVING YEAST

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When budding yeast (S. cerevisiae) are subjected to glucose restriction, they switch from the log stage of growth to the stationary stage. At this point, the membrane of the vacuole (an organelle) undergoes liquid-liquid phase separation into large, micron-scale domains. Two aspects of this phase separation are notable: the vacuole is inside a living cell, and the cell is at its usual growth temperature. As in model membranes, the vacuole membrane can be forced to mix at higher temperatures, and this miscibility phase transition is reversible. Yeast actively regulate the membrane transition holding it about 15C above the yeast growth temperature. The yeast's shift from the log state to the stationary stage is associated with a change in the lipidome of the vacuole membrane. We find that the fraction of phosphatidylcholine lipids doubles, and that these PC-lipids have higher melting temperatures. A perplexing observation is that in every population of yeast, some fraction (typically $\geq 20\%$) of vacuole membranes do not appear to phase separate, which may be related to the yeast's replicative age.

MEMBRANE MECHANICAL EFFECT OF SMALL CHANGES IN LIPID AND STEROL CHEMICAL STRUCTURE

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The likelihood of fusion and fission of bilayer vesicles depends on the spontaneous curvature of their lipid composition. Cholesterol has a very strong negative spontaneous curvature, suggesting a strong affinity for the negative curvature of fusion and fission pores that are intermediates in reshaping. Yet it thickens the hydrocarbon region of bilayers, which are thinned in fusion intermediates. As a proxy for fusion and fission likelihood, we model the shape and spatially resolved lipid composition of highly curved fusion/fission intermediates using molecular dynamics simulations. Our simulations show that this thickening effect of cholesterol is stronger than the curvature effect, leading to cholesterol exclusion from a small pore. Here we test additional sterols (upregulated in developmental diseases) to further quantify the connection between sterol structure and membrane reshaping. The thickening effect of sterols is implicated as a molecular determinant of the stability of (cholesterol enriched) ordered phases that coexist with disordered (cholesterol depleted) phases. The thin fusion pore neck is analogous to a fixed region of disordered phase. General anesthetics (such as short, <14 carbon n-alcohols) lower the transition temperature of lateral phase separation with magnitude correlated with anesthetic potency. Therefore, we test the influence of a series of general anesthetics on pores, connecting their spontaneous curvature and thickness preference to pore geometry.

EXPLOITING PLASMA MEMBRANE HETEROGENEITY TO DELINEATE MOLECULAR MECHANISMS OF GPCR POTENCY AND EFFICACY

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Ankur Gupta, Jens Carstensen, Christopher G. Shuttle, Gabriele Kockelkoren, Dimitrios Stamou*G protein-coupled receptors (GPCRs), integral membrane proteins, mediate essential physiological processes and are primary drug targets. Their activation is strongly influenced by the nanoscale heterogeneity of the plasma membrane, including local lipid composition, membrane curvature, and lateral organization. These factors regulate GPCR spatial distribution, conformational states, and ligand interactions, suggesting a direct coupling between membrane complexity and receptor activation. Despite this complexity, early-stage drug discovery primarily relies on potency and efficacy assessments from downstream signaling assays (e.g., cAMP production), which provide averaged readouts. However, GPCRs populate multiple inactive and active states. Thus, each of them can potentially contribute uniquely to ligand efficacy and pharmacological outcomes. To address this gap, we developed a novel method integrating 3D fluorescence microscopy and conformational biosensors while leveraging the coupling between spatial membrane heterogeneity and GPCR activation to map the spatial distribution of GPCR activity (equivalent to conformational) states at the plasma membrane of live-cell. Using the β1 adrenergic receptor (β1AR) as a model, we quantified ligand-induced redistribution of receptor states and determined state-specific potency and efficacy. Strikingly, conventional potency and efficacy metrics are not simple averages of individual receptor states but are instead driven by specific conformations, which appear to be driven by the nanoscale membrane organization. This study highlights the necessity of incorporating membrane complexity into drug screening assays, providing a refined framework for GPCR pharmacology, with the potential to improve detection of true positives and minimizing false negatives. By acknowledging the functional relevance of membrane organization, our approach enables more precise ligand characterization and optimization for therapeutic development in biologically relevant environments.

SYNERGY BETWEEN MEMBRANE CURVATURE AND AREA EXPANSION IN A MECHANISM OF PLASMA MEMBRANE REPAIR

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The plasma membrane of eukaryotic cells is susceptible to perforation as induced by mechanical or molecular perturbations and holes must be sealed rapidly to ensure survival. Membrane repair involves binding of specific proteins to the damage site activated by the influx of calcium. The mechanism of repair is incompletely known and a matter of significant interest, not least because diseases including several cancer types are associated with abnormal repair. Annexin proteins are necessary but not sufficient components of repair which act by inducing membrane curvature and creating a neck around the hole (Boye et. al. DOI: 10.1038/s41467-017-01743-6). We search for conditions required to form a constricted neck as a transition state to hole closure. Applying a dual approach composed of theory and model membrane experiments, we identify membrane area expansion during repair as a key player, acting in synergy with spontaneous curvature. With variational calculus of the Helfrich energy functional, the shape equations for a curved membrane near a hole are formulated and solved (Klenow et. al. DOI: 10.1016/j.bpj.2024.05.027). A state-diagram of neck shapes is produced, describing the evolution in neck morphology with respect to the available (excess) membrane area. Results show that supply of extra membrane area after damage is linked to the formation of narrow necks with potential importance in repair. Based on UV-laser injury studies in MCF7 cancer cells, the calcium and phospholipid-binding protein kinase C alpha (PKCα) is identified as a key player in plasma membrane repair. Using planar membrane patches with free edges we show that PKCα can induce a significant membrane area expansion of up to 40% at nanomolar protein concentrations. We propose that PKCα-induced expansion, coupled with annexin-mediated curvature, facilitates repair. Our findings highlight membrane area regulation as a key player in plasma membrane repair.

BEYOND SAFFMAN–DELBRÜCK THEORY: DIFFUSION OF MEMBRANE PROTEIN AGGREGATES

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The celebrated Saffman–Delbrück theory established the logarithmic dependence of the diffusion coefficient of transmembrane proteins on their radius. The model succeeded by realizing that diffusion in a membrane inherently requires consideration of the membrane-surrounding fluids. Since its inception, many corrections and extensions of the model have been proposed. However, it remains restricted to individual membrane proteins, even though membrane proteins often form oligomers or aggregates. In this talk/poster, we propose a new theory for the diffusion coefficients of membrane protein aggregates like those formed by Linker for the Activation of T-cells (LAT) and epidermal growth factor receptor (EGFR). To this end, we first visualize the flow field that a diffusing protein induces in a membrane and show that the high viscosity of lipid membranes leads to long-range hydrodynamic coupling. This indicates that hydrodynamic effects are highly relevant for the dynamics of proteins in crowded environments. Subsequently, we use Kirkwood-Risemann theory to self-consistently capture the internal hydrodynamic coupling of protein aggregates and determine their effective diffusion coefficients. From this theory, we also obtain an expression for the hydrodynamic radius that characterizes the diffusion of protein aggregates. By applying the theory to various aggregation models like diffusion-limited aggregation, we then demonstrate that the Saffman–Delbrück model is approximately valid for protein aggregates if the protein radius is replaced by the hydrodynamic radius. Lastly, we discuss the implications of these results for protein aggregation mechanisms and how they can inform single-particle tracking measurements.

THE PHASOR APPROACH FOR STUDYING CELLULAR COMPLEXITY WITH DAN PROBES IN MEMBRANES AND CELLULAR CROWDING: PERSUADING LUIS CONSTITUTED A REMARKABLE JOURNEY SPANNING OVER A DECADE OF FRIENDSHIP.

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Dimethylamino-naphthalene probes, specifically LAURDAN and ACDAN, have been employed to investigate membrane and cellular crowding across various scales for several decades. Since the 1990s, Enrico Gratton and Tiziana Parasassi have introduced the Generalized Polarization (GP) function to quantify the spectral shift experienced by DAN probes. With the emergence of bioimaging in the early 2000s and for more than twenty years, GP has served as a crucial tool for elucidating the complex yet rich photophysics that DAN probes can offer in vitro, in cellular contexts, and in vivo systems. Beginning in the late 2000s, with the advent of Fluorescence Lifetime Imaging Microscopy (FLIM) and Hyperspectral Imaging (HSI), novel opportunities have arisen to dissect the intricate photophysics of DAN probes. The phasor approach for FLIM and HSI provides a unique, model-free methodology that facilitates straightforward analysis of FLIM and HSI from complex systems through simple principles and a robust mathematical foundation. However, GP continues to be extensively utilized, and phasors have not yet been fully embraced as fundamental tools to enhance the understanding of cellular complexity with DAN probes. This presentation will discuss over a decade of research in the domain of phasors for FLIM and HSI, examining their value in addressing in vitro, in cellulo, and in vivo complexities involving DAN probes. Furthermore, leveraging my profound friendship with Luis, I will share cherished anecdotes and discussions that I hold dear, commemorating a remarkable scientist and friend who left us too early.

THE SELECTIVITY OF THE ANTIMICROBIAL PEPTIDE MP1 IS CONTROLLED BY MULTIPLE MEMBRANE FACTORS.

L. Stefania Vargas-Velez[1]; Iván Felsztyna[1]; **Natalia Wilke**; **– Lipotype Sponsored Speaker**
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MP1 is a short-sequence antimicrobial peptide with a low positive charge. This peptide disrupts bacterial membranes at low concentrations, leaving red blood cell membranes unaffected.Experiments with synthetic membranes suggest that the presence of cholesterol and the lower proportion of charged lipids in mammalian membranes are the main factors contributing to this selectivity.However, the peptide's activity depends on the bacterial strain, and therefore factors other than the presence of cholesterol and anionic lipids govern its action.Our goal was to identify all possible membrane factors that affect the peptide's activity against different organisms, in order to fully understand how membrane properties govern peptide-induced membrane disruption.We studied the biophysical properties of three different bacterial strains with varying sensitivity to MP1 and found that no single property of cell membranes explains responses to MP1. Instead, surface charge, lateral diffusion, and compressibility together explain the cellular response to the peptide. To understand this phenomenon in a simpler system, the peptide's activity and affinity for membranes composed of phosphatidylcholine were evaluated. A dependence on acyl chain length and saturation was observed. Fluid membranes with unsaturated lipids were more resistant to peptide-induced leakage than those with saturated lipids. Furthermore, MP1 does not interact with membranes in the gel state, indicating a very subtle regulation of the peptide's action by acyl chain characteristics. This process is currently being studied using molecular dynamics simulations to better explain the experimental results.

IN-SITU STRUCTURE OF THE BACTERIAL OUTER MEMBRANE PROTEIN A

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Outer membranes of Gram-negative bacteria contain transmembrane proteins embedded in a uniquely asymmetric lipid environment. Whereas the inner leaflet of outer membranes is composed of regular phospholipids, the outer leaflet consists almost exclusively of lipopolysaccharides (LPS). To date, no methods exist to experimentally mimic the complex native lipid environment of bacterial outer membranes. Consequently, studies characterizing the structure and function of outer membrane proteins rely on membrane-mimetic systems or artificially reconstituted bilayers. However, a growing number of studies indicate that membrane composition directly influences the structural and functional states of integral membrane proteins. To address this crucial issue, we recently developed a method to manipulate the protein content of bacterial outer membrane vesicles (OMVs). These vesicles are prepared to contain selected outer membrane proteins at high density and circumvent the limitations of established methods. Using engineered OMVs, we characterize the prototypic outer membrane protein A (OmpA) from Klebsiella pneumoniae for the first time within the unaltered asymmetric bacterial membrane environment at atomic resolution, employing proton-detected ultra-fast solid-state NMR spectroscopy. Our methodology allows extensive assignment and structure determination of OmpA's transmembrane region, as well as site-specific comparisons with OmpA reconstituted in lipid bilayers. Supported by dynamics measurements across multiple timescales, we find that OmpA adopts a stable, extended fold within the native membrane environment, exhibiting substantial differences from structures observed in membrane-mimetic systems. This fold is stabilized through direct interactions with LPS, which, in turn, may contribute to membrane integrity by reinforcing the functional role of OmpA in the Gram-negative cell envelope. Our work thus provides a blueprint for in situ structural biology of membrane proteins in their native environments.

PLASMA MEMBRANE CHOLESTEROL ASYMMETRY REGULATES CELLULAR CHOLESTEROL HOMEOSTASIS

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The plasma membrane is the interface between the external world and a cell's internal chemistry, and must therefore facilitate a multitude of parallel, tightly regulated tasks. To achieve this functional complexity, mammalian cells produce hundreds of lipid species, nearly all of which are asymmetrically distributed between the two leaflets of the plasma membrane bilayer. Further, the various organellar membranes have vastly different lipid compositions that are essential for their functions, and thus must be homeostatically maintained. 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. 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 area and mechanical stresses. Through computational and experimental approaches, we find that the combination of cholesterol's preference for the more saturated exoplasmic lipids and the overabundance of lipids in the cytoplasmic leaflet yields a major enrichment of cholesterol in the exoplasmic leaflet. We show that this transbilayer distribution can be altered by manipulating the determinants of cholesterol asymmetry, namely the disparity in phospholipid composition and abundance between leaflets. Specifically, addition of phospholipids to the outer leaflet redistributes cholesterol to the cytosolic leaflet of the plasma membrane. This redistribution leads to cholesterol's recognition by cytoplasmic sensor proteins (i.e. GRAMD), which transfer it to the endoplasmic reticulum for esterification into cholesterol esters and storage in lipid droplets. Thus, transbilayer cholesterol asymmetry is a major contributor to cellular cholesterol homeostasis.

CONDENSATE PRONE BULK MIXTURES CAN PREWET MEMBRANES

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Many macromolecular components in cells have a thermodynamic propensity to phase separate into coexisting liquid phases, sometimes termed condensates. In a few cases these condensates present as roughly spherical three-dimensional droplets even within cells. But often, while components will form three dimensional droplets when isolated at high concentrations, in the cellular context they are present at far lower concentration, and the domains that they form are localized to other structures, like the plasma membrane. Here I will argue that many of these domains are likely generalized prewet phases, stable only due to interactions with biological surfaces that contain their own transitions. Classical prewetting occurs when bulk mixtures tuned near to phase coexistence undergo thermodynamic surface transitions at a suitable surface even though they are outside of true bulk coexistence. But for classical surfaces the regime of prewetting is very narrow. Here I will focus on prewetting to the plasma membrane, a two-dimension fluid which has its own propensity to phase separate into coexisting two-dimensional liquids, with many cell types sitting near a critical point of this transition. With a combination of theory, simulation and experiment I will show that the fluid nature of the membrane along with its own propensity to phase separate vastly enlarges the prewetting regime.

FLOW, FRICTION, AND ELASTICITY: THEORETICAL INSIGHTS INTO MEMBRANE MECHANICS

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I will describe two theoretical results that arise from the mechanical properties of membranes. First, in the context of flow, we show theoretically how molecular rotors—molecules that adopt twisted conformations upon photoexcitation—can be harnessed to measure interleaflet friction. Second, focusing on membrane elasticity, we demonstrate using theory and experiments how the coupling between flow and elasticity can lead to either attraction or repulsion of particles moving near a membrane. These findings highlight the crucial role of mechanical interactions in governing membrane behavior and suggest new strategies for probing membrane dynamics.

USING MOLECULAR FIELD THEORIES TO STUDY PLASMA MEMBRANE COMPLEXITY

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In this talk, we will introduce molecular field theory (MFT) as an alternative to other computational techniques to study plasma membrane complexity. MFT provides a theoretical foundation to quantify thermodynamic stability, transport properties, and emergent structural behavior across length scales, linking molecular interactions to macroscopic material behavior. Starting from related density functional theories and self-consistent field theories, we will explain the theoretical underpinnings of MFT while then presenting applications of MFT to elucidate plasma membrane behavior from a molecular perspective. Plasma membrane complexity, including protein recruitment and spatial organization, is motivated by either highly selective recognition of specific target membrane components or nonspecific attraction to general physical properties of the membrane, such as charge, lipid heterogeneity, and curvature. As one specific example of using MFT, we discuss the interactions between proteins and lipid membranes from a comprehensive examination of how features of the membrane and its lipid constituents, including lipid composition, headgroup size, degree of tail saturation, tail length (membrane thickness), and membrane geometry, affect the adsorption ability and spatial inhomogeneities of the proteins.

LONG-RANGE MEMBRANE VISCOSITY OF LIVING CELL

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The viscosity of the plasma membrane in living cell is a crucial biophysical parameter that regulates cellular functions. We categorize the plasma membrane viscosity into short-range and long-range viscosities based on the spatial scale of the cellular processes they influence. Short-range viscosity originates from the nanometer-scale diffusion of membrane molecules, regulating signal transduction and membrane transport. It has been reported that short-range viscosity in living cells is comparable to, or at most 10 times greater than, that of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) membranes. In contrast, cell-scale (micrometer scale) membrane flow, driven by a flow of the actin cytoskeleton, occurs in dynamic processes such as cell migration and cell division. We shall refer to the resistance to this cell-scale membrane flow as long-range viscosity. While transmembrane proteins anchored to the actin cytoskeleton have minimal effect on short-range viscosity, they are likely to affect long-range viscosity. Thus, short- and long-range viscosities in the living cell should differ significantly. In this study, to reveal the difference from the short-range viscosity, we measure the long-range viscosity by applying an external point force to plasma membrane of C.elegans early embryo using a microinjection technique. In intact cells, no membrane flow was induced by the applied point force. However, when actin polymerization was inhibited, a point force induced a pair of vortex flows across the plasma membrane. By comparing the vortex flow pattern with a hydrodynamic model, we found that the long-range viscosity in living cells was four orders of magnitude greater than both DOPC membranes and the short-range viscosity in living cells. This drastic viscosity increase was attributed to the structure of actin filaments attached to transmembrane proteins after polymerization inhibition. We conclude that the remaining actin cytoskeleton and its associated transmembrane proteins contribute to the increased long-range viscosity.

THE MEMBRANE OF STAPHYLOCOCCUS AUREUS: MODULATION OF MEMBRANE BIOPHYSICAL PROPERTIES AND THE INHIBITION OF ANTIMICROBIAL PEPTIDE ACTIVITY

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Staphylococcus aureus is a human pathogen responsible for a wide range of life-threatening diseases. Due to the emergence of Methicillin-resistant S. aureus strains, there is an urgent need to develop alternative treatments. Within this context, antimicrobial peptides have emerged as potential alternatives, where their main mode of action is the formation of pores in the bacterial membrane. Although, many antimicrobial peptides show pore forming activity in simple lipid model systems, only a few have reached clinical use. This points to a gap between biophysical studies and implementation at the clinical level. To help bridge this gap, our work focuses on understanding the lipid composition of S. aureus membranes, and how this influences antimicrobial peptide activity, For this, a lipidomic investigation through mass spectroscopy coupled to HPLC is conducted to understand how S. aureus lipid composition varies under different environmental conditions. Total lipid extracts and model systems are then studied using fluorescence spectroscopy and FTIR to understand the implications of composition on membrane biophysical properties. Calcein release experiments are used to monitor antimicrobial peptide activity. We find that S. aureus lipid components such as cardiolipin and staphyloxanthin play a crucial role in increasing resistance to pore forming activity for a variety of antimicrobial peptides. The content of these lipids varies significantly depending on the growth state of S. aureus, which will significantly influence the effectiveness of antimicrobial peptides under different infection states. We also find that these lipid components have drastically different inhibitory levels depending on the antimicrobial peptide that is being tested. These results may help determine more precisely the effectiveness of candidate antimicrobial peptides under clinical conditions.

PRESSURE-DEPENDENT TENSION PROPAGATION IN CRUMPLED CELL MEMBRANES

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Propagation of membrane tension mediates mechanical signal transduction along surfaces of live cells and sets the time scale of mechanical equilibration of cell membranes. In stark contrast to the earlier expectations, recent studies in several cell types and under different conditions revealed a strikingly wide variation range of the tension propagation speeds including extremely low ones. The latter suggests a possibility of long-living inhomogeneities of membrane tension crucially affecting mechano-sensitive membrane processes. We propose a mechanism of tension propagation in membranes crumpled by the contractile cortical cytoskeleton, where the pace of the tension propagation is controlled by the intracellular pressure and the degree of the membrane crumpling. The tension spreading is mediated by the membrane flow between the crumples. We provide experimental support for the suggested mechanism by monitoring the rate of tension propagation in cells exposed to external media of different osmolarities.

THE ROLE OF LIPID ENVIRONMENT IN MEMBRANE PROTEIN SIGNALING AND CATALYSIS

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Diego de Mendoza Institute of Molecular and Cellular Biology of Rosario (IBR-CONICET-UNR), Ocampo y Esmeralda, 2000, Rosario, Argentina. Membrane proteins play essential roles in numerous cellular processes, often featuring hydrophobic α-helices that interact with the lipid bilayer. These interactions make the organization of membrane protein helices highly sensitive to lipid properties such as fluidity and hydrophobic thickness. In response, helices may adapt in ways that influence overall protein structure and function. In this talk, I will explore how the lipid environment regulates the signaling state of a bacterial transmembrane protein and how the bilayer modulates the catalytic activity and substrate channeling of a bacterial peripheral membrane protein.

STAPHYLOXANTHIN MODIFIES THE STRUCTURE OF BACTERIAL-MODEL PHOSPHOGLYCEROL MEMBRANES

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Staphyloxanthin (STX) is a carotenoid responsible for the scavenging of radicals across the membrane of Staphylococcus aureus. STX has also been recently linked to the antimicrobial resistance of this bacterium through largely-unknown molecular mechanisms. To shed light into this issue, in this work, we used an integrative approach, combining molecular dynamics simulations, X-ray scattering, and Laurdan, DPH, and infrared measurements. We structurally characterized the behavior of bacterial model phosphoglycerol (PG) membranes containing STX. STX forms conglomerates that span both leaflets, significantly modifying the membrane thickness, the lipid packing density and the ordering of the surrounding PG lipids. STX is therefore suggested to confer an increased mechanical resistance to the membranes that contain it. In consequence, our work highlights a possible new function of STX, beyond radical scavenging, which is directly related to the increased resistance of Staphylococcus aureus to antibiotics.

SOFT DYNAMIC CHANNELS IN LIQUID ORDERED LIPID MEMBRANE

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Over the last few decades, extensive investigations on spatial and dynamic heterogeneity in plasma membrane has been carried out on carefully reconstituted model membranes and cell derived membranes. However, characterising heterogeneity at molecular scales is often challenging due to the experimentally inaccessible time- and length-scales of these emergent behaviours. In this context, computer simulations can provide insights into molecular-level interactions and mechanisms leading to membrane heterogeneity and associated functions. To that end, we have developed a new framework using non-affine displacement (NAD) and 3D-packing defects calculations to faithfully capture the extent of local membrane order in simulated heterogeneous bilayers [1]. With these tools in hand, I shall discuss recent results where we investigated spatial and temporal organisation on microseconds trajectories of liquid-ordered bilayer systems at all-atom resolution (DPPC/DOPC/CHOL: 0.55:0.15:0.30 ; 40 nm x 40 nm with total 5600 lipids, 2 million atom system). Interestingly, the lateral organisation exhibits noticeable heterogeneity despite its liquid-ordered nature. Our analysis reveals soft channels within the tightly packed membrane reminiscent of the classical two-component Kob-Andersen glass-forming models. Hence, we characterised them using classical glass physics markers for dynamic heterogeneities such as overlap, four-point susceptibility, van Hove and intermediate scattering functions to quantify the multiple time scales underlying the ordered lipid dynamics. Our analyses suggest that highly ordered membrane systems (lipid nanodomains, rafts) may exhibit glass-like dynamics with highly fluid channels within them that facilitate anomalous molecular encounters for biological functions [2]. [1] (2024) Madhusmita Tripathy* and Anand Srivastava*, "Non-affine deformation analysis and 3D packing defects: A new way to probe membrane heterogeneity in molecular simulations", Invited contribution to Methods in Enzymology book series (Biophysical Approaches for the Study of Membrane Structure), Editors: Tobais Baumgart and Markus Deserno) (https://linkinghub.elsevier.com/retrieve/pii/S0076687924000958) [2] (2024) Harini Sureshkumar, Sahithya Iyer, Atreyee Banerjee, Pratyush Poduval, Edward Lyman, Anand Srivastava*, “Signatures of glassy dynamics in highly ordered lipid bilayers with emergence of soft dynamic channels”, bioRxiv link: https://biorxiv.org/cgi/content/short/2024.11.22.624930v1 (Under consideration)

TRACKING LIPIDS THROUGH PHYSICAL AND CHEMICAL SPACE

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Eukaryotic cells produce over 1000 different lipid species which tune organelle membrane properties, control signalling and store energy. How lipid species are selectively sorted between organelles to maintain specific membrane identities is largely unknown due to the difficulty to image lipid transport in cells. Here, we measured transport and metabolism of individual lipid species in mammalian cells using time-resolved fluorescence imaging of bifunctional lipid probes in combination with ultra-high resolution mass spectrometry and mathematical modelling. Quantification of lipid flux between organelles revealed that directional, non-vesicular lipid transport is responsible for fast, species-selective lipid sorting compared to slow, unspecific vesicular membrane trafficking. Using genetic perturbations, we found that coupling between active lipid flipping and passive non-vesicular transport is a mechanism for directional lipid transport. Comparison of metabolic conversion and transport rates showed that non-vesicular transport dominates the organelle distribution of lipids while species-specific phospholipid metabolism controls neutral lipid accumulation. Our results provide the first quantitative map of retrograde lipid flux in cells. We anticipate that our pipeline for quantitative mapping of lipid flux through physical and chemical space in cells will boost our understanding of lipids in cell biology and disease.

REGULATION OF PLASMA MEMBRANE ORGANIZATION BY LIPID TAIL LENGTHS

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Lipids that constitute cellular membranes have a large diversity in structures, and exist at various ratios depending on the organism, cell type, organelle, or submembrane region. Understanding the biological importance of membrane lipid composition in cellular contexts requires tools to manipulate lipid composition as precisely as possible. We use CRISPR-Cas9-based multiplex mutations to induce reductions of whole lipid classes, or to tune structural details of specific lipids. We also develop lipidomics approaches that allow us to assess the precision of our manipulation. Using these tools, we disrupted genes regulating sphingolipid chain length, unsaturation, and head group compositions. This revealed that sphingolipid chain length is critical for the localization of phosphatidylserine in the opposite leaflet of the plasma membrane, suggesting a mechanism to couple components of the two leaflets. Detailed analysis of the structural requirements for this sphingolipid-phosphatidylserine interaction suggested that sphingolipid head group did not play important roles, while monounsaturated very-long chain sphingolipids were more potent in regulating phosphatidylserine component. This suggests lipid structure-dependent coupling mechanism in the plasma membrane, which could have a broader impact on the organization of proteins too.

CRITICAL BEHAVIOR IN MULTICOMPONENT MIXTURES WITH STRUCTURED INTERACTIONS: FROM TUNING TO FUNCTION

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No Abstract

ON THE LIPID DEPENDENCE OF BACTERIAL MECHANOSENSITIVE CHANNELS GATING

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For bacterial mechanosensitive channels that function as turgor-adjusting osmolyte release valves, membrane tension serves as the primary activating stimulus. Since tension is transmitted to the gate through the surrounding lipid bilayer, the presence or absence of certain lipid species can be consequential. In MscS, lipids serve a dual purpose; they not only transmit tension to the peripheral domains but can also intercalate between the helices, separate the gate, and thus drive inactivation. In this study, we characterize the lipid dependence of chromosome-encoded MscS and MscL in E. coli strains with genetically altered lipid compositions. We utilize two previously generated strains lacking one or two primary lipid species (PE, PG, or CL) and an engineered strain that is highly enriched in cardiolipin (CL) due to the presence of hyperactive cardiolipin synthase ClsA. We assess the behavior of these channels using patch-clamp techniques and quantify the relative tension midpoints, closing rates, inactivation depth, and the rate of recovery to the closed state. Additionally, we measure the osmotic survival of lipid-deficient strains, testing the channel function at the cellular level. We find that MscS and MscL both tolerate the absence of specific lipid species in terms of opening and closing. However, the lack of CL reduces the active MscS population relative to MscL, decreases the closing rate, increases the likelihood of MscS inactivation, and slows the recovery process. Importantly, PG and CL co-purify with MscS when gently isolated in the Glyco-DIBMA polymer. MD simulations indicate that PG and CL tend to localize near the cytoplasmic side of the MscS protein interface and partition into the crevices. We discuss the effect of CL on MscS kinetics in relation to its properties. The data underscores the robustness of the osmolyte release system and highlights the importance of cardiolipin for the adaptive behavior of MscS.

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THE LIPID BILAYER MODULATES MEMBRANE PROTEIN ENERGETICS

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The lipid bilayer has a dramatic change in its chemical composition across the axis normal to its surface, predominantly due to the million-fold change in the water concentration over a distance of only a few angstroms. This steep water gradient affects the solvation- and backbone-hydrogen bond energies of membrane proteins embedded within this gradient. We have been investigating how this chemical property of the bilayer affects the magnitudes of the sidechain transfer free energies. We experimentally measure these energies using protein folding experiments, and we compliment these studies with all-atom molecular dynamics simulations to obtain atomistic detail on how the protein and bilayer energetically respond to amino acid sequence variants. Through this work, we have developed a novel hydrophobicity scale that uses a native transmembrane protein and phospholipid bilayer as the environment and have discovered an empirical correlation between the surface area of the nonpolar side chain, the transfer free energies, and the local water concentration in the membrane that allows for the nonpolar solvation to be accurately estimated at any location in the bilayer. Currently we are working on determining how much energy it costs to introduce polar side chains into the bilayer and how they are accommodated by changes in the lipid.

MECHANISMS OF LIPID-DRIVEN GPCR ACTIVATION

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Biophysical and biological studies increasingly recognize membrane lipids as endogenous regulators of GPCR signaling, but structural mechanisms behind these observations are incomplete. Using an integrative structural biology approach, featuring NMR spectroscopy, we demonstrate that membrane phospholipids and sterols modulate GPCR conformational equilibria, with a magnitude comparable to that of drug efficacy. Integrating NMR data with functional assays and molecular modeling, we propose a molecular mechanism for lipid-driven GPCR complex formation.This work prompted a broader evaluation of membrane mimetics, revealing limitations in preserving native receptor-lipid interactions. Our NMR and biophysical data illustrate how common mimetics can alter or obscure these interactions. Based on these findings, we engineered new mimetics that better retain physiologically relevant receptor-lipid interactions.

PROTEIN-LIPID INTERACTIONS IN COMPLEX CROWDED MEMBRANES

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Protein-lipid interactions are key regulators of membrane protein function, and can be integral to membrane structure and dynamic organisation. However, it can be challenging to observe protein-lipid interactions at a molecular scale, especially within membranes of physiological complexity. Here I will show how coarse-grained molecular dynamics simulations can be used to untangle the complex interplay between multiple simultaneous lipid interactions with membrane proteins, and to unpick the contribution of protein-lipid interactions to membrane behaviour and membrane function.

A CELL-BASED SCRAMBLING ASSAY REVEALS PHOSPHOLIPID HEADGROUP PREFERENCE OF TMEM16F ON THE PLASMA MEMBRANE

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The asymmetric resting distribution of the three major phospholipid classes on the plasma membrane of mammalian cells, with phosphatidylserine and phosphatidylethanolamine mostly on the inner leaflet, and phosphatidylcholine mostly on the outer leaflet, is maintained by several classes of ATP-dependent flippases and floppases that exhibit head group selectivity. Upon signaling cues, this asymmetry is dissipated by several families of ATP-independent phospholipid scramblases, thus allowing cells to respond to stimuli and adapt to the physiological context. The prevailing view in the field is that phospholipid scramblases on the plasma membrane act without headgroup preference. However, this paradigm has not been rigorously examined due to limitations in the existing detection modalities. We devised a cell-based phospholipid scrambling assay that utilizes the fluorescence polarization of nitrobenzodiazole-labeled phospholipids to monitor phospholipid scrambling in their native environment. We discovered that the plasma membrane-residing calcium-activated phospholipid scramblase TMEM16F preferentially acts on phosphatidylserine and phosphatidylcholine but not phosphatidylethanolamine. This is the first reported demonstration of phospholipid headgroup preference of a phospholipid scramblase on the plasma membrane. Additional efforts to understand the mechanisms conferring the headgroup preference for TMEM16F are ongoing, as is the search for calcium-activated phospholipid scramblases with headgroup preference different from that of TMEM16F.

ACTIVE STRESSES FUNCTIONALLY SHAPE THE LOCAL COMPOSITION OF THE LIVING CELL MEMBRANE

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A eukaryotic cell interfaces with the external milieu constantly decoding signals in the form of chemical and mechanical inputs and responding, almost instantly. These cues are interpreted by membrane receptors embedded in the plasma membrane. One such membrane receptor, the integrin receptor, receives chemical and mechanical signals from the extracellular matrix. Chemical cues activate Rho A-dependent signalling cascades generating actomyosin stresses in the cell whereas mechanical cues activate mechano-transducers. By studying the impact of the integrin receptor activation on membrane organization we find that the activation of these two pathways result in the creation of localized mesoscale liquid-ordered (*lo*) membrane domains consisting of nanoclusters of outer-leaflet localized GPI-anchored proteins and inner-leaflet lipids, necessary for this response. These membrane domains encode information about the chemical and mechanical nature of the substrate, regulating crucial aspects of integrin receptor function including cell spreading and migration. This level of regulated organization in a fluid membrane bilayer is only possible due to its engagement with an energy consuming medley of myosin proteins at or near the plasma membrane, draped over a cortical actin meshwork. This active actin-membrane composite behaves as a mechano-responsive medium, serving to integrate chemical and physical cues presented at the cell periphery for the regulation of cell physiology.

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TO SEE OR NOT TO SEE: LATERAL ORGANIZATION OF BIOLOGICAL MEMBRANES

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This is how a review of Luis Bagatolli (LB) was initiated in 2006 (Biochim. et Biophys. Acta 1758 (2006) 1541), where the many possibilities to explore the lateral structure of giant vesicle model membranes by fluorescence imaging techniques were unfolded. However, the successes with visualizing lateral structure of giant vesicles were difficult to apply directly to biological membranes which caused a vivid debate in the community with LB as an engaged participant. From a viewpoint of basic physics modelling of lipid membrane cooperative phenomena we discuss some fundamental obstacles for the translation of results on lateral structure of giant vesicles to biological membranes. The modelling indicate that the unique physical properties of lipid bilayer membranes hamper the experimental characterization of the lateral structure of biomembranes, while they may be harnessed for biological functions. We argue that aspects of the observed complexity of the lateral structure of biological membranes are captured by the complexity of the simple membrane models.

INTERSTITIAL HYDRODYNAMIC INSTABILITIES SCULPT CELL ADHESIONS

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Linker dynamics is not the only parameter that controls cell adhesion. Using biomimetic experiments and theoretical modelling, we show that hydrodynamic instabilities in the thin interstitial fluid layer can lead to patterning of the membrane adhesion and to the formation of membrane blisters in two very different biological contexts. In the first system, we show that increased hydraulic pressure arising from osmotic disbalance across the lipid membrane induces membrane blistering that plays a crucial role in the lumen formation of embryos. In the second system, we show that conformational changes in the glycocalyx arising from area changes in the underlying membrane trigger similar hydrodynamic instabilities that can destabilize the adhesion contacts. Our work points to the universal, yet under-appreciated role of interstitial hydrodynamics in regulating cell adhesion.

MEMBRANE COMPLEXITY IN ENVELOPED VIRAL ENTRY: MULTIFACTORIAL EFFECTS AND HINTS OF EMERGENT PHENOMENA

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Enveloped viruses often gain entry to cells via membrane fusion with either the plasma membrane or endocytic compartments. Separating the effect of membrane properties versus membrane trafficking effects can be challenging in live cells. We therefore perform single-virus binding and fusion experiments on both cell-derived and synthetic membranes, correlating the results with live-cell experiments. Using these tools, we aim to understand for which viruses and which mechanistic steps in binding and fusion cell membrane properties and lateral organization play a role. For influenza virus, which enters via endosomes, we show that most of the difference in infectious efficiency in endosomes versus plasma membrane can be attributed to a difference in membrane permissiveness to full fusion. Interestingly, these differences cannot simply be reconstituted by adding a major endosomal lipid to plasma membrane, although moderate cholesterol extraction has a definite, if complex, effect on fusion and cell infection that is distinct from changes to membrane asymmetry. Combined with prior data from our group and others on the effects of cholesterol in viral and synthetic membranes, these results suggest multifactorial roles of cell membrane components in modulating membrane materials properties versus membrane lateral organization and ways that both of those can regulate viral binding and fusion. Viral entry well illustrates the complexity of physiological membranes because it depends on membrane materials properties and on membrane phase behavior but often in nontrivial ways.

STOCHASTIC SIGNALING REACTIONS ON THE MEMBRANE SURFACE

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A majority of biological signal transduction reactions occur on the membrane surface. Beyond the lipids themselves, the membrane surface creates a reaction environment that entangles mechanical and spatial features with the chemical reactions themselves. This can lead to a rich array of complex and sometimes quite unexpected behaviors. I will discuss several distinct biological signaling systems, which share interesting stochastic phenomena enables by the membrane interface. From single molecule sensitivity in T cells to spontaneous symmetry breaking and cellular polarization, we will see essential roles for the membrane beyond the lipids.

SHEDDING NEW LIGHT ON MITOCHONDRIAL PERMEABILIZATION IN APOPTOSIS

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Mitochondrial permeabilization is a key event in the apoptotic pathway and defines the cell's commitment to death. The executioner members of the BCL2 family member, most importantly BAX and BAK, open a pore at the mitochondrial outer membrane that releases apoptotic factors for caspase activation, which accelerate cell death execution. Subsequently, the mitochondrial inner membrane is also permeabilized, which releases mtDNA and can initiate inflammatory signaling under low caspase activity. I will discuss our recent findings using advanced microscopy strategies to understand how the permeabilization of mitochondrial membranes controls cell death and inflammation.

REGULATION MECHANISMS OF RECEPTOR AND SIGNALING MOLECULE ACTIVITIES IN LIPID DOMAINS AS REVEALED BY SINGLE-MOLECULE IMAGING AND SUPER-RESOLUTION MICROSCOPY

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Intracellular molecules do not operate synchronously but rather function through an inherently stochastic process due to their Brownian motion. Individual molecules engage in interactions lasting less than a second, with the proportion of interacting molecules seldom exceeding 10%. For example, single-molecule observations have revealed that the oncogene product K-Ras is activated via GTP binding for less than 0.5 seconds, with the fraction of activated molecules remaining below 10%. To elucidate the mechanisms underlying such transient and rare molecular events in living cells, we developed an ultrafast single-molecule imaging system, achieving acquisition rates of up to 20,000 frames per second (Suzuki et al., Nat. Chem. Biol., 2012; Komura et al., Nat. Chem. Biol., 2016; Fujiwara et al., J. Cell Biol., 2023). Furthermore, by using the technique of high-speed single-molecule imaging, we advanced high-speed dual-color PALM/dSTORM super-resolution microscopy and analytical methodologies, enabling the acquisition of pseudo-real-time movies in living cells (Kenmoku et al., Nat. Commun. 2024; Hirosawa et al., Nat. Commun. in press). Using these state-of-the-art imaging techniques, we recently uncovered the nanoscale (~100 nm in diameter) clustering of signaling molecules such as K-Ras and STING in distinct lipid domains of the plasma membrane and trans-Golgi network, respectively, in living cells. Our results indicate that both lipid-lipid and protein-lipid interactions play critical roles in the formation of these molecular clusters. Additionally, we elucidated the mechanisms by which these signaling molecules interact with the downstream effectors in lipid domains. In this presentation, I will introduce these imaging techniques and discuss recent advances in our research.

PEPTIDE TRANSLOCATION ACROSS ASYMMETRIC PHOSPHOLIPID MEMBRANES

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Cells are separated from their surroundings by a semi-permeable cytoplasmic membrane. Peptides with specific properties are able to spontaneously cross this barrier and act as drugs or drug carriers. Typically, the permeation of these peptides is studied using symmetric model membranes, even though actual cell membranes are usually asymmetric. It is commonly thought that the permeability of an asymmetric membrane can be approximated from the permeabilities of the corresponding symmetric membranes. Using computer simulations with both coarse-grained and atomistic force fields, we calculated the free energy profiles for the passage of model amphiphilic peptides and a lipid across various symmetric and asymmetric membranes. Our results consistently demonstrate that while the free energy profiles for asymmetric membranes with a small differential stress align with symmetric ones in the region of lipid headgroups. However, the profiles differ around the membrane centre. In this region, the free energy for the asymmetric membrane transitions between the profiles corresponding to two symmetric membranes composed of individual leaflets. We show that peptide permeability through an asymmetric membrane cannot always be predicted from the permeabilities of the symmetric membranes. This suggests that using symmetric membranes does not provide an accurate representation of peptide translocation across asymmetric membranes.

INTRINSIC DISORDER AS AN ORGANIZING PRINCIPLE FOR MEMBRANE BIOLOGY

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As the gateway for cellular entry and communication, the surface of the cell holds the answersto critical questions in biology and medicine, while simultaneously providing inspiration forengineered materials and systems. Recent progress in our lab and others illustrates thatnetworks composed of proteins with a high degree of intrinsic disorder may provide thenecessary flexibility to facilitate efficient assembly of functional protein complexes at membranesurfaces. In particular, we found that a flexible network of disordered proteins helps to catalyzethe assembly of endocytic structures at the plasma membrane. This understanding providesnew insight into the optimal design of therapeutic carriers that harness endocytosis for cellularentry. More broadly, our lab seeks to understand and mimic the ability of biological membranesto spontaneously reorganize in response to diverse cues. This remarkable capacity for self organization,which is largely absent in man-made materials, holds great promise for the designof responsive, cell-like therapeutic systems.

SHAPING MEMBRANES WITH(OUT) PROTEINS: HOW SIMPLE PHYSICOCHEMICAL FACTORS DRIVE CURVATURE AND REMODELING

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Cell membranes exhibit a remarkable range of curvatures, commonly attributed to specialized proteins. This talk will demonstrate that curvature can arise from fundamental physicochemical asymmetries, which may play an important role in defining organelle shapes. Using giant unilamellar vesicles (GUVs) as an artificial cell model, we explore several mechanisms of curvature generation, including asymmetric ion distributions, insertion and desorption of gangliosides, and polymer (PEG) adsorption. Additionally, we will show how molecularly crowded solutions and protein condensates can drive membrane wetting, leading to vesicle budding, tubulation, formation of double-membrane sheets and micro- and nano-scale restructuring. These examples demonstrate that membranes can be remodeled even in the absence of specific curvature-inducing proteins or active cellular processes, highlighting the importance of intrinsic physicochemical forces in membrane organization and transformation.

FLOWERS, PETALS & BUDS: MEMBRANE REMODELING VIA ASSOCIATIVE AND SEGREGATIVE PHASE SEPARATION

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Living cells orchestrate a myriad of biochemical reactions within a highly complex and crowded intracellular environment. A major factor responsible for such seamless assembly are the preferential interactions between the constituent macromolecules, either associative or segregative, that can drive de-mixing to produce co-existing phases and thus provide a dynamic intracellular compartmentalization. But how these two types of interactions influence each other as well as the confinement is still largely unknown. This makes it crucial to understand de-mixing of biomolecules within cell-mimicking vesicles and further apply the resulting molecular interactions to engineer increasingly complex synthetic cells.In this talk, I will showcase our recent attempts in using multiple phase separation systems in liposomes to gain spatiotemporal control over the localization of membraneless organelles. We show that the interplay between segregative and associative phase separation can lead to rich dynamics between the two phases and the soft confinement itself. Using on-chip microfluidic systems, we encapsulate the associative and segregative components in cell-sized vesicles and trigger their phase separation to create hierarchical structures that act as molecular recruiters, membrane targeting agents, and initiators of condensation. The obtained multiphase architecture provides an isolated microenvironment for the condensates, restricting their molecular communication as well as diffusive motion, and leading to budding-like behavior at the lipid membrane. In conclusion, we propose segregative phase separation as a universal condensate regulation strategy in managing molecular distribution, condensate location, as well as membrane interaction.We believe our approach will facilitate controlling the behavior of membraneless organelles within synthetic cells by providing an effective condensate regulation strategy and further allowing tunable interactions with the lipid membrane.

PRESSURE DEPENDENT ELASTIC CONSTANTS OF MEMBRANES

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Environments like the deep sea are characterized by low temperature (near freezing) and high pressure, with pressure increasing by 1 bar for every 10 m depth. This presents a substantial challenge for cell membranes because the membrane is the most pressure-sensitive material in the cell, with a compressibility ten times that of a folded soluble protein. Recently, Winnikoff et al. showed that a class of invertebrates called ctenophores synthesizes an extraordinary amount of plasmalogen to adapt to the high pressure of the deep ocean and suggest a "homeocurvature" mechanism—ctenophores adjust their lipidomes to maintain critical material properties within a narrow range, to preserve deformability at high pressure. Here, we use high-pressure (Hi-P) MD simulations to systematically study the pressure dependence of plasmalogens, comparing the spontaneous curvature under pressure of ester, ether, and plasmalogen-linked phospholipids. A comparison with Hi-P SAXS measurements suggests that ester and ether lipids have qualitatively correct pressure dependence. At the same time, simulations of the plasmalogen membranes show that the monolayer is too flat compared to the others. Here, we consider several explanations, including the pressure dependence of headgroup hydration and the conformational landscape of the plasmalogen linkage.

HOPANOIDS AND THE EVOLUTION OF LIPID ORDERING

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The cell membrane must balance mechanical stability with fluidity to function as both a barrier and an organizational platform. Key to this balance is the ordering of hydrocarbon chains and the packing of lipids. Many eukaryotes synthesize sterols, which are uniquely capable of modulating the lipid order to decouple membrane stability from fluidity. Ancient sterol analogs known as hopanoids are found in many bacteria and proposed as ancestral ordering lipids. The juxtaposition of sterols and hopanoids in extant organisms prompts us to ask why both pathways persist, especially in light of their convergent ability to order lipids. In this work, simulations, monolayer experiments, and cellular assays show that hopanoids and sterols order unsaturated phospholipids differently based on the position of double bonds in the phospholipid acyl chain. We find that cholesterol and diplopterol's methyl group distributions lead to distinct effects on unsaturated lipids. In Mesoplasma florum, diplopterol's constrained ordering capacity reduces membrane resistance to osmotic stress, unlike cholesterol. These findings suggest that cholesterol's broader lipid-ordering ability may have facilitated the exploration of a more diverse lipidomic landscape in eukaryotic membranes.

BIOPHYSICAL PROPERTIES OF CELLS AND NANOSCALE BIOPARTICLES AS NEW BI-OMARKERS IN HEALTH AND DISEASE

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Remodelling of our cells as response to environmental changes is essential for their survival and function. Although numerous studies aimed at finding protein markers during such cellular processes, there is a major gap in our understanding of how collective biophysical properties of the cells (such as stiffness, membrane fluidity, viscosity etc) alter during these crucial biological processes. Similarly, our understanding of how biophysical properties of cells change in diseases is also limited. To gain a thorough mechanistic perception of cellular processes and diseases, it is essential to fill this gap and have a clear and quantitative picture of biophysical remodelling of the cells. We and others have made extensive effort to unravel the biophysical aspects of cells in a quantitative manner. To achieve this, we developed advanced imaging approaches that could reveal the molecular details with very high spatiotemporal resolution. These technologies allowed us to see how biophysical properties of cells play crucial roles for signalling from molecular to cellular level. Although these technologies were extremely useful to study biophysical aspects of cellular life at the molecular level, their low sampling (one cell at a time) has been a major obstacle to apply them to medical problems that require measuring thousands of cells. This can be overcome with high throughput methodologies that can robustly report on the ensemble biophysical properties of cells which require reliable reporters and instruments. Thus, while developing advanced instrumentation, we also develop reliable probes to quantify different biophysical properties of cells. Here, I will discuss our approach from probe development to high throughput biophysical analysis.

BIOPHYSICAL BASIS FOR CAVEOLAE FORMATION AND FUNCTION

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Almost 70 years ago, numerous 65-nm diameter vesicles, now known as caveolae, were observed closely nestled against the plasma membrane of endothelial cells in capillaries of multiple tissues. Subsequent studies revealed similar flask-shaped invaginations on the plasma membrane of cells throughout the body and identified oligomers of the integral membrane protein caveolin-1 (CAV1) as an essential structural component of caveolae. Caveolins and caveolae are now recognized as key regulators of physiology, with cellular functions ranging from signaling and lipid homeostasis to mechanosensing and mechanoprotection. Yet, despite decades of study, the molecular mechanisms by which caveolins and caveolae operate remain elusive.Recently, we determined the first cryo-EM structure of CAV1. Unexpectedly, the structure revealed that CAV1 assembles into a disc-shaped amphipathic complex of 11 tightly packed protomers, organized into a flat, membrane-interacting “cap” with a central β-barrel facing the cytoplasm. Furthermore, the complex contains an extended hydrophobic interface, suggesting that CAV1 interacts with lipids and deforms membranes in unusual ways. Here, I will discuss how computational, biochemical, and cell-based studies inspired by this structure are shedding new light on the fundamental elements that define caveolins, the mechanisms by which they shape membranes and organize lipids to form caveolae, and their role as dynamic scaffolds for signaling proteins.

A FUNCTIONALIZED GM1 STRUCTURAL LIBRARY REVEALS DISTINCT MEMBRANE ORGANIZATION

Stefanie S. Schmieder, Waldemar Kulig, Cecilie Soennichsen, Ilpo Vattulainen, Wayne I. Lencer

Each cell exhibits a diversity of ceramide acyl chain structures in their sphingolipids, which likely influence the sphingolipid's membrane behavior and function. To address this question, we synthesized a functionalized library of GM1 glycosphingolipids with natural and synthetic acyl chain structures to quantitatively assess their membrane behavior and environment. Using both, super-resolution microscopy, single particle tracking and molecular dynamics simulations we defined 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 enables interaction of the acyl chain with cholesterol and therefore incorporation of the lipid into membrane nanodomains. This motif drives distinct subcellular sorting of these GM1 species into exclusively the lysosomal pathway. Perturbations of the C14* motif by e.g. cis-unsaturation disrupts acyl-chain cholesterol interaction leading to exclusion from membrane nanodomains for these GM1 species. GM1 species without C14* motif display membrane dynamics similar to transferrin receptor and enter, upon endocytosis, into endosomal sorting tubules of the recycling, retrograde and transcytotic pathways. Further functionalization of these two different types of GM1 species (with and without C14* motif) enabled the visualization of their respective membrane nano-environments. These results define the structural motives in sphingolipids underlying their overall membrane organization.

POSTER ABSTRACTS

Monday, July 7, 2025
Poster Session I
15:00 – 17:00
University of Copenhagen

Below are the formal presentations for Monday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd Number Boards Present 15:00 – 16:00 | Even Number Boards Present 16:00 – 17:00

Barrera, Francisco	**1-POS**	**Board 1**
Brocklehurst, James	**2-POS**	**Board 2**
Cervantes, Marcos	**5-POS**	**Board 3**
Chen, Ariel	**6-POS**	**Board 4**
Frey, Shelli	**9-POS**	**Board 5**
Geißler, Katharina	**10-POS**	**Board 6**
Hitaishi, Prashant	**13-POS**	**Board 7**
Hossein, Amirali	**14-POS**	**Board 8**
Imai, Masayuki	**17-POS**	**Board 9**
Inanc, Arda	**18-POS**	**Board 10**
Kurisu, Minoru	**21-POS**	**Board 11**
Lazaridis, Themis	**22-POS**	**Board 12**
Lotfipour Nasudivar, Sara	**25-POS**	**Board 13**
Machta, Benjamin	**26-POS**	**Board 14**
Moller, Elissa	**29-POS**	**Board 15**
Mori, Toshiki	**30-POS**	**Board 16**
Perez-Salas, Ursula	**33-POS**	**Board 17**
Pesen Inanc, Tuna	**34-POS**	**Board 18**
Ramirez, Ricard	**37-POS**	**Board 19**
Razmazma, Hafez	**38-POS**	**Board 20**
Schmieder, Stefanie	**41-POS**	**Board 21**
Schott-Verdugo, Stephan	**42-POS**	**Board 22**
Simonsen, Adam Cohen	**45-POS**	**Board 23**
Srivastava, Anand	**46-POS**	**Board 24**
Thoma, Johannes	**49-POS**	**Board 25**
Tuomivaara, Sami	**50-POS**	**Board 26**
Villamil, Ana	**53-POS**	**Board 27**
Wittenberg, Nathan	**54-POS**	**Board 28**

Posters should be set up on the morning of Monday, July 7 and removed by 18:00. All uncollected posters will be discarded.

1-POS Board 1

POLYSACCHARIDE-COATED MEMBRANE MODEL SYSTEMS

Teresa Rodríguez-García[1]; Loretta Akakpo[2]; Sadie L Nickles[3]; Ryan J Schuck[1]; Daiane S Alves[1]; Katherine G Schaefer[3]; Frederick A Heberle[2]; Gavin M King[3,4]; **Francisco N. Barrera**[1];
[1]University of Tennessee, Department of Biochemistry & Cellular and Molecular Biology, Knoxville, TN, USA
[2]University of Tennessee, Department of Chemistry, Knoxville, TN, USA
[3]University of Missouri, Department of Physics and Astronomy, Columbia, MO, USA
[4]University of Missouri, Department of Biochemistry, Columbia, MO, USA

The plasma membrane of eukaryotic cells is coated by the glycocalyx, which is formed by glycosylation of lipids and proteins at the extracellular side of the membrane. The glycocalyx plays a structural role that protects cell integrity and participates in mechanosensing and other processes. However, our understanding of glycocalyx function is hampered by the lack of suitable model systems to perform biophysical investigation. Here, we describe the engineering of asymmetric glycoliposomes, where lipid vesicles were chemically conjugated at the outer surface with glycosaminoglycans (GAGs). GAGs, also known as mucopolysaccharides, are long and linear polysaccharides consisting of repeating two-sugar units. We used the abundant GAG chondroitin sulfate, which was modified with a thiol group. Liposomes were doped with headgroup-modified maleimide lipids, which allowed thiol-maleimide conjugation of the chondroitin sulfate molecule on a phospholipid. In order to verify the attachment of the GAGs to the membrane, we used an environmentally sensitive fluorophore tagged on lipid headgroups. Studies were extended to coated giant unilamellar vesicles, which were investigated by confocal microscopy to reveal the polysaccharide distribution on the membrane. Finally, atomic force microscopy revealed that chondroitin sulfate coating of supported lipid bilayers yields a rough membrane surface. To summarize, this work highlights the development of a facile protocol for the coating with chondroitin sulfate of the three main types of model membrane systems: large and giant unilamellar vesicles, and supported lipid bilayers. The use of chondroitin sulfated coated membranes will allow more physiologically-relevant membrane transport assays, to better mimic the binding of proteins and peptides to the cell surface, and to determine how glycan asymmetry influences lipid asymmetry.

1-POS Board 1

POLYSACCHARIDE-COATED MEMBRANE MODEL SYSTEMS

Teresa Rodríguez-García[1]; Loretta Akakpo[2]; Sadie L Nickles[3]; Ryan J Schuck[1]; Daiane S Alves[1]; Katherine G Schaefer[3]; Frederick A Heberle[2]; Gavin M King[3,4]; **Francisco N. Barrera**[1];
[1]University of Tennessee, Department of Biochemistry & Cellular and Molecular Biology, Knoxville, TN, USA
[2]University of Tennessee, Department of Chemistry, Knoxville, TN, USA
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2-POS Board 2

INFLUENCE OF CHOLESTEROL CONTENT ON THE HYDRATION OF PHOSPHATIDYLCHOLINE BILAYERS

James Brocklehurst[1]; Simon Connell[2]; Michael Rappolt[1];
[1]University of Leeds, School of Food Science and Nutrition, Leeds, United Kingdom
[2]University of Leeds, School of Physics and Astronomy, Leeds, United Kingdom

The binary phase diagram of dimyristoylphosphatidylcholine (DMPC)/cholesterol has been widely studied for a better understanding of cholesterol's role in regulating the fluidity of plasma cell membranes. Less is known about the molecular interplay between lipid, cholesterol, including additionally the confined water at the bilayer/water interface. Applying the recently published Three-Water Layer model, extracted structural parameters and membrane hydration data improve our understanding of the hydration behaviour of liquid disordered, L_d, and liquid ordered, L_o, phases. DMPC/cholesterol dispersions spanning 0-35 mol% cholesterol were analysed, using small-angle X-ray scattering over a 15-60 °C temperature range. The data were analysed using a global fitting method, wherein the electron density profile was refined with a three Gaussian model, mimicking the lipid headgroup, the methyl trough region, and the cholesterol insertion within the lipid leaflets. Dilatometry was performed over the same temperature range to obtain volumetric data. Key structural parameters of the bilayer concern the Gibbs dividing surface, the headgroup-to-headgroup distance, the headgroup extension, hydrocarbon chain length, and area per lipid. Additionally, three water layers are discerned, relating to bound water at the headgroup, perturbed water, and unperturbed water. General difference of the hydration properties in the L_o and L_d phases show that the overall water layer thickness in the L_d phase is dominated by the perturbed water layer, while the L_o phase displays a thinner perturbed water layer, but is dominated by a larger free water core instead. Generally, the thickness of the perturbed water layer increases with temperature and membrane fluidity, whereas the thickness of the free water core exhibits the opposite trend. The relative position of the cholesterol within the bilayer, depends on both the hydration and structural properties of the membrane, displaying the deepest insertion in the miscibility gap.

5-POS Board 3

PROBING MEMBRANE CHARACTERISTICS THAT IMPACT BINDING AND FUSION KINETICS IN SARS-COV-2 ENTRY

Marcos Cervantes[1,2]; Peter M Kasson[1,2,3]; Tobin Hess[3];
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The viral entry process for SARS-CoV-2 is mediated by the viral spike glycoprotein and the canonical entry point involves binding to the ACE2 receptor expressed on the surface of cells. Subsequent activation of the spike glycoprotein via a suitable protease facilitates eventual viral-host membrane fusion. Previously, we have shown that membrane fusion to artificial liposomes can proceed in the absence of ACE2 receptor engagement as long as appropriate attachment factors and protease are present, albeit at a slower rate compared to fusion in plasma membrane vesicles. Kinetic analysis has revealed that this slower, ACE2-independent process contains at least 2 rate-limiting steps, and that engagement with soluble receptor removes at least one of these kinetic barriers in synthetic liposomes. However, the fastest kinetics observed in plasma membrane vesicles are not fully reconstituted using soluble receptor, hinting that there remains an unidentified biochemical factor influencing the entry process. Here, we attempt to identify these contributions from the plasma membrane by probing how both host membrane organization and stoichiometric arrangement of the ACE2 receptor affects viral binding and subsequent fusion. We probe the former by altering lipid compositions and geometries in both synthetic liposomes and plasma membrane vesicles. The latter is investigated by single-molecule counting and stoichiometric analysis of ACE2 clustering effects using fluorescent microscopy techniques. Initial results suggest that moderate changes to plasma membrane cholesterol content do not affect fusion rates and that fusion displays a non-trivial dependence on ACE2 surface display, which we dissect. From this we aim to identify the exact factors that influence the viral entry process of SARS-CoV-2 as well as establish a platform for probing those factors that contribute to this process from the host plasma membrane.

6-POS Board 4

STRATEGY FOR GENERATING GIANT UNILAMELLAR VESICLES (GUVS) WITH WELL-CONTROLLED SIZE USING THE MODIFIED CDICE METHOD

Ariel Chen[1]; Shachar Gat[1]; Lior Ohana[1]; Evgenee Yekymov[1]; Yoav Tsori[1]; Anne Bernheim-Groswasser[1,2];
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[2]Ben Gurion University of the Negev, Ilse Kats Institute for Nanoscale Science and Technology, Be'er Sheva, Israel

Giant unilamellar vesicles (GUVs) provide a model system for understanding cellular processes and serve as a platform for the construction of artificial cells with biomimetic functions. Among the various methods developed for producing GUVs, the modified continuous droplet interface crossing encapsulation (cDICE) method offers several advantages: it is rapid and inexpensive, and demonstrates high production yields and encapsulation efficiency. However, the size distribution of the generated GUVs is highly polydisperse and poorly controlled, presenting a main drawback in using this method for the development of artificial cells. Here, we propose a strategy where we optimize the conditions for achieving effective GUV size selection. By systematically investigating critical parameters—the rotation time (t_ROT), angular frequency (ω), and inner solution density (ρ_I)—we demonstrate their role as useful control parameters for optimizing size sorting in the system. Our data show that as the rotational time decreases, the distribution shifts toward higher GUV diameters, with fewer observed GUVs but a higher frequency of larger ones. Similarly, we find that as ω decreases, the distribution shifts toward higher GUV diameters with improved size sorting, accompanied by a reduction in the total yield. Investigating the effect of inner solution density (ρ_I), our data demonstrate that as the inner solution density decreases, the distribution similarly shifts toward larger GUV diameters, again with fewer overall vesicles but a higher frequency of larger ones. Importantly, encapsulation efficiency remained high (>80%), even when encapsulating complex biological systems, including preformed actin filaments and actomyosin networks. This high encapsulation efficiency underscores the robustness of the modified cDICE method for encapsulating diverse biological systems. Overall, by providing a robust methodology for GUV sorting, our optimized strategy offers considerable advantages for researchers in developing cell-mimicking compartments for synthetic biology applications, as well as in designing bioreactors for biomedical and gene therapy applications.

9-POS Board 5

INVESTIGATING THE MEMBRANE CURVATURE SENSING ABILITY OF THE N-TERMINAL DOMAIN OF HUNTINGTIN

Jordyn M Markle[1]; Neha Nanajkar[2]; Abhilash Sahoo[3]; Silvina Matysiak[4]; **Shelli L Frey**[1];
[1]Gettysburg College, Chemistry, Gettysburg, PA, USA
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Huntington's disease (HD) is an inherited neurodegenerative disorder associated with motor and cognitive decline, caused by a mutation in the poly-glutamine (polyQ) region near the N-terminus of the huntingtin (htt) protein. Expansion of the polyQ region results in the disease that is characterized by oligomeric and fibrillar aggregates of mutated protein. The first 17 amino acids (Nt17) of htt, which are adjacent to the polyQ tract, function as a lipid-binding domain, facilitated by the formation of an amphipathic α-helix. There is increasing evidence that lipid interactions may play a role in the toxic gain of function associated with the htt polyQ expansion, as membrane-related changes, including structural abnormalities of several organelles, are observed in HD. Given the uneven and curved shapes of organelles, it is important to examine the mechanistic preferences that drive the preferential partitioning of Nt17 to curved membranes. To better understand the role of the cell membrane environment in the interaction and aggregation of htt, circular dichroism, fluorescence microscopy, and coarse-grained molecular dynamics were employed to measure the association of Nt17 with phospholipid vesicles and subsequent effects throughout time. In zwitterionic curved membranes, sensing was driven by the bulky sidechains of phenylalanine residues, which are able to sense lipid packing defects in the curved regions of the membrane. However, in a mixture of zwitterionic and anionic lipids, curvature sensing is affected by the anionic lipid content, implying the surface charge of membranes affects the curvature sensing process. Salt screening experiments suggest a balance between the electrostatic and hydrophobic interactions that governs the extent to which Nt17 can sense physiologically relevant regions of curvature.

10-POS Board 6

VAULT ASSOCIATES WITH MEMBRANES IN SITU

Katharina Geißler[1,3]; Jan Philipp Kreysing[1,3]; Yuning Wang[1,3]; Patrick C Hoffmann[1]; Desislava Glushkova[1,3]; Gerhard Hummer [2,4]; Martin Beck[1,5];
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[2]Max Planck Institute of Biophysics, Theoretical Biophysics, Frankfurt am Main, Germany
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[4]Goethe University, Institute of Biophysics, Frankfurt am Main, Germany
[5]Goethe University, Institute of Biochemistry, Frankfurt am Main, Germany

The eukaryotic Vault particle is a giant ribonucleoprotein complex that assembles into an iconic cage-like structure. Yet, despite their abundance, unique appearance, potential therapeutic use, and four decades of research, their cellular function remains obscure. Thus, we set out to delineate Vault's subcellular context by cryo-electron tomography. We found that a minor fraction of Vault particles transiently associates to ER membranes in situ and solve the structure of cytosolic and membrane-associated particles by subtomogram averaging. We find that membrane binding occurs at a defined region of the Vault, indicating specific interaction. When comparing Vault's membrane association to members of the phylogenetically related SPFH protein family, substantial differences are apparent. Our data suggest a noncanonical membrane binding mechanism requiring massive conformational rearrangements, potentially explaining why Vault's membrane binding has been overlooked previously. Additionally, our dataset reveals that both soluble and membrane-bound Vaults occasionally encapsulate ribosomes with specific relative orientations. While membrane-bound Vaults encapsulate ribosomes in a translation-competent state, soluble Vaults enclose ribosomes with a different relative orientation, pointing towards a functional relationship between Vaults and ribosomes. Our findings suggest an unanticipated role of Vault in membrane quality control or as a ribosome carrier and urge further experimental investigations to elucidate the function of this enigmatic particle.

13-POS Board 7

REVERSIBLE PHOTO-SWITCHING DYNAMICS OF AZOBENZENE GLYCOCONJUGATES IN CHOLESTEROL-MODIFIED LIPID MEMBRANES

Prashant Hitaishi[1]; Svenja C Hövelmann[1,2,3]; Nicolas Hayen[1]; Philipp Jordt[1]; Ella Diebell[1]; Julia Kobus[1]; Otto C Lippmann[1,5]; Chen Shen[3]; Thisbe Lindhorst[4]; Bridget Murphy[1,2];
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[2]University of Kiel, Ruprecht Haensel Laboratory, Kiel, Germany
[3]Deutsches Elektronen Synchrotron, Hamburg, Germany
[4]University of Kiel, Otto Diels-Institut für Organische Chemie, Kiel, Germany
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Reversible photo-switching molecules have applications in smart surfaces and biomedical technologies, particularly in light-triggered drug delivery. Light offers spatial, temporal, and intensity control, making it a potential external stimulus as compared to chemical or biological triggers. Azobenzene glycoconjugates (Azo) undergo reversible trans–cis isomerization upon alternating UV and visible light exposure, enabling light-mediated control of molecular conformation and membrane properties [1]. Our recent work has investigated the light-responsive behavior of Azo embedded in model lipid monolayers composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) [2]. We demonstrated that Azo integration results in reversible, reproducible bidirectional photo-switching. Notably, light-induced switching in DPPC monolayers revealed an additional phase transition associated with shifts in surface pressure and layer thickness, marking a crossover point between molecular states [3]. To better mimic biological membranes, we extended this study to DPPC membranes with varying cholesterol content (0, 15, 30 mol%). Cholesterol is known to modulate membrane rigidity, permeability, and promote the formation of lateral heterogeneities such as liquid-ordered domains. These microdomains significantly influence membrane dynamics and function. Our findings reveal that Azo molecules exhibit distinct excitation and relaxation dynamics depending on the cholesterol concentration, suggesting altered local environments and phase behavior. The interplay between Azo photo-isomerization and cholesterol-induced membrane organization provides insight into the molecular mechanisms underlying light-responsive behavior in complex lipid systems. This work contributes to understanding the dynamic membrane controlled through external stimuli and supports the potential of Azo-based systems for responsive biomaterials and therapeutic applications.References:Reise, Franziska, et al. "Photoswitchable glycolipid mimetics: synthesis and photochromic properties of glycoazobenzene amphiphiles." Chemistry–A European Journal 24.66 (2018): 17497-17505.Hövelmann, Svenja C., et al. "Photoinduced bidirectional mesophase transition in vesicles containing azobenzene amphiphiles." IUCrJ 11.4 (2024): 486-493.Warias, Jonas E., et al. "Photoinduced bidirectional switching in lipid membranes containing azobenzene glycolipids." Scientific reports 13.1 (2023): 11480.

14-POS Board 8

INVESTIGATING CURVATURE SENSITIVITY OF MEMBRANE-ASSOCIATED PROTEINS

Amirali Hossein[1]; Jay Dadhania[1]; Noah Englander[1]; Alexander J Sodt[1];
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Membrane-associated proteins play a crucial role in determining the mechanical response of biological membranes, and as such, elucidating the nature of the strong specific interactions of these proteins with the surrounding lipids is as vital as understanding the role of the lipids themselves. Using an extension of our previously introduced method of dynamically sampling lipid-curvature coupling in molecular dynamics simulations, we investigate curvature sensitivity of a number of peripheral and integral proteins such as the tandem C2 domains of Synaptotagmin (Syt) isoforms, which are involved in membrane fusion through the SNARE complex, the amphipathic helix of IFITM3, a protein involved in antiviral activity against a variety of enveloped viruses, as well as a coarse-grained model of the autophagosomal protein ATG9A. This work contrasts the advantages and disadvantages of dynamical sampling of curvature coupling with the traditional method of characterizing the average membrane deformation around a protein. A critical element is the dynamic correlation of protein-lipid interactions with curvature, which is straightforward for our curvature coupling approach.

17-POS Board 9

MORPHOGENESIS OF CANCER ORGANOIDS REVEALED BY PHASE FIELD MODEL

Toshikaze Chiba[1]; Kotaro Kawamura[1]; Tatsuaki Tsuruyama[2]; **Masayuki Imai**[1];
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Tumors show characteristic morphologies based on their pathological state, and these morphologies provide crucial information for pathological diagnosis. Therefore, clarifying the mechanical and physiological parameters that govern tumor morphogenesis and their relationship to the gene expression information of cancer cells is important for improving the reliability of pathological diagnoses. In this study, we aim to extract mechanical and physiological parameters that can reproduce the morphological characteristics of primary and metastatic ovarian cancer organoids by simulations using a phase field model. In the Phase field model calculations, the organoids are represented as a cell cluster surrounding lumens, focusing on parameters such as surface tension, adhesive force, lumen pressure, cell division cycle, and cell polarity to control morphology, and we examined the effects of these parameters on morphology.Observed morphologies of ovarian cancer organoids revealed a significant difference: in primary cancer organoids, the lumen structure appeared at the early proliferation stage (4-cell stage), while in metastatic cancer organoids, the lumen was only observed after the organoids grew to the 40-cell stage. Therefore, we conducted phase field model simulations of the organoid morphogenesis with varying parameters, to examine the relationship between the lumen structure formation and parameters. The results showed that below a certain lumen pressure, the lumen is crushed and disappears; above this pressure, the lumen can exist stably, but if the division cycle is prolonged, the stability of the lumen is compromised, and the organoid structure collapses. Furthermore, when the adhesive force weakens, the organoids cannot withstand the lumen pressure and split into disparate cells. The differences in the lumen structure formation process observed in experiments can be explained by the balance between lumen pressure and the cell cycle.

18-POS Board 10

RAMAN SPECTROSCOPY AND OPTICAL TWEEZERS FOR ASSESSING HEAT-INDUCED MECHANOCHEMICAL ALTERATIONS IN HUMAN RED BLOOD CELLS

Arda Inanc[1,2]; Tuna Pesen[1,2]; Sena Saglam[1]; Bora Akgun[1,2];
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We investigated the heat-induced damage in red blood cells (RBCs) using Raman spectroscopy and optical tweezers. Our results reveal a significant decrease in the deformability index at elevated temperatures, indicating increased stiffness and reduced mechanical stability. Meanwhile, Raman spectral analysis shows distinct differences in key molecular bands, including porphyrin, protein, and lipid-associated peaks, reflecting structural alterations in hemoglobin and membrane components. These findings provide a comprehensive perspective on RBC damage under heat stress by correlating mechanical stiffening with molecular level transformations. The study highlights the interplay between biomechanical and biochemical changes in thermally stressed RBCs, offering insights into heat-induced cellular dysfunction.

21-POS Board 11

OSMOTIC SPAWNING VESICLE

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Cell division is one of the essential cellular functions, but it has been challenging to artificially reconstruct it starting from simple lipid vesicles. In this study, we present a casade vesicle division system, where a single vesicle under hypertonic stress spontaneously produces multiple daughter vesicles by repeating membrane budding and fission. Giant unilamellar vesicles (GUVs) were composed of bis (2-ethylhexyl) sulfosuccinate (AOT) and cholesterol (Chol). The GUVs encapsulate a 20 mM NaH_2PO_4 solution containing membrane-impermeable osmolytes, sucrose, ranging from 5–500 mM. The binary AOT + Chol GUVs were then immersed in a 20 mM NaH_2PO_4 solution containing membrane-permeable osmolytes, fructose, at the same concentration as the encapsulated sucrose. This simple experimental setup enabled an asymmetry in membrane permeabilities of osmolytes and subsequently imposed osmotic tension on the vesicle membranes. When the external bulk solution contained AOT molecules, the binary AOT + Chol GUVs incorporated additional AOT molecules from the bulk solution to relax the membrane tension stress. In previously reported phospholipid vesicle systems, such an osmotically tensed GUVs resulted in vesicle inflation, maintaining their spherical shapes. However, in our binary AOT + Chol GUVs system, the mother GUVs repeatedly form small membrane buds and subsequently undergo division over several hundred seconds, resulting in the production of approximately 30–300 daughter GUVs from a single mother GUV. The observed morphological change of GUVs is well described by the mechanical balance between membrane bending, membrane tension, and osmotic pressure difference based on the spontaneous curvature model. This osmotic spawning behavior of GUVs does not rely on chemical reactions or functional macromolecules. Therefore, this cascade division system will be compatible with various chemical systems and has the potential to implement proliferation ability in artificial cells, drug delivery systems, and protocells simply by modifying their membrane compositions and osmolytes.

22-POS Board 12

LIPID COMPOSITION EFFECTS ON THE MEMBRANE-BOUND STRUCTURE OF THE CAVEOLIN 8S COMPLEX

Sayyid Y Vasquez Rodriguez[1]; **Themis Lazaridis**[1];
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The protein caveolin-1 (CAV1) is essential in the generation of caveolae, cup-like invaginations in the plasma membrane, but the mechanism of its action remains unclear. A recent cryo-EM structure showed an 11-mer of CAV1 (the 8S complex) forming a disk with a flat membrane-facing surface, raising the question of how a flat complex is able to generate membrane curvature. We previously conducted implicit-solvent molecular dynamics simulations, which showed the 8S complex adopting a conical shape, with its outer ridge deep inside the implicit membrane. These results suggested a scaffolding-type mechanism for curvature generation by the 8S complex. We then conducted all-atom simulations, which also showed the complex taking a conical shape. Starting deep inside the bilayer, displacing the proximal leaflet, led to the distal leaflet lipids adsorbed onto the 8S concave surface. To characterize the curvature generating potential of 8S and the role of specific lipids, we are currently conducting simulations in nanodiscs with different lipid compositions.

25-POS Board 13

Abstract Content:

MEMBRANE DAMAGE INDUCED BY LIPID PEROXIDATION

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Ferroptosis is a form of regulated cell death triggered by the excessive accumulation of lipid peroxides in the plasma membrane that exceeds the capacity of the cellular antioxidant defence system. This oxidation predominantly affects long-chain polyunsaturated fatty acids (PUFAs) within the plasma membrane and leads to significant alterations in its biophysical properties. A major unanswered question in the field of ferroptosis is how PUFA peroxidation disrupts plasma membrane integrity, leading to membrane permeabilization and cell death. In model membranes, oxidation induced by the Fenton reaction led to increased permeability in vesicles, which correlated kinetically with malondialdehyde formation. In a supported lipid bilayer, we observed with atomic force microscopy (AFM) altered lateral organization, a reduction in lipid phase mismatch, and decreased circularity of ordered domains. AFM force spectroscopy measurements further revealed a destabilized bilayer, indicated by a decreased breakthrough force in piercing experiments. We will further expand our findings of lipid oxidation-induced interaction changes in the lipid bilayer through future experiments on ferroptotic plasma membrane sheets. Additionally, we will determine mechanical properties such as the bending modulus and compare these in cell membranes to those observed in simple model membrane systems.

26-POS Board 14

CONDENSATE PRONE BULK MIXTURES CAN PREWET MEMBRANES

Benjamin B. Machta[1]; Mason Rouches[1,2]; Yousef Bagheri[3]; Sarah Veatch[3];
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Many macromolecular components in cells have a thermodynamic propensity to phase separate into coexisting liquid phases, sometimes termed condensates. In a few cases these condensates present as roughly spherical three-dimensional droplets even within cells. But often, while components will form three dimensional droplets when isolated at high concentrations, in the cellular context they are present at far lower concentration, and the domains that they form are localized to other structures, like the plasma membrane. Here I will argue that many of these domains are likely generalized prewet phases, stable only due to interactions with biological surfaces that contain their own transitions. Classical prewetting occurs when bulk mixtures tuned near to phase coexistence undergo thermodynamic surface transitions at a suitable surface even though they are outside of true bulk coexistence. But for classical surfaces the regime of prewetting is very narrow. Here I will focus on prewetting to the plasma membrane, a two-dimension fluid which has its own propensity to phase separate into coexisting two-dimensional liquids, with many cell types sitting near a critical point of this transition. With a combination of theory, simulation and experiment I will show that the fluid nature of the membrane along with its own propensity to phase separate vastly enlarges the prewetting regime.

29-POS Board 15

POLYMER-EXTRACTED STRUCTURE OF MSCS REVEALS MECHANISM OF INACTIVATION

Elissa Moller[1,2,3]; Madolyn Britt[1,2]; Fei Zhou[1]; Hyojik Yang[4]; Andriy Anishkin[2]; Robert Ernst[4]; Juan Vanegas[5]; Doreen Matthies[1]; Sergei Sukharev[2,3];
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Membrane protein structure determination is technically challenging and further complicated by the removal or displacement of lipids, which can result in non-native conformations or a strong preference for certain states at the exclusion of others. This is especially applicable to mechanosensitive channels (MSC's) that evolved to gate in response to subtle changes in membrane tension in the lipid bilayer. E. coli MscS, a model system for MSC gating, is an inner membrane protein that opens when external osmolarity changes cause water influx and stretches the membrane. The efflux of osmolytes through these channels reduces the osmotic gradient and prevents cell lysis, enabling bacteria to colonize osmotically challenging host environments and survive transmission through fresh water. As a tension sensor, MscS is very sensitive and highly adaptive. It readily opens under super-threshold tension and closes upon tension reduction, but under lower tensions, it slowly inactivates and can only recover after tension release. Existing cryo-EM structures do not explain the entire functional gating cycle of open, closed, and inactivated states. A central question in the field has been the assignment of the frequently observed non-conductive conformation to either a closed or inactivated state. In this study we solved a 3 Å cryo-EM structure of MscS in native nanodiscs obtained via extraction with the novel Glyco-DIBMA polymer, eliminating the detergent solubilization and lipid removal step common to all prior structures. We observe densities of endogenous phospholipids between the transmembrane helices, stabilized by electrostatics interactions. Through mutations we examine the functional effects of their destabilization, illustrating a novel lipid-mediated inactivation mechanism based on an uncoupling of the peripheral tension-sensing helices from the gate. The use of this polymer increased the predictive power of our cryo-EM structure, allowing us to associate the solved conformation with the inactivated state of the multi-state MSC MscS.

30-POS Board 16

REGULATION MECHANISMS OF K-RAS SIGNAL TRANSDUCTION BY LIPID DOMAINS AS REVEALED BY HIGH-SPEED SUPER-RESOLUTION MICROSCOPY

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Lipids in the outer leaflet of the cell plasma membrane (PM) have been supposed to form signaling platforms, such as lipid rafts. However, the distribution and domain organization of lipids in the inner leaflet remain elusive. To elucidate this, we visualized lipid distribution using super-resolution microscopy to determine whether inner leaflet lipids form distinct domains. Our analysis revealed that all 6 lipid species examined transiently formed small domains of less than 100 nm in diameter. Next, we investigated whether these lipid domains function as signaling platforms for K-Ras, a pivotal small G protein that associates with the inner leaflet the PM via a farnesyl-cysteine-methyl-ester anchor. Previous studies have suggested that K-Ras interacts with phosphatidylserine to form nanoclusters essential for downstream signal transduction (Zhou et al. Science, 2015; Cell, 2017). However, the precise mechanisms by which lipid domains regulate K-Ras activity and their interactions with downstream signaling molecules remain unknown. To address this issue, we aimed to simultaneously visualize K-Ras or BRAF, a key effector of K-Ras, alongside lipid domains in living cell PMs. We found that, upon activation, K-Ras underwent dynamic transitions between lipid domains of distinct compositions. Furthermore, by combining super-resolution microscopy of lipid domains with single-molecule imaging of BRAF, we observed that BRAF was exclusively recruited to specific lipid domains. These findings offer unprecedented insights into the spatial regulation of K-Ras signaling.

33-POS Board 17

UNEXPECTED ASYMMETRIC DISTRIBUTION OF CHOLESTEROL AND PHOSPHOLIPIDS IN EQUILIBRIUM MODEL MEMBRANES

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Lipid compositional asymmetry across the leaflets of the plasma membrane is an ubiquitous feature in eukaryoticcells. How this asymmetry is maintained is thought to be primarily controlled by active transport of lipids between leaflets. Thisstrategy is facilitated by the fact that long-tail phospholipids and sphingolipids diffuse through the lipid bilayer slowly—takingmany hours or days. However, a lipid like cholesterol—which is the most abundant lipid in the plasma membrane of animalcells—has been harder to pinpoint in terms of its favored side. In this work we show that, when a saturated lipid is added toa mix of the unsaturated lipid palmitoyl-oleoyl-phosphatidylcholine (POPC) and cholesterol, both cholesterol and the long-tailphospholipids organize asymmetrically across the membrane's leaflets naturally. In these extruded unilamellar vesicles, mostcholesterol as well as the saturated lipid—dipalmitoylphosphatidylcholine or sphingomyelin—segregated to the inner leafletwhile POPC preferentially localized in the outer leaflet. This asymmetric arrangement generated a slight phospholipid numberimbalance favoring the outer leaflet and thus opposite to where cholesterol and the saturated lipids preferentially partitioned.These results were obtained using magic-angle spinning nuclear magnetic resonance (MAS NMR) in combination with small-angle neutron scattering (SANS) using isotope labeling to differentiate lipid species. We suggest that sidedness in membranescan be driven by thermodynamic processes. In addition, our MAS NMR results show that the lower bound for cholesterol's flip-flop half-time at 45 C is 10 ms, which is at least two orders of magnitude slower than current MD simulations predict. This resultstands in stark contrast to previous work that suggested that cholesterol's flip-flop half-time at 37 C has an upper bound of 10 ms.

34-POS Board 18

THE EffECT OF REPETITIVE STRETCHING ON THE DEFORMABILITY OF HUMAN RED BLOOD CELLS

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The red blood cell (RBC) cytoskeleton plays a pivotal role in maintaining the cell's mechanical integrity and ensuring physiological functions. As RBCs traverse microcapillaries narrower than their diameter, their highly deformable cytoskeleton enables them to withstand significant mechanical stress while preserving cell integrity. Disruption of this delicate balance is linked to various pathological conditions such as sickle cell disease, malaria, and diabetes mellitus. To understand how cytoskeletal mechanics change under mechanical stress, we investigated the mechanical response of RBCs subjected to repetitive stretching-relaxation cycles using optical tweezers. By applying biaxial stretch-relaxation cycles we assessed how the RBC cytoskeleton responds to sustained mechanical load. Our results reveal that RBCs become progressively stiffer with each cycle, eventually reaching a state of complete rigidity. Moreover, prolonged stretching induced non-uniform morphological changes, including localized dark regions and extreme elongation. These findings highlight the cytoskeleton's role in RBC mechanical resilience and provide insights into how mechanical stress may compromise cellular function in disease conditions.

37-POS Board 19

2DANALYSIS: AN OPEN-SOURCE PROJECT TO STUDY COMPLEX LIPID MEMBRANES AND THEIR INTERACTIONS WITH BIOPOLYMERS

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Molecular simulations expand our ability to study the interplay of biomolecules in complex environments. Biological membranes, composed of diverse lipids with varying physicochemical properties, are highly dynamic structures that modulate cellular functions. Proteins, nucleic acids, glycans, and bio-compatible polymers interact with these membranes and other surfaces, influencing molecular organization and function. The biophysical and mechanical properties of lipid membranes vary across the plane perpendicular to the normal, making two-dimensional analysis a highly suitable approach for capturing these variations. Similarly, biopolymers interacting with surfaces experience restricted degrees of freedom, making 2D projections a natural choice for quantifying their interactions. 2Danalysis is a computational toolbox designed to project membrane and biopolymer properties onto a two-dimensional plane, enabling the characterization of molecular interactions and spatial correlations that define lipid-lipid, lipid-biopolymer, and biopolymer-surface signatures. The toolbox contains two hubs implemented using MDAKits architecture, one for membranes and one for biopolymers, that can be used independently or together. Here, case studies and tutorials in GitHub introduce the user to the toolbox capabilities and suggest additional analysis that benefits from the generated outputs. These analyses include order parameters, lipid tail splay angles, membrane thickness, and packing defects; all of which can be readily applied to multiple replicates with diverse lipid compositions, facilitating streamlined pipeline development for membrane studies.

38-POS Board 20

SCRAMBLING PATHWAYS IN THE GHRELIN RECEPTOR ARE MODULATED BY MEMBRANE LIPID COMPOSITION

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G protein-coupled receptors (GPCRs) are best known for their central roles in signal transduction, although recent studies have revealed that certain Class A GPCRs may also exhibit lipid scramblase activity [1], expanding our understanding of their functional versatility. In this work, we explore the Ghrelin receptor (GHSR), a highly constitutively active GPCR involved in energy balance and hormonal regulation, and its unexpected ability to mediate bidirectional phospholipid translocation. Using coarse-grained molecular dynamics simulations (MARTINI3 force field), we show that both active and inactive conformations of GHSR facilitate lipid flip-flop through three distinct membrane-spanning pathways: primarily between transmembrane helices TM5 and TM6, secondarily via TM2–4, and additionally between TM4 and TM5. These helices form a groove that could facilitate lipid scrambling via a similar credit card mechanism as identified for other scramblases [1]. Simulations of the naturally occurring GHSR mutant A204E, known to affect basal signaling, showed a marked disruption of lipid translocation pathways, in agreement with experimental observations of reduced scramblase activity. Notably, the presence of cholesterol strongly suppresses scrambling by occupying key translocation pathways, indicating a direct regulatory role for membrane composition, consistent with its established effect on GHSR's canonical signaling. These computational predictions were supported by fluorescence-based assays in proteoliposomes containing purified GHSR and physiologically relevant lipids such as cholesterol, PIP2, ceramides, POPC, POPE, and POPG, confirming GHSR as a scramblase sensitive to membrane composition, particularly cholesterol. Our findings underscore the complex interplay between membrane proteins and the lipid environment and provide insight into how membrane complexity modulates the multifunctionality of GPCRs in living systems.[1] Khelashvili, George, and Anant K. Menon. "Phospholipid scrambling by G protein–coupled receptors." Annual review of biophysics 51, no. 1 (2022): 39-61.

41-POS Board 21

A FUNCTIONALIZED GM1 STRUCTURAL LIBRARY REVEALS DISTINCT MEMBRANE ORGANIZATION

Stefanie S. Schmieder, Waldemar Kulig, Cecilie Soennichsen, Ilpo Vattulainen, Wayne I. Lencer

Each cell exhibits a diversity of ceramide acyl chain structures in their sphingolipids, which likely influence the sphingolipid's membrane behavior and function. To address this question, we synthesized a functionalized library of GM1 glycosphingolipids with natural and synthetic acyl chain structures to quantitatively assess their membrane behavior and environment. Using both, super-resolution microscopy, single particle tracking and molecular dynamics simulations we defined 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 enables interaction of the acyl chain with cholesterol and therefore incorporation of the lipid into membrane nanodomains. This motif drives distinct subcellular sorting of these GM1 species into exclusively the lysosomal pathway. Perturbations of the C14* motif by e.g. cis-unsaturation disrupts acyl-chain cholesterol interaction leading to exclusion from membrane nanodomains for these GM1 species. GM1 species without C14* motif display membrane dynamics similar to transferrin receptor and enter, upon endocytosis, into endosomal sorting tubules of the recycling, retrograde and transcytotic pathways. Further functionalization of these two different types of GM1 species (with and without C14* motif) enabled the visualization of their respective membrane nano-environments. These results define the structural motives in sphingolipids underlying their overall membrane organization.

42-POS Board 22

MOLECULAR DYNAMICS INSIGHTS ON HOW THE BACTERIAL ESCRT-III PSPA COMPLEX INDUCES MEMBRANE TUBULATION

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[4]Heinrich Heine University Düsseldorf, Department of Biology, Düsseldorf, Germany
[5]Heinrich Heine University Düsseldorf, Institute for Pharmaceutical and Medicinal Chemistry, Düsseldorf, Germany

Upon phage infection, many bacterial cells activate the so-called phage-shock protein (Psp) response, which has been shown to help maintain the integrity and repair the cellular membrane. The exact mechanism by which the Psp response, and in particular its main effector PspA helps to maintain membrane integrity is not clearly understood. Furthermore, although cryo-EM experiments show that interactions of the protein with the membrane lead to tubulated membranes in the lumen of the Synechocystis PspA complex, and to shifts of the complex diameter distribution towards wider assemblies, it is not clear how these interactions are formed, why the size distribution is shifted, or how the membrane is tubulated. By performing molecular dynamics simulations and free energy computations of the N-terminal amphipathic helix interaction with the membrane surface, we determined that the helix strongly interacts with negatively charged lipids, folds into a helix upon surface binding, and kinks upon partial unbinding, with a calculated free energy of -6.32±2.07 kcal mol^{-1}. All-atom simulations of a 290 Å-diameter PspA complex shows how it is able to retain the membrane within its lumen, with the Helfrich curvature energy landscape suggesting it is one of the narrowest assemblies able to sustain such an interaction. Overall, we propose a compensating mechanism, by which the favorable and increasing number of N-terminal helices interacting with the membrane surface drives the unfavorable tubulation and narrowing of the membrane into the lumen of the complex. These results provide an initial understanding at the molecular level of the vesicular membrane remodeling mechanism of action of PspA in bacteria.

45-POS Board 23

SYNERGY BETWEEN MEMBRANE CURVATURE AND AREA EXPANSION IN A MECHANISM OF PLASMA MEMBRANE REPAIR

Martin Berg Klenow[3]; Anne Sofie Busk Heitmann[1]; Sabina Elmi[1]; Ellen Juel Pørtner[3]; Jesper Nylandsted[1,2]; **Adam Cohen Simonsen**[3];
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The plasma membrane of eukaryotic cells is susceptible to perforation as induced by mechanical or molecular perturbations and holes must be sealed rapidly to ensure survival. Membrane repair involves binding of specific proteins to the damage site activated by the influx of calcium. The mechanism of repair is incompletely known and a matter of significant interest, not least because diseases including several cancer types are associated with abnormal repair. Annexin proteins are necessary but not sufficient components of repair which act by inducing membrane curvature and creating a neck around the hole (Boye et. al. DOI: 10.1038/s41467-017-01743-6). We search for conditions required to form a constricted neck as a transition state to hole closure. Applying a dual approach composed of theory and model membrane experiments, we identify membrane area expansion during repair as a key player, acting in synergy with spontaneous curvature. With variational calculus of the Helfrich energy functional, the shape equations for a curved membrane near a hole are formulated and solved (Klenow et. al. DOI: 10.1016/j.bpj.2024.05.027). A state-diagram of neck shapes is produced, describing the evolution in neck morphology with respect to the available (excess) membrane area. Results show that supply of extra membrane area after damage is linked to the formation of narrow necks with potential importance in repair. Based on UV-laser injury studies in MCF7 cancer cells, the calcium and phospholipid-binding protein kinase C alpha (PKCα) is identified as a key player in plasma membrane repair. Using planar membrane patches with free edges we show that PKCα can induce a significant membrane area expansion of up to 40% at nanomolar protein concentrations. We propose that PKCα-induced expansion, coupled with annexin-mediated curvature, facilitates repair. Our findings highlight membrane area regulation as a key player in plasma membrane repair.

46-POS Board 24

DEGREE AND POSITION OF PROTONATION ON PIP LIPIDS DICTATES MEMBRANE ASSOCIATION OF AKT1

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The phosphatidylinositol-3,4,5-triphosphate (PIP3) lipid can exist in four distinct ionization states with overall eight different protonation states. Similarly, three different ionization states (and four protonation states) are possible for phosphatidylinositol-4,5-diphosphate (PIP2) lipids. The membrane micro-environment dictates the relative populations of the ionization states of these lipids, which in turn affects the membrane association behavior of peripheral membrane proteins. However, it is non-trivial to accurately obtain the relative populations of the protonation states of the lipids due to the ensemble-averaged low-resolution binding-assay readouts from the experiments. In this work, with the pleckstrin homology domain of AKT1 protein (AKT1-PHD) as a model system, we use atomic-scale molecular dynamics (MD) simulations and umbrella sampling calculations to explore the effect of protonation states of the phosphatidylinositol lipids on AKT1-PHD dissociation free energy of membrane binding. We find that the binding geometries and the dissociation free-energies have remarkable variations with changes in the ionization/protonation states of the lipids, which highlight the role of environmental factors such as membrane composition, local pH, and electrostatics environment in regulating the PI3K-AKT signaling pathways. Finally, using an integrative modeling machine-learning based approach, we provide a framework to estimate the relative populations of individual protonation states of PIP3 lipids for our system. Towards that, we apply Bayesian Inferencing approach that integrates the information from ssNMR and ITC-based average experimental KD with the simulation-generated KD values for each protonation state and provides us with the relative population of each of the protonation state of the lipid to match with the averaged experimental KD values.

49-POS Board 25

IN-SITU STRUCTURE OF THE BACTERIAL OUTER MEMBRANE PROTEIN A

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Outer membranes of Gram-negative bacteria contain transmembrane proteins embedded in a uniquely asymmetric lipid environment. Whereas the inner leaflet of outer membranes is composed of regular phospholipids, the outer leaflet consists almost exclusively of lipopolysaccharides (LPS). To date, no methods exist to experimentally mimic the complex native lipid environment of bacterial outer membranes. Consequently, studies characterizing the structure and function of outer membrane proteins rely on membrane-mimetic systems or artificially reconstituted bilayers. However, a growing number of studies indicate that membrane composition directly influences the structural and functional states of integral membrane proteins. To address this crucial issue, we recently developed a method to manipulate the protein content of bacterial outer membrane vesicles (OMVs). These vesicles are prepared to contain selected outer membrane proteins at high density and circumvent the limitations of established methods. Using engineered OMVs, we characterize the prototypic outer membrane protein A (OmpA) from Klebsiella pneumoniae for the first time within the unaltered asymmetric bacterial membrane environment at atomic resolution, employing proton-detected ultra-fast solid-state NMR spectroscopy. Our methodology allows extensive assignment and structure determination of OmpA's transmembrane region, as well as site-specific comparisons with OmpA reconstituted in lipid bilayers. Supported by dynamics measurements across multiple timescales, we find that OmpA adopts a stable, extended fold within the native membrane environment, exhibiting substantial differences from structures observed in membrane-mimetic systems. This fold is stabilized through direct interactions with LPS, which, in turn, may contribute to membrane integrity by reinforcing the functional role of OmpA in the Gram-negative cell envelope. Our work thus provides a blueprint for in situ structural biology of membrane proteins in their native environments.

50-POS Board 26

A CELL-BASED SCRAMBLING ASSAY REVEALS PHOSPHOLIPID HEADGROUP PREFERENCE OF TMEM16F ON THE PLASMA MEMBRANE

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The asymmetric resting distribution of the three major phospholipid classes on the plasma membrane of mammalian cells, with phosphatidylserine and phosphatidylethanolamine mostly on the inner leaflet, and phosphatidylcholine mostly on the outer leaflet, is maintained by several classes of ATP-dependent flippases and floppases that exhibit head group selectivity. Upon signaling cues, this asymmetry is dissipated by several families of ATP-independent phospholipid scramblases, thus allowing cells to respond to stimuli and adapt to the physiological context. The prevailing view in the field is that phospholipid scramblases on the plasma membrane act without headgroup preference. However, this paradigm has not been rigorously examined due to limitations in the existing detection modalities. We devised a cell-based phospholipid scrambling assay that utilizes the fluorescence polarization of nitrobenzodiazole-labeled phospholipids to monitor phospholipid scrambling in their native environment. We discovered that the plasma membrane-residing calcium-activated phospholipid scramblase TMEM16F preferentially acts on phosphatidylserine and phosphatidylcholine but not phosphatidylethanolamine. This is the first reported demonstration of phospholipid headgroup preference of a phospholipid scramblase on the plasma membrane. Additional efforts to understand the mechanisms conferring the headgroup preference for TMEM16F are ongoing, as is the search for calcium-activated phospholipid scramblases with headgroup preference different from that of TMEM16F.

53-POS Board 27

HOW SPHINGOMYELIN AFFECTS THE KINETICS OF VIRAL MEMBRANE FUSION

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Sphingomyelin imparts specific properties to biological membranes. It has a high propensity to form lamellar structures and plays a major role in membrane fluidity giving rise to liquid order phases in the presence of cholesterol. Although sphingomyelin is specially stable from a chemical perspective it is converted to ceramide by the action of sphingomyelinase. This amplifies the possibilities of physical changes in the membrane properties which are in turn powerful signaling factors at the physiological level. The objective of this study was to assess the effects of sphingomyelin in viral membrane fusion. Using fluorescence microscopy and single particle analysis, we compared the binding and the fusion kinetics of influenza virus to target membranes containing different amounts of sphingomyelin. The main effect of sphingomyelin in the target membrane composition was a differential binding of the virus, which in turn impacted the efficiency of the fusion process. These results are consistent with previous work showing that cholesterol induces the clustering of receptors at the target membrane and the well-established formation of membrane domains by sphingomyelin and cholesterol.

54-POS Board 28

ENGINEERING PLANAR GRAM-NEGATIVE OUTER MEMBRANE MIMICS USING BACTERIAL OUTER MEMBRANE VESICLES

Nathan J. Wittenberg[1]; Aarshi Singh[1]; Meishan Wu[2]; Tiffany Ye[1]; Angela Brown[2];
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[2]Lehigh University, Chemical & Biomolecular Engineering, Bethlehem, PA, USA

New methods to integrate the gram-negative outer membrane with surface-based sensors may accelerate the discovery and development of new antibiotics. The objective of this work was to devise an approach to planarize outer membrane vesicles (OMVs) from gram-negative bacteria (Aggregatibacter actinomycetemcomitans) by forming hybrid vesicles that readily rupture on glass and silica substrates. These hybrid vesicles, called OM-hybrids, are formed by mixing OMVs with POPC/PEG-PE (99.5/0.5 mol %) liposomes and subjecting them mixture to multiple freeze-thaw cycles. Fluorescence dequenching assays demonstrate membrane mixing between the liposomes and OMVs, and the mean diameter of OM-hybrids lies between those of the liposomes and OMVs. Once formed, the OM-hybrids adsorb and rupture on glass substrates to form an outer membrane supported bilayer (OM-SB). Without hybridization OMVs adsorb and remain intact on these substrates. We examined the OM-SBs with FRAP and found that the lipid diffusion coefficient in these membranes is significantly lower than in supported bilayers formed POPC/PEG-PE. The formation of OM-SBs on silica was monitored with QCM-D. The OM-SBs had larger final frequency shifts (more mass) and larger final dissipation shifts (higher viscoelasticity) than POPC/PEG-PE supported bilayers. Using fluorescence imaging assays, we determined that the OM-SBs retain many of the biomolecular components associated with the OMV membranes, including proteins and DNA, indicating that the freeze-thaw hybridization method is relatively gentle. Finally, the interaction of a cyclic peptide antibiotic polymyxin B with the OM-SBs was examined. We found that polymyxin B significantly disrupts the structure of OM-SBs, while POPC/PEG-PE supported bilayers are unaffected by polymyxin B. In conclusion, fusion of OMVs with liposomes gives rise to hybrid vesicles that readily form supported bilayers on sensor surfaces. The lipids in the supported bilayers are laterally mobile, and the bilayers also possess the components of the native OMVs.

Tuesday, July 8, 2025
Poster Session II
15:00 – 17:00
University of Copenhagen

Below are the formal presentations for Tuesday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd Number Boards Present 15:00 – 16:00 | Even Number Boards Present 16:00 – 17:00

Brown, Chelsea	**3-POS**	**Board 1**
Casalis, Loredana	**4-POS**	**Board 2**
Chung, Jean	**7-POS**	**Board 3**
Dommer, Abigail	**8-POS**	**Board 4**
Goggin, Beatrix	**11-POS**	**Board 5**
Hakami Zanjani, Ali Asghar	**12-POS**	**Board 6**
Hsiao, Chwan-Deng	**15-POS**	**Board 7**
Hyeon, Changbong	**16-POS**	**Board 8**
Javed, Sadaf	**19-POS**	**Board 9**
Khandelia, Himanshu	**20-POS**	**Board 10**
Levental, Kandice	**23-POS**	**Board 11**
Lopes, Laura	**24-POS**	**Board 12**
Maglic, Jasmin	**27-POS**	**Board 13**
Milshteyn, Daniel	**28-POS**	**Board 14**
Omar, Yannick Azhri Din	**31-POS**	**Board 15**
Pedrera, Lohans	**32-POS**	**Board 16**
Rai, Vishwesh	**35-POS**	**Board 17**
Rai, Vishwesh	**36-POS**	**Board 18**
Reagle, Tyler	**39-POS**	**Board 19**
Sakuma, Yuka	**40-POS**	**Board 20**
Shamaprasad, Parashara	**43-POS**	**Board 21**
Sharma Deep, Karan	**44-POS**	**Board 22**
Stuer, Alexandra	**47-POS**	**Board 23**
Sukharev, Sergei	**48-POS**	**Board 24**
Vanegas, Juan	**51-POS**	**Board 25**
Vargas Urbano, Sasiri	**52-POS**	**Board 26**
Yu, Hao	**55-POS**	**Board 27**

Posters should be set up on the morning of Tuesday, July 8 and removed by 18:00. All uncollected posters will be discarded.

3-POS Board 1

AN INTEGRATIVE MODELLING APPROACH TO THE MITOCHONDRIAL CRISTAE

Chelsea M Brown[1]; Marieke S Westendorp[1]; Rubi Zarmiento-Garcia[1]; Jan A Stevens[1]; Bart M Bruininks[1]; Sarah L Rouse[2]; Siewert J Marrink[1]; Tsjerk A Wassenaar[1,3];
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The curved morphology of the inner membrane is a signature feature of mitochondria; the invaginations are called cristae, which house membrane proteins that are essential to mitochondrial function. As computational methods improve, more aspects of mitochondria have been studied in silico, but combining features into one representative model remains challenging. The objective of this work was to create an initial model of a human mitochondrial crista, including appropriate lipid composition and curvature, and the most relevant membrane protein complexes. To this end, we collated structures of 13 protein complexes (with 113 unique chains) using experimentally resolved structures alongside AlphaFold models and biochemical data for validation. These were then embedded in membranes with lipid compositions and curvatures to reflect experimentally measured values. This model was assembled and simulated using the Martini 3 forcefield to assess stability and analyse initial characteristics. We gained initial insights into the effect of the system complexity on lipid sorting and protein-protein contact. Alongside the simulation results, this work provides a framework to combine experimental data from cryo-EM, biochemical studies, structure modelling and molecular simulations to create a system that captures the complexity of biological membranes in situ. Overall, we present an initial 'living' model of a human mitochondrial crista, intended to be built upon and improved as our understanding, methodology and resources develop.

4-POS Board 2

STRUCTURAL INVESTIGATION OF HETEROGENEOUS BIOMIMETIC MEMBRANE MODELS AND THEIR INTERACTION WITH EXTRACELLULAR VESICLES

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Objective: Lipid phase separation and membrane fluidity play a relevant role in plasma membrane biological functions. We propose here a versatile model membrane platform to mimic lipid heterogeneity of native membranes, by mixing different phospho- and sphingo-lipid components to promote phase-separated, ordered domains, which resemble the structure of lipid rafts. Our models encompass solid-ordered (S_o) and liquid-ordered (L_o) phases, embedded in a liquid-disordered matrix (L_d). Working prevalently on supported and suspended lipid bilayer, we aim at translating lipid heterogeneity in terms of biophysical properties conferred to the membrane. Methods: for a comprehensive study, we synergically combined atomic force microscopy, nano-indentation, fluorescence microscopy, differential scanning calorimetry and neutron scattering to gain information on the membrane's transverse and lateral organization, as well as on its thermotropic and mechanical behaviour, for different multi-components lipid mixtures, keeping cholesterol concentration as a key variable. Results: our novel platform allowed to study the molecular mechanisms and the physical properties that govern lipid-bilayer functions, and how they rule the interaction with cell-cell communication relevant players, as extracellular vesicles (EVs) of different origin. In the L_o-L_d system, we found that upon increasing cholesterol percentage, the fluidity of the sole L_o phase increased, driving EVs selectively to different domains. On the S_o-L_d model, where transition of the ordered domains to the liquid phase has longer time scales with respect to L_o domains, the interaction with EVs appeared un-preferential, occurring at all lipid phases with high fusogeicity and fast exchange of lipids. Conclusions: the model membrane platform developed here, allowed to unravel and disentangle the different lipid contributions of the biomimetic interfaces, described in terms of physical parameters, to the EVs fusion mechanisms. Capitalizing on what learned from these simpler systems, we are currently tackling more complex , protein-containing membranes, as the bilayers derived from giant plasma membrane vesicles.

7-POS Board 3

MEMBRANE-DEPENDENT ASSEMBLY OF BRUTON'S TYROSINE KINASE MEDIATED BY PRR AND SH3 DOMAINS

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Cellular membranes provide a unique platform for interactions that drive emergent behaviors in protein dynamics and cellular signaling, distinct from those observed in solution. We investigated the proline-rich region (PRR) and Src Homology 3 (SH3) domains of Bruton's tyrosine kinase (Btk) and its phase separation driven by the weak interactions of regulatory domains at membrane surfaces. In this work, we demonstrate that membrane localization amplifies weak PRR-SH3 interactions, enabling the formation of higher-order assemblies and phase separated condensates. These assemblies, previously undescribed by solution state studies, are supported by reductions in the lateral diffusion of membrane-bound Btk molecules, and the stabilization of reversible condensates at the membrane surface. Constructs containing the native PRR and SH3 domains reliably formed membrane-associated clusters, while mutation or deletion of these domains lessened changes in diffusion and impaired condensate formation. Our findings establish the membrane as an essential mediator of PRR-SH3-driven phase separation in Btk, advancing knowledge of membrane-specific regulation in signaling protein dynamics.

8-POS Board 4

WHEN LIPIDS GO AIRBORNE: MULTISCALE MODELING OF BIOLOGICAL AEROSOLS

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Scrutiny over the role of biological aerosols (bioaerosols) in the transport of infectious disease has increased significantly in the years since the COVID-19 pandemic. Respiratory aerosols, emitted from the fluid lining of the respiratory tract, can carry infectious pathogens such as SARS-CoV-2, influenza, and M. tuberculosis, and remain aloft in unventilated spaces for hours. But beyond their roles in the spread of disease, bioaerosols are ubiquitous in the natural environment. Originating in surface waters (e.g., oceans, rivers, and wastewater runoff) as well as soils, plants, and animals, bioaerosols have widespread systemic effects on public health, ecosystem biodiversity and stability, and climate change. Here, the phase state and morphology of aqueous biological aerosols are studied with atomistic and coarse-grain molecular dynamics simulations. In addition to describing the particular challenges of modeling lipids at air-water interfaces, we describe a workflow for constructing stable, complex submicron bioaerosol models for molecular simulation, and specifically investigate the roles of (a) fatty acids in sea spray aerosols, and (b) lung surfactant in respiratory aerosols. We discuss how the internal and external heterogeneity of bioaerosols may suggest molecular mechanisms for the protection of delicate biological matter (e.g., enzymes or viruses) from atmospheric stressors. This work sets the stage for future studies of bioaerosols in the transfer of infectious disease, long-range transport of genes and antimicrobial resistance, and regulation of critical climate processes.

11-POS Board 5

BIASED ALLOSTERIC MODULATION OF CB_1R: IN SILICO STUDIES IN A MODEL BLOOD-BRAIN BARRIER MEMBRANE

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The cannabinoid 1 receptor (CB_1R) is the most abundant receptor in the endocannabinoid system[1]. Its implication in a range of biological processes such as pain, immunity, and mood make it a desirable drug target. The therapeutic potential of CB_1R has been historically limited due to psychoactive effects arising from its activation. Biased allosteric modulators are a class of drug that act by directing a receptor's response towards a specific signalling pathway in the presence of an endogenous ligand. This is thought to be achieved through certain receptor conformations having higher stabilities in presence of the allosteric modulator[2]. In 2015, a biased allosteric modulator of CB_1R, ZCZ011, was reported to reduce neuropathic pain with no psychoactive effects in mouse models[3]. Administration of ZCZ011 alone produced no response in assays, but co-administration with endocannabinoid 2-AG led to the transient peak of ERK1/2 phosphorylation becoming sustained[4]. Molecular dynamics simulations were undertaken to further understanding of ZCZ011 interaction with CB_1R in the presence of 2-AG. Simulations were carried out in a model blood brain barrier membrane with lipid composition taken from human brain microvascular endothelial cell (HBMEC) lipid composition. This work contributes to mechanistic understanding of ZCZ011 action and more broadly adds to the growing body of knowledge surrounding biased allosteric modulators. Additionally, the use of a complex membrane model that reflects in vitro models provides an opportunity for more robust comparison between in silico and lab results, facilitating greater capacity for the two methods to direct and inform each other. (1) Lu, H.-C.; Mackie, K. Biol. Psychiatry 2016(2) Slosky, L. Trends Pharmacol. Sci. 2021(3) Ignatowska-Jankowska, B. Neuropsychopharmacology 2015(4) Green, H. M. Br. J. Pharmacol. 2024

12-POS Board 6

INVESTIGATING ANNEXIN'S ROLE IN PLASMA MEMBRANE REPAIR THROUGH MOLECULAR DYNAMICS SIMULATIONS

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Cell membranes are constantly subjected to stress and damage, requiring rapid and efficient repair mechanisms to maintain cellular integrity. Annexins, a family of calcium-dependent membrane-binding proteins, play a pivotal role in plasma membrane resealing. Despite their crucial role in membrane repair, the molecular mechanisms underlying annexin function remain poorly understood. We use molecular dynamics (MD) simulations to explore annexin-lipid interactions, providing new insights into the biophysical basis of membrane repair.Our investigation focuses on how annexin trimers, such as ANXA4 and ANXA5, contribute to negative membrane curvature, a key factor in plasma membrane resealing. Through atomic-level simulations of annexin-membrane interactions, we show how cholesterol and anionic lipid headgroups modulate annexin-induced curvature, which in turn influences annexin recruitment to membrane rupture sites. Additionally, we engineered ANXA3 to form trimeric structures, similar to ANXA4 and ANXA5, to investigate the structural determinants essential for efficient membrane repair. To quantify annexin-induced curvature, we developed a Python code to compute the mean curvature induced by proteins upon binding to the membrane. This code is available at https://github.com/AliAHZanjani/MemCurv.Integrating MD simulations with experimental validation, we construct a high-resolution model illustrating how protein-lipid interactions drive membrane repair. Furthermore, we explore the potential for pharmacological modulation of these interactions to impair membrane repair, offering therapeutic implications for cancer treatments where disrupting membrane integrity can induce cell death.Our findings provide a refined molecular-level understanding of membrane repair, presenting novel insights into how cells preserve structural stability under stress, and lay the groundwork for future therapeutic strategies targeting membrane repair pathways.This work builds on contributions to the following studies:Hakami Zanjani et al. (2024), The Journal of Physical Chemistry B.Hakami Zanjani et al. (2023), Biophysical Journal.Florentsen et al. (2021), Soft Matter.Heitmann et al. (2021), Journal of Biological Chemistry.Bendix et al. (2020), Cells.

15-POS Board 7

INSIGHTS INTO NITRATE TRANSPORT MODULATION BY THR101 PHOSPHORYLATION AND HIS356 PROTONATION IN ATNRT1.1

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AtNRT1.1 (also known as CHL1/NPF6.3) is characterized by its dual-affinity transport mechanism for nitrate, which enables plants to efficiently acquire nitrate under various environmental conditions and is regulated by Thr101 phosphorylation. A central question regarding the function of NRT1.1 is how it recognizes and transports nitrate. Although phosphorylation is known to modulate the affinity of transporters, its precise molecular mechanism remains unresolved. In addition, previous study revealed that His356 plays a crucial role in nitrate binding; however, there has been limited discussion on how transport is regulated. To address this question, we employed molecular dynamics (MD) simulations to explore the dynamic conformational transitions underlying nitrate transport and affinity switching. Our study reveals a protonation-dependent gating mechanism centered on His356 that dynamically regulates nitrate transport in protonated and deprotonated states. Furthermore, we demonstrate that Thr101 phosphorylation induces significant conformational rearrangements, primarily in transmembrane helices TM2 and TM4, thereby enhancing the flexibility of the transporter and affecting its functional state. These structural modifications provide new insights into how AtNRT1.1 dynamically adjusts its trafficking mode, offering a new mechanistic explanation for dual-affinity switching.

16-POS Board 8

POLYMER BRUSH-INDUCED DEPLETION INTERACTIONS AND CLUSTERING OF SIGNALING PROTEINS

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When cylindrical inclusions, representing signaling membrane proteins in plasma membranes, are embedded in mobile polymer brushes in good solvents, an imbalance in the osmotic pressure of polymer chains, characterized by short-range steric repulsion, induces depletion attraction between the inclusions. By employing a theoretical model based on the Asakura–Oosawa (AO) theory and molecular dynamics simulations, we explore the polymer brush-induced depletion interaction with varying Sukharev ting density and brush height. The depletion attraction between inclusions increases with the brush height when the brush is shorter than the inclusion; however, when brush polymers extend beyond the inclusion height at high grafting densities, the repulsion between the polymer chains partially offsets the attraction. Consequently, the brush polymer-induced depletion attraction displays non-monotonic variation with the brush height. The blob concept of brush polymers incorporated into the AO theory proves most effective in quantitatively explaining the simulation results, in particular, the depletion layer thickness and the strength of depletion attraction. Furthermore, we find that increasing branchness of brush polymer, which mimics the degree of glycosylation in glycoprotein, enhances the formation of protein clusters. Our study suggests brush-induced depletion interaction as one of many molecular mechanisms underlying recent experimental observations of integrin nanocluster formation and signaling in glycocalyx.

19-POS Board 9

RESILIENT MEMBRANIZED COACERVATES FORMED THROUGH SPONTANEOUS WRAPPING OF HEAT-DESTABILIZED LIPID BILAYERS AROUND COACERVATE DROPLETS AS PROTOCELL MODELS

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Cellular membranes and the membraneless biocondensates spatially organize cells and work together to drive cellular processes. Separately, these two kinds of compartments fail to fully mimic a cell, with its dense, biomolecule-rich cytosol protected with a semi-permeable membrane. Here, we present a new method to create membranized coacervates (MCs) for coacervates with any surface charge and a wide range of phospholipid membrane compositions. MCs are formed when liposomes, destabilized using heat and divalent ions, are mixed with coacervate dispersions. The MC membranes form an effective barrier against small molecules, protecting the coacervate core from extreme pH (2–10) and salt concentrations (up to 0.5 M), while the coacervate matrix supports the membrane during hypotonic and hypertonic conditions and repeated freeze-thaw cycles. Therefore, MCs exhibit enhanced stability than both coacervates and liposomes. MC membranes are unilamellar and fluid with densely packed lipids. We show that the MC surface is peppered with coacervate polymers protruding out from the core at varying degrees, resulting in highly heterogenous populations. We try to exploit MC surface complexity to predict and mediate inter- and intra-population MC interactions. Finally, we present postulates for MC interactions based on our observations. MCs can help us understand how stable primitive cells might have emerged, communicated, and evolved and build advanced synthetic cells with enhanced stability and selectivity.

20-POS Board 10

FUN OUTCOMES OF ELECTROMECHANICAL COUPLING IN MEMBRANES, SUCH AS TEMPERATURE SPIKES DURING THE PASSING OF THE ACTION POTENTIAL AND MEMBRANES BENT BY TRANSMEMBRANE ELECTRICAL POTENTIAL

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When an action potential passes through a neuron, heat is first produced and then reabsorbed by the neuronal membrane, resulting in a small measurable temperature spike. Here, we describe the thermodynamics and molecular features of the heat production using coarse-grained molecular dynamics. We study a simple lipid bilayer membrane surrounded by physiological salt solution with and without an external electric field, which represents an imbalanced charge across the membrane. We show that the temperature increases significantly upon removal of the electric field under constant pressure conditions. The potential energy converted to heat is initially stored mainly in the imbalanced ion distribution across the membrane and the elastic energy of the membrane has only a minor role to play. We demonstrate that the mechanism of heat production involves interaction between ions as well as lipid headgroup dipoles while the interactions between polar water molecules and lipid headgroup dipoles absorbs a considerable portion of such produced heat upon removal of the electric field. We also describe other interesting electrostatic phenomena near membranes, such as the coupling between membrane curvature and transmembrane electrical potential, and the ability of single transmembrane dipolar helices to sense transmembrane electrical potential.

23-POS Board 11

PLASMA MEMBRANE CHOLESTEROL ASYMMETRY REGULATES CELLULAR CHOLESTEROL HOMEOSTASIS

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The plasma membrane is the interface between the external world and a cell's internal chemistry, and must therefore facilitate a multitude of parallel, tightly regulated tasks. To achieve this functional complexity, mammalian cells produce hundreds of lipid species, nearly all of which are asymmetrically distributed between the two leaflets of the plasma membrane bilayer. Further, the various organellar membranes have vastly different lipid compositions that are essential for their functions, and thus must be homeostatically maintained. 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. 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 area and mechanical stresses. Through computational and experimental approaches, we find that the combination of cholesterol's preference for the more saturated exoplasmic lipids and the overabundance of lipids in the cytoplasmic leaflet yields a major enrichment of cholesterol in the exoplasmic leaflet. We show that this transbilayer distribution can be altered by manipulating the determinants of cholesterol asymmetry, namely the disparity in phospholipid composition and abundance between leaflets. Specifically, addition of phospholipids to the outer leaflet redistributes cholesterol to the cytosolic leaflet of the plasma membrane. This redistribution leads to cholesterol's recognition by cytoplasmic sensor proteins (i.e. GRAMD), which transfer it to the endoplasmic reticulum for esterification into cholesterol esters and storage in lipid droplets. Thus, transbilayer cholesterol asymmetry is a major contributor to cellular cholesterol homeostasis.

24-POS Board 12

INFLUENCE OF STEROL CHEMICAL STRUCTURE ON MEMBRANE ORDER

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Sterols are present in membranes of different organisms, including bacteria, fungi, mammals and plants, and play an important role in membrane dynamics, regulating order-disorder. This balance controls many cellular functions, as more order leads to more thick and rigid membranes, while less order induces more flexible and thin membranes, the latter more prone to fusion, for example. Despite the importance of membrane order for biological function, the study of its molecular mechanism is still unknown. In my work I investigated how small differences in molecular structure induces different membrane order. To this goal I worked with precursors of ergosterol, yeast's sterol, that demand a long metabolic pathway. Experiments performed by collaborators show that small differences in ring and tail chemical structure lead to qualitative differences in phase separation of the vacuole. While order appears important, the final yeast sterol (ergosterol) is not the strongest ordering sterol of the pathway, but instead promotes clear phase separation in the yeast vacuole, as opposed to a gel. I applied a molecular dynamics approach to validate the molecular mechanism of sterol ordering in these experiments. The simulations are run using ergosterol and other five of its precursors with a 20% concentration in DMPC. The agreement of the highly simplified molecular model with the yeast phenotype strongly suggests the yeast sterol function can be understood from its interaction with saturated acyl chains like those of DMPC. The simulations demonstrate that the ordering effect is linked to the enrichment of a well- defined pool of highly ordered chains.

27-POS Board 13

HOW PLASMA MEMBRANE CURVATURE SHAPES GLP-1R SIGNALLING BIAS

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The plasma membrane (PM) forms a complex curvature landscape. Upon close inspection, even seemingly smooth membrane sections exhibit variations in their topography that can affect signal transduction, protein clustering, and vesicle formation. However, due to the difficulty in constructing large and physiologically accurate model systems, curvature remains an under-appreciated modulator of protein organisation. Using confocal microscopy in live cells, we recently demonstrated how the formation of G-protein coupled receptor (GPCR) nanodomains is strongly regulated by shallow PM curvature ($> 1 \mu m^{-1}$). Here, we expand on these findings by investigating how curvature is tied to GPCR signalling. Using the glucagon-like peptide-1 receptor (GLP-1R), we find that its two most important signalling pathways, the G-protein and the β-arrestin pathway, form spatially separated signalling domains in opposing curvature environments. We observe that activation via agonists biased towards a pathway modulates the receptor distribution between these curvature environments. Based on these data, we model the activation response using three components, of which one is strongly associated with the clathrin-driven internalisation pathway. Interestingly, receptor reorganisation upon activation is not limited to GLP-1R, indicating that this curvature-driven regulatory mechanism applies to GPCRs in general. Our findings demonstrate that signalling bias is spatially heterogenous and provide a yet unexplored insight into how PM curvature encodes signal transduction and cell function.

28-POS Board 14

HOMEOCURVATURE REGULATION OF THE YEAST LIPIDOME UNDER HYDROSTATIC PRESSURE

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Cell membranes are composed of both bilayer and non-bilayer phospholipids, with the high curvature shape of the latter required to support membrane dynamics and the function of membrane proteins. Phospholipid metabolism has been proposed to maintain key membrane properties, like membrane fluidity in response to temperature changes (homeoviscosity). Based on analysis of ctenophore membranes from deep-ocean environments, we recently proposed that homeocurvature regulation of non-bilayer lipids could also influence lipid metabolism. Here, we show that yeast grown under high-pressure laboratory conditions regulate lipidome curvature through the synthesis of phosphatidylinositol (PI). We first show that artificial manipulation of the lipidome curvature – driven via the phosphatidylethanolamine (PE) to phosphatidylcholine (PC) ratio – affects high-pressure growth and viability. We then observe that the membranes of wild-type cells show increased propensity to form non-lamellar phases after extended pressure incubations. This change in lipid phase behavior corresponds with increased levels of PI, a phospholipid whose curvature had not been previously characterized. We report that PI is a non-bilayer phospholipid, with a negative spontaneous curvature intermediate to that of PE and PC. Accounting for PI, we show that mean lipidome curvature is buffered in response to pressure in two distantly-related yeast, but not in bacterial cells with less phospholipid diversity. These results indicate that there may be conserved mechanisms in eukaryotic lipid metabolism to maintain the shape of phospholipids within a viable domain.

31-POS Board 15

BEYOND SAFFMAN–DELBRÜCK THEORY: DIFFUSION OF MEMBRANE PROTEIN AGGREGATES

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The celebrated Saffman–Delbrück theory established the logarithmic dependence of the diffusion coefficient of transmembrane proteins on their radius. The model succeeded by realizing that diffusion in a membrane inherently requires consideration of the membrane-surrounding fluids. Since its inception, many corrections and extensions of the model have been proposed. However, it remains restricted to individual membrane proteins, even though membrane proteins often form oligomers or aggregates. In this talk/poster, we propose a new theory for the diffusion coefficients of membrane protein aggregates like those formed by Linker for the Activation of T-cells (LAT) and epidermal growth factor receptor (EGFR). To this end, we first visualize the flow field that a diffusing protein induces in a membrane and show that the high viscosity of lipid membranes leads to long-range hydrodynamic coupling. This indicates that hydrodynamic effects are highly relevant for the dynamics of proteins in crowded environments. Subsequently, we use Kirkwood-Risemann theory to self-consistently capture the internal hydrodynamic coupling of protein aggregates and determine their effective diffusion coefficients. From this theory, we also obtain an expression for the hydrodynamic radius that characterizes the diffusion of protein aggregates. By applying the theory to various aggregation models like diffusion-limited aggregation, we then demonstrate that the Saffman–Delbrück model is approximately valid for protein aggregates if the protein radius is replaced by the hydrodynamic radius. Lastly, we discuss the implications of these results for protein aggregation mechanisms and how they can inform single-particle tracking measurements.

32-POS Board 16

BIOPHYSICAL MEMBRANE PROPERTIES ARE AFFECTED DURING FERROPTOSIS EXECUTION

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Ferroptosis is a form of regulated necrosis characterized by the iron-dependent accumulation of lipid peroxides within cellular membranes. However, how lipid oxidation affects membrane biophysical properties and how these alterations contribute to the opening of membrane pores are major questions in the field. To address these questions, we combined quantitative live-cell confocal microscopy with several biophysical methods to systematically study changes in membrane biophysical properties in ferroptotic cells in connection to membrane permeabilization and cell death. We found that ferroptosis induces the reorganization of the plasma membrane into a more compartmentalized environment where Glycosylphosphatidyl-inositol green fluorescent protein (GPI-GFP) cluster into immobile regions. We also found that lipid peroxidation promotes lipid flip-flop, which is associated with the formation of lipidic pores characterized by fusion of the two bilayer leaflets and the increase of the transbilayer movement of the lipid by lateral diffusion. Interestingly, and in contrast to the fast exposition of phosphatidylserine (PS) to the outer leaflet of the plasma membrane during apoptosis, the appearance of PS in ferroptotic cells is a late event concomitant with the complete plasma membrane breakdown. We also detected temporal changes in membrane mechanics leading to reduced tension in the permeabilized state of the membrane. Finally, we found that ferroptotic cells exhibit increased membrane polarity, which may be associated with local changes in membrane fluidity and/or remodelling of membrane components. Taken together, these findings indicate that lipid oxidation alters the structural organization of lipids in the plasma membrane consistent with changes in lateral segregation, leading to loss of permeability barrier function. Pore opening allows water entry and swelling, which affects membrane mechanical properties such as membrane tension, domain packing and membrane stiffness prior to cell bursting.

35-POS Board 17

SPHINGOMYELIN-RICH LIPID MEMBRANES ENHANCE CYTOLYSIN A ACTIVITY BY FACILITATING THE MONOMER-TO-PROTOMER CONFORMATIONAL TRANSITION

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Lipid components of the plasma membrane play crucial roles in regulating the biological functions of membrane proteins, which are ultimately governed by lipid-induced conformational dynamics. In addition to membrane proteins, pore-forming toxins (PFTs) secreted by various pathogenic bacteria exploit the lipids in target cell membranes to achieve their active forms through lipid-assisted conformational transitions. Cytolysin A, an α-PFT secreted by a pathogenic strain of E. coli as a water-soluble monomer, undergoes conformational changes upon interaction with lipid membranes, ultimately forming a dodecameric transmembrane pore. This structure disrupts ionic balance, modulates intracellular signalling, and eventually leads to cell death. Although recent studies establish the role of cholesterol in facilitating the conformational transitions, the same remains to be studied for multi-component lipid membranes. Most studies have been confined to detergent-based or simplified membrane systems, which do not accurately replicate the complexity of real cell membranes. The cell membrane comprises various lipid components, with phosphatidylcholine (PC), sphingomyelin (SM), and cholesterol (CHOL) being the primary ones. In this study, we combine an ensemble of biophysical techniques with molecular dynamics simulations to investigate the conformational dynamics and activity of the protein when introduced to simple two-component (POPC-CHOL) and three-component (POPC-SM-CHOL) membrane systems. Our results reveal that the SM-rich membrane enhances the protein's lytic activity by – 1. sensitizing the early conformational melting, and 2. facilitating refolding of the beta tongue, a critical membrane-inserted motif of Cytolysin A. Molecular dynamics simulations indicate that the POPC-SM-CHOL complex interacts with and stabilizes both flanks of the beta tongue intermediate, facilitating the refolding process. These findings provide new insights into how membrane components influence the conformational dynamics of pore-forming proteins, with implications for establishing design-property relationships for general bistable biomolecules intended for membrane-specific cellular targeting.

36-POS Board 18

BEHAVIOUR OF POLYMERIC CROWDER INFLUENCES THE MOBILITY OF BIOMOLECULES ON LIPID MEMBRANES

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Cell and organelle membranes are decorated with proteins and glycolipids, where the high density of intramembrane domains and their associated ectodomains and glycolipid headgroups critically regulate biological processes such as molecular assembly, recognition, and signalling. Despite the recognized importance of molecular crowding, understanding how crowded environments created by ectodomains or headgroups influence the dynamics of similar membrane molecules with protruding ectodomains remains challenging. Efforts to recreate crowding conditions in vitro frequently utilize simplified models that do not adequately reflect the complexity of cell membranes. For example, although transmembrane crowding effects have been investigated, the distinct impact of ectodomain crowding on the movement and interactions of other molecules with ectodomains is still not well comprehended. Similarly, polymer-grafted lipids have been used to mimic crowding, but the effects of polymer configuration on the dynamics of protruding molecules are not fully resolved. Here, we show that PEG-lipid-induced crowding, which mimics ectodomain and headgroup crowding, significantly alters the diffusion of membrane-associated molecules with protruding domains. Using single particle tracking on supported lipid bilayers, we demonstrate that these molecules experience dramatic, non-monotonic changes in mobility as the PEG grafting density transitions from low to high. Notably, diffusion coefficients decrease at intermediate PEG concentrations, followed by an increase before declining again at higher PEG concentrations. We attribute some of the initial reduction in mobility to substrate-induced drag experienced by the PEG polymer. In contrast, lipid-anchored tracers without protruding domains maintain relatively stable mobility. Lipid components like cholesterol also modulate molecular diffusion in a density-dependent manner. These findings provide new insights into how ectodomain and headgroup crowding influence the mobility of membrane-associated biomolecules, with implications for understanding cellular membrane organization, designing drug delivery systems, and developing biomimetic sensing platforms.

39-POS Board 19

DIFFERENTIAL TENSION INCURRED BY LIPID NUMBER ASYMMETRY MODULATES THE CHEMICAL ACTIVITY OF LIPIDS, BIASING THEIR BIOCHEMICAL AVAILABILITY

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A molecule's chemical activity reflects its thermodynamic/kinetic propensity to participate in a biochemical process or diffuse from one location to another (I.e. its availability). For example, in the case of a lipid in a bilayer, the lipid's chemical activity must govern its tendency to i) bind to a biosensor domain, ii) flux across a membrane contact site, or iii) solvate a transmembrane protein domain. Though several studies have investigated the chemical activity of cholesterol, there have been fewer studies focusing on those of the diacyl lipids constructing the bilayer matrix of most cellular membranes. To address this gap in understanding, we present an expression for the chemical activity of bilayer-forming lipids and use it to experimentally estimate this quantity in the outer leaflets of POPC bilayers harboring an asymmetry in lipid abundance across the two leaflets of the bilayer (I.e. lipid number asymmetry). Motivated by curvature-elastic theory of lipid membranes, we propose that the chemical activities of those lipids are determined to a large extent by lateral stretching/compression of said lipids within a leaflet. With this theoretical insight, we use experimental determinations of lipid chemical activity to calculate the mechanical stresses in lipid bilayers harboring unequal lateral tensions between their leaflets (I.e. differential stress). At constant vesicle volume, the thermodynamic and mechanical consequences of lipid number asymmetry depend on whether it is generated through flip-flop or asymmetric extraction/insertion of lipid. Noting this distinction, we calculate the differential stress at the point of vesicle collapse in bulk cyclodextrin-vesicle mixtures (where lipid number asymmetry is generated via asymmetric extraction). Altogether, this contribution 1) deepens the connection between experiment and simulation/theory on asymmetric membranes and 2) systematizes efforts to uncover the role of lipid asymmetry (and differential stress) in membrane biology.

40-POS Board 20

LONG-RANGE MEMBRANE VISCOSITY OF LIVING CELL

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The viscosity of the plasma membrane in living cell is a crucial biophysical parameter that regulates cellular functions. We categorize the plasma membrane viscosity into short-range and long-range viscosities based on the spatial scale of the cellular processes they influence. Short-range viscosity originates from the nanometer-scale diffusion of membrane molecules, regulating signal transduction and membrane transport. It has been reported that short-range viscosity in living cells is comparable to, or at most 10 times greater than, that of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) membranes. In contrast, cell-scale (micrometer scale) membrane flow, driven by a flow of the actin cytoskeleton, occurs in dynamic processes such as cell migration and cell division. We shall refer to the resistance to this cell-scale membrane flow as long-range viscosity. While transmembrane proteins anchored to the actin cytoskeleton have minimal effect on short-range viscosity, they are likely to affect long-range viscosity. Thus, short- and long-range viscosities in the living cell should differ significantly. In this study, to reveal the difference from the short-range viscosity, we measure the long-range viscosity by applying an external point force to plasma membrane of C.elegans early embryo using a microinjection technique. In intact cells, no membrane flow was induced by the applied point force. However, when actin polymerization was inhibited, a point force induced a pair of vortex flows across the plasma membrane. By comparing the vortex flow pattern with a hydrodynamic model, we found that the long-range viscosity in living cells was four orders of magnitude greater than both DOPC membranes and the short-range viscosity in living cells. This drastic viscosity increase was attributed to the structure of actin filaments attached to transmembrane proteins after polymerization inhibition. We conclude that the remaining actin cytoskeleton and its associated transmembrane proteins contribute to the increased long-range viscosity.

43-POS Board 21

INVESTIGATING THE EFFECT OF SPHINGOMYELIN COMPOSITION ON CHOLESTEROL FLIP-FLOP USING MACHINE-LEARNING-BASED ENHANCED SAMPLING METHODS

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Owing to its high mobility and comparatively small hydrophilic group, cholesterol flip-flop between the leaflets of a plasma membrane (PM) is much faster (by several orders of magnitude) than phospholipid flip-flop. In the PM, cholesterol flip-flop has recently been suggested to regulate mechanical tension, supporting a dramatic abundance asymmetry of the phospholipids (1). While several computational and experimental studies have found that cholesterol flip flop is heavily dependent on local lipid composition, the effect of sphingolipids composition on the molecular-scale mechanism and rate of flip flop is still unknown. This is a critical knowledge gap, as the outer leaflet of the PM is greatly enriched in sphingolipids as compared to the inner leaflet. In this work, we investigate the influence of sphingolipid composition on the rate and mechanism of cholesterol flip-flop in model membrane systems containing POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), sphingomyelin, and cholesterol using molecular dynamics simulations. Using a series of prior simulations, a dimensionality reduction method was used to learn a low dimensional latent space that describes a cholesterol translocation event (2). Well-tempered metadynamics is then applied to these collective variables in order to obtain good sampling of cholesterol flip flop events in sphingolipid-rich membranes. Doktorova, M., J.L. Symons, X. Zhang, H.-Y. Wang, J. Schlegel, J.H. Lorent, F.A. Heberle, E. Sezgin, E. Lyman, K.R. Levental, and I. Levental. 2023. Cell Membranes Sustain Phospholipid Imbalance Via Cholesterol Asymmetry. bioRxiv. 2023.07.30.551157.Wang, D., and P. Tiwary. 2021. State predictive information bottleneck. J. Chem. Phys. 154:134111.

44-POS Board 22

INVESTIGATING MEMBRANE ASYMMETRY USING CRYO-EM AND MOLECULAR DYNAMICS SIMULATIONS

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The two leaflets of biological membranes can differ in both lipid composition and relative lipid abundance. Such bilayer asymmetries influence membrane properties and cellular functions, but the underlying molecular mechanisms are poorly understood. Abundance asymmetry, which occurs when there is an imbalance in the total number of lipids in the two leaflets, has proven particularly difficult to detect and characterize. Here, to begin to address this problem, we present an integrated experimental and computational framework to investigate lipid number asymmetry that arises naturally in very small vesicles. We used cryo-electron microscopy (cryo-EM) to characterize interleaflet lipid distributions in individual vesicles. We identified features in the intensity profiles across the bilayer that are sensitive to the number of lipids in each leaflet and confirmed the metric by analyzing large sets of liposomes with different diameters. At the same time, we use atomistic molecular dynamics (MD) to generate synthetic cryo-EM intensity profiles of simulated bilayers with varied number asymmetry for cross-validation with experimental data. Results from in vitro and in silico studies across various lipid compositions show similar trends with increasing number asymmetry but differences in absolute values, likely due to different interleaflet packing densities in the flat simulated bilayers and curved experimental membranes. This study thus provides a framework for systematic investigation of lipid number asymmetry and its biophysical effects in experimental systems.

47-POS Board 23

HARNESSING SIMULATIONS TO STUDY THE BARRIER PROPERTIES OF THE LPP OF THE LIPID MATRIX OF THE STRATUM CORNEUM

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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 different lipids in a gel-like state. In dermatology or cosmetics, the performance of a particular active ingredient, an 'active', requires understanding how and whether the active penetrates the lipid matrix. The complexity (composition and structure) and slow dynamics of the matrix create challenges that hinder computational studies to investigate molecule permeation across the skin with atomistic resolution. The lipid matrix is found in two coexisting phases. We study the more complex Long Periodicity Phase (LPP), consisting of two asymmetric bilayers sandwiching a monolayer slab.We use Anton2 and MD simulations to understand the kinetics and dynamics during permeation in the lipid region. Anton2 allows us to probe the energy barrier as well as the diffusion of molecules at higher resolution. Using large data sets of long production runs we study permeability and barrier properties of the LPP as well as the structure and dynamics of the lipid structure itself. The heterogeneity of the LPP gives rise to regions with different properties that seem to influence the path of the active crossing the structure, such as intra-bilayer flip-flop or inter-bilayer transfer. The more rigid and dense outer leaflets of the asymmetric bilayers demonstrate a higher energetic barrier, which reduces significantly for the more fluid-like and mobile inner leaflets. Cholesterol is observed to have higher intra-bilayer flip-flop rates suggesting easier diffusion through the fluid-like tail-tail region in the centre of the bilayer. In contrast, for Fatty Acids, inter-bilayer transfer dominates due to the polarity of the headgroups interacting with other polar groups in the headgroup region.

48-POS Board 24

ON THE LIPID DEPENDENCE OF BACTERIAL MECHANOSENSITIVE CHANNELS GATING

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For bacterial mechanosensitive channels that function as turgor-adjusting osmolyte release valves, membrane tension serves as the primary activating stimulus. Since tension is transmitted to the gate through the surrounding lipid bilayer, the presence or absence of certain lipid species can be consequential. In MscS, lipids serve a dual purpose; they not only transmit tension to the peripheral domains but can also intercalate between the helices, separate the gate, and thus drive inactivation. In this study, we characterize the lipid dependence of chromosome-encoded MscS and MscL in E. coli strains with genetically altered lipid compositions. We utilize two previously generated strains lacking one or two primary lipid species (PE, PG, or CL) and an engineered strain that is highly enriched in cardiolipin (CL) due to the presence of hyperactive cardiolipin synthase ClsA. We assess the behavior of these channels using patch-clamp techniques and quantify the relative tension midpoints, closing rates, inactivation depth, and the rate of recovery to the closed state. Additionally, we measure the osmotic survival of lipid-deficient strains, testing the channel function at the cellular level. We find that MscS and MscL both tolerate the absence of specific lipid species in terms of opening and closing. However, the lack of CL reduces the active MscS population relative to MscL, decreases the closing rate, increases the likelihood of MscS inactivation, and slows the recovery process. Importantly, PG and CL co-purify with MscS when gently isolated in the Glyco-DIBMA polymer. MD simulations indicate that PG and CL tend to localize near the cytoplasmic side of the MscS protein interface and partition into the crevices. We discuss the effect of CL on MscS kinetics in relation to its properties. The data underscores the robustness of the osmolyte release system and highlights the importance of cardiolipin for the adaptive behavior of MscS.

51-POS Board 25

UNRAVELING THE MICROSCOPIC ORIGIN OF MEMBRANE MODULI THROUGH LOCAL ELASTICITY PROFILES

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Numerous cellular processes such as membrane fission and fusion depend on the energetics of elastic deformation which include in-plane stretching and out-of-plane bending. These modes of deformation have macroscopic moduli associated with them that are typically measured experimentally or estimated computationally through various methods. The recent curvature-tilt theory of Terzi, Ergüder, and Deserno suggests that there may be at least five macroscopic moduli needed to describe out-of-plane membrane deformations including bending, Gaussian curvature, tilt, splay-tilt, and twist. Within that theory, these moduli have well-defined microscopic origins through various integral moments of microscopic quantities such as the local Young's modulus. Here, we use the stress-stress fluctuation method to obtain local elasticity profiles from molecular dynamics simulations of coarse-grained (CG) MARTINI lipid membranes. We use these elasticity profiles to estimate locally-defined quantities such as the Young's, area, and shear moduli of the CG membranes. We show that the results from the stress-stress fluctuation formula are in excellent agreement with other methods such as explicit deformation and area fluctuation. We compare the integral moments of the microscopic moduli to values of the macroscopic moduli obtained through analysis of power spectra of large fluctuating membrane simulations. Our results provide for the first time a direct estimation of these important microscopic moduli, which determine the elastic response of a membrane due to any type of deformation.

52-POS Board 26

PRESSURE DEPENDENT ELASTIC CONSTANTS OF MEMBRANES

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Environments like the deep sea are characterized by low temperature (near freezing) and high pressure, with pressure increasing by 1 bar for every 10 m depth. This presents a substantial challenge for cell membranes because the membrane is the most pressure-sensitive material in the cell, with a compressibility ten times that of a folded soluble protein. Recently, Winnikoff et al. showed that a class of invertebrates called ctenophores synthesizes an extraordinary amount of plasmalogen to adapt to the high pressure of the deep ocean and suggest a “homeocurvature” mechanism—ctenophores adjust their lipidomes to maintain critical material properties within a narrow range, to preserve deformability at high pressure. Here, we use high-pressure (Hi-P) MD simulations to systematically study the pressure dependence of plasmalogens, comparing the spontaneous curvature under pressure of ester, ether, and plasmalogen-linked phospholipids. A comparison with Hi-P SAXS measurements suggests that ester and ether lipids have qualitatively correct pressure dependence. At the same time, simulations of the plasmalogen membranes show that the monolayer is too flat compared to the others. Here, we consider several explanations, including the pressure dependence of headgroup hydration and the conformational landscape of the plasmalogen linkage.

55-POS Board 27

STERIC PACKING MODES STABILIZING MEMBRANE PROTEIN FOLDING AND ASSEMBLY REVEALED BY AFM-BASED FORCE SPECTROSCOPY

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The essential features 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 understand how dominant interactions between specific residues stabilizing these proteins and how various forces collaborate to establish universal folding principles. 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. Analyzing unfolding pathways initiated from both N- and C-termini provided complementary perspectives that collectively elucidated a comprehensive interaction network that stabilizes the protein. We identified inter-helical side chain packing interactions as a key determinant of DGK stability. These interactions exhibited characteristics consistent with the classical knobs-into-holes packing common in α-helical interfaces. Notably, we identified previously unrecognized helix-helix interaction patterns within DGK, that were also observed across resolved structures of other proteins. Mutagenesis creating packing defects induced dramatic decrease to the mechano-stability of unfolding intermediates and also to the thermo-stability of DGK trimer, in good agreement with our interaction analysis. Our findings provide a detailed, residue-level perspective on the molecular determinants governing the mechanical and thermal stability of membrane proteins. The accurate intermediate structural assignment established and key transmembrane interaction modes revealed here may substantially expand the scope of single molecule studies and facilitate more accurate design of membrane proteins.