Biophysical Society Conferences

Membrane Fusion and Budding

Estes Park, Colorado | October 5-10, 2025

Biophysical Society

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Biophysical Journal

Organizing Committee

Arun Anantharam, University of Toledo, USA

Michelle Knowles, University of Denver, USA

Ling-Gang Wu, National Institutes of Health (NIH), USA

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October 2025

Dear Colleagues,

We would like to welcome you to the Biophysical Society Conference entitled, *Membrane Fusion and Budding*. This meeting will bring together trainees, emerging scientists, and leaders in the fields of membrane fusion and budding over the course of four days. The topics will cover many subfields of the fusion and budding disciplines, including, but not limited to, fusion and budding at synapses and neuroendocrine cells (exocytosis and endocytosis), in relation to the function of membrane-bound organelles like mitochondria and extracellular vesicles, and in the context of host/virus interactions, cell-cell fusion, and autophagy. Approaches to study these processes range from organisms and cellular systems to reconstituted systems and computational modeling of membrane fusion and budding.

Overall, this conference will feature 24 posters, 62 lectures, and bring together over 100 scientists from a wide range of backgrounds and expertise. We hope that this meeting will not only provide a place to share your recent findings, but also to help promote new collaborations, helpful discussions, and future connections.

We invite you all to actively take part in the discussions following each talk, the poster sessions, and the informal exchanges that will be possible during the coffee breaks, free time, and meals. We also hope that you will also enjoy the beautiful city of Estes Park!

We would like to thank our generous sponsors: The National Institute of Neurological Disorders and Stroke, NIH and Leica for their support of this meeting.

The Organizing Committee
Arun Anantharam
Michelle Knowles
Ling-Gang Wu

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

Venue check-in to obtain your room key will be located at the Front Desk at the Holiday Inn Estes Park, 101 South Saint Vrain Avenue, Estes Park, CO 80517.

The BPS Registration Desk, to pick up your badge and meeting materials, will be located in the Ballroom Front Foyer during the following times:

Sunday, October 5	5:00 PM – 8:30 PM
Monday, October 6	8:00 AM – 4:00 PM
Tuesday, October 7	8:30 AM – 4:00 PM
Wednesday, October 8	8:30 AM – 2:30 PM
Thursday, October 9	8:30 AM – 4:00 PM

Instructions for Presentations

(1) Presentation Facilities:

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

(2) Poster Session:

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

2) A display board measuring 5 feet wide (152.4 cm) and 3.4 feet tall (103.6 cm) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as listed in the E-book.

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

4) There will be formal poster presentations on Monday and Tuesday. Poster presentation times vary by day, so please refer to the daily schedule for your formal presentation date and time. Ninety (90) minutes have been allotted for poster presentations each day. Presenting authors with odd-numbered poster boards should present during the first 45 minutes, and those with even-numbered poster boards should present during the last 45 minutes.

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

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

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

Meals, Coffee Breaks, and Socials
Breakfasts and luncheons will be served in Ballroom Salon DEF. Thursday evening's dinner will also be held in Ballroom Salon DEF. Coffee Breaks will be held in the Front Foyer. The evening socials scheduled for Sunday-Tuesday will be held in the Front Foyer.

Smoking
Please be advised that the Holiday Inn Estes Park Hotel is a non-smoking facility.

Name Badges
Name badges will be given to you when you check-in at the Registration Desk in the Ballroom Front 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.

On-Site Contact Information
If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from October 5-9 during registration hours.

In case of emergency, you may contact the following:

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

Erica Bellavia
Phone: 571-435-7669
Email: ebellavia@biophysics.org

Membrane Fusion and Budding

Estes Park, Colorado
October 5-10, 2025

All scientific sessions will be held in Ballroom ABC unless otherwise noted.

PROGRAM

Sunday, October 5, 2025

5:00 PM – 8:30 PM	**Registration/Information**	**Front Foyer**
7:00 PM – 7:10 PM	**Welcome and Opening Remarks** Michelle Knowles, University of Denver, USA	
Session I	**Presynapse Formation, Plasticity, and Release Mechanisms** Michelle Knowles, University of Denver, USA	
7:10 PM – 7:35 PM	David DiGregorio, University of Colorado School of Medicine, USA ***Diversity of Short-Term Plasticity: A Synaptic Mechanism for Sculpting Neuronal Activity***	
7:35 PM – 8:00 PM	Xuelin Lou, Medical College of Wisconsin, USA ***β-Cell Membrane Trafficking: Dynamin-Regulated Autolysosome Fission***	
8:00 PM – 8:50 PM	Volker Haucke, Leibniz-FMP, Germany **Plenary Talk:** ***How the Presynapse Forms and Functions***	
9:00 PM – 10:30 PM	**Social Gathering**	**Front Foyer**

Monday, October 6, 2025

7:30 AM – 8:30 AM	**Breakfast**	**Ballroom DEF**
8:00 AM – 4:00 PM	**Registration/Information**	**Front Foyer**
Session II	**Endocytic Protein Mechanisms and Functions** Jihong Bai, Fred Hutch Cancer Center, USA, Chair	
8:30 AM – 8:55 AM	Jihong Bai, Fred Hutch Cancer Center, USA ***Synaptic Vesicle Endocytosis: Roles of Amphipathic Motifs***	
8:55 AM – 9:20 AM	Avital Rodal, Brandeis University, USA ***Distinct and Overlapping Functions of Drosophila Dynamin PRD Isoforms***	
9:20 AM – 9:35 AM	Feng-Ching Tsai, Curie Institute, France* ***Asymmetric Branched Actin Assembly Driven By N-Wasp Around a Membrane Neck Leads to Neck Breakage***	
9:35 AM – 10:00 AM	Schuyler van Engelenburg, University of Denver, USA	

	Pushing the Envelope of Superresolution Microscopy to Study Enveloped Virus Budding	
10:00 AM – 10:45 AM	**Coffee & Networking Break**	**Front Foyer**
Session III	**Exocytosis: Protein Functions and Fusion Pore Structure** Volker Kiessling, University of Virginia, USA, Chair	
10:45 AM – 11:10 AM	Jacqueline Burré, Cornell Medical College, USA ***Molecular Mechanisms Underlying Disease-Causing Mutations in SNAP-25***	
11:10 AM – 11:35 AM	Jiajie Diao, University of Cincinnati, USA ***MUNC18 Modulates Syntaxin Phase Separation for Exocytosis***	
11:35 AM – 12:00 PM	Volker Kiessling, University of Virginia, USA ***Insights into the Lipid-Protein and Protein-Protein Interactions Guiding SNARE Mediated Membrane Fusion***	
12:00 PM – 12:25 PM	Frederic Meunier, University of Queensland, Australia ***Building the Fusion Pore at the Nanoscale: A Sisyphean View***	
12:25 PM – 1:30 PM	**Lunch**	**Ballroom DEF**
Session IV	**Synaptotagmin Functions** Jeff Knight, University of Colorado, Denver, USA, Chair	
1:30 PM – 1:55 PM	Jeff Knight, University of Colorado, Denver, USA ***Carbonyl Reactivity of Synaptotagmins and Related Secretory Pathway Proteins***	
1:55 PM – 2:20 PM	Shyam Krishnakumar, Yale University, USA ***Differential Tuning of Complexin Clamping Mechanism Across Species***	
2:20 PM – 2:45 PM	Noreen Reist, Colorado State University, USA ***Synaptotagmin in Health and Disease***	
2:45 PM – 4:15 PM	**Poster Session I**	**Front Foyer**
4:15 PM – 7:30 PM	**Free Time/Dinner on Own**	
Session V	**Synaptic Vesicle Recycling, Plasticity, and Neurological Disorders** Jeremy Dittman, Weill Cornell Medical College, USA, Chair	
7:30 PM – 7:55 PM	Robert Edwards, UCSF Weill Institute for Neurosciences, USA ***A Prysynaptic Form of Homeostatic Plasticity***	
7:55 PM – 8:45 PM	Timothy Ryan, Cornell Medical College, USA **Plenary Talk:** ***Synaptic Vesicle Recycling: A Metabolic Vulnerability Associated with Neurodegeneration***	
8:45 PM – 10:15 PM	**Social Gathering** **Front Foyer**	

Tuesday, October 7, 2025

7:30 AM – 8:30 AM	**Breakfast** **Ballroom DEF**	
8:30 AM – 4:00 PM	**Registration/Information**	**Front Foyer**
Session VI	**Synaptic Vesicle Exocytosis and Endocytosis** Jason Vevea, St. Judes Children's Research Hospital, USA, Chair	
8:30 AM – 8:55 AM	Jason Vevea, St. Judes Children's Research Hospital, USA ***Age-Dependent Molecular Changes in the Synaptic Vesicle***	
8:55 AM – 9:20 AM	Wallace B. Thoreson, University of Nebraska Medical Center, USA ***Glutamate Release at Ribbon Synapses of Rod Photoreceptor Cells***	
9:20 AM – 9:45 AM	Henrique von Gersdorff, The Vollum Institute, USA ***Ultrafast Endocytosis at an Inhibitory Synapse***	
9:45 AM – 10:00 AM	Dennis Weingarten, The Vollum Institute, USA* ***Synaptotagmin-3 And -7 Dynamically Regulate the Readily Releasable Pool***	
10:00 AM – 10:15 AM	Grant Walters, Vanderbilt University, USA* ***ATP Dependent Regulation of Spontaneous Neurotransmission***	
10:15 AM – 10:45 AM	**Coffee Break**	**Front Foyer**
10:45 AM – 12:15 PM	**Networking Break**	
12:15 PM – 1:30 PM	**Lunch**	**Ballroom DEF**
Session VI I	**Virus Entry and Budding – CANCELLED**	
Session VIII	**Fusion Pore and Release Regulation** Michelle Knowles, University of Denver, USA, Chair	
1:30 PM – 1:45 PM	H. Robe Guy, Amyloid Research Consultant, USA* ***Amyloid Beta 42 Mechanosensitive Sieve Models of Fusion Pores***	
1:45 PM – 2:10 PM	Heidi HH, Vanderbilt University, USA ***Role of GPCRs in Regulation of Hormone and Neurotransmitter Release***	
2 :10 PM – 3:40 PM	**Poster Session II**	**Front Foyer**
3:40 PM – 7:30 PM	**Free Time/Dinner on Own**	
Session IX	**Mechanical Forces and Molecular Mechanisms for Vesicle Formation and Fission** Arun Anantharam, University of Toledo, USA, Chair	
7:30 PM – 7:55 PM	Jürgen Klingauf, University of Münster, Germany ***The Readily Retrievable Pool of Synaptic Vesicles***	
7:55 PM – 8:20 PM	Rajesh Ramachandran, Case Western Reserve University, USA ***Structural Insights into Curvature-Sorted Dynamin Pleckstrin-Homology Domain (PHD)-Phospholipid Interactions During Endocytic Vesicle Scission***	
8:20 PM – 9:10 PM	David Drubin, University of California, Berkeley, USA	

Plenary Talk: ***Harnessing Actin Assembly Forces for Endocytic Vesicle Formation***

9:10 PM – 10:30 PM **Social Gathering**
Front Foyer

Wednesday, October 8, 2025

7:30 AM – 8:30 AM **Breakfast**
Ballroom DEF

8:30 AM – 4:00 PM **Registration/Information** **Front Foyer**

Session X **Mitochondria and Golgi Membrane Dynamics**
Ann Wehman, Texas A&M University, USA, Chair

8:30 AM – 8:55 AM Ya-Wen Liu, National Taiwan University College of Medicine, Taiwan
Lipid Logic: How Cardiolipin Conversion Enables Selective Mitochondrial Fusion

8:55 AM – 9:20 AM Joseph Hoolachan, City of Hope, USA
Beyond Exocytosis: SNARE-Mediated Hijacking at the Mitochondria

9:20 AM – 9:45 AM Halil Aydin, NYU Pain Research Center, New York University, USA
Cardiolipin Dynamics Promote Mitochondrial Membrane Remodeling

9:45 AM – 10:00 AM Chase Amos, Yale University, USA*
The Bridge-Like Lipid Transfer Protein VPS13D Controls Membrane Dynamics at the Trans Golgi/TGN

10:00 AM – 10:15 AM Maohan Su, Yale University*
Golgi Stack Is Organized by a Precise Tetraplex of Rim Golgin Proteins

10:15 AM – 10:45 AM **Coffee Break** **Front Foyer**

Session XI **Lipid Functions in Protein Assembly, Pore Formation, and Synapse Function**
Sarah Veatch, University of Michigan, USA, Chair

10:45 AM – 11:10 AM Sarah Veatch, University of Michigan, USA
Lipids Facilitate Protein Assembly at Membranes Through Prewetting

11:10 AM – 11:35 AM Huan Bao, University of Virginia School of Medicine, USA
Designer Nanodiscs to Probe and Reprogram Membrane Fusion and Pore Formation

11:35 AM – 12:00 PM Ruth Heidelberger, McGovern Medical School at UTHealth Houston, USA
Roles of Syntaxin3 at Retinal Ribbon-Style Synapses

12:00 PM – 1:30 PM **Lunch** **Ballroom DEF**

Session XII **Fusion, Fission, Membrane Contact, and Cell Migration**
Matthew Brody, University of Michigan Medical School, USA, Chair

1:30 PM – 1:45 PM	Weronika Tomaka, University of Virginia, USA* ***MUNC18 and Membrane Lipids Cooperate to Regulate the Lateral Distribution of Syntaxin-1A in the Plasma Membrane***
1:45 PM – 2:10 PM	Matthew Brody, University of Michigan Medical School, USA ***Regulation of Atrial Natriuretic Peptide Secretion by the RAB3 Cycle***
2:10 PM – 2:35 PM	Anna Shnyrova, Biofisika Institute, Spain ***Fusion Mediated by Fission Protein Machinery***
2:35 PM – 7:30 PM	**Free Time/Dinner on Own**
Session XIII	**Endocytosis, Dense-Core Vesicle Fusion and Neuronal Endosome-Lysosome** Michael B. Hoppa, Dartmouth University, USA, Chair
7:30 PM – 7:55 PM	Michael B. Hoppa, Dartmouth University, USA ***KV2-Mediated Presynaptic ER/PM Junctions Organize Calcium Signaling and Regulate Vesicle Fusion***
7:55 PM – 8:20 PM	Lois Weisman, University of Michigan, USA ***Pikfyve Promotes Cell Migration via Control of a Negative Regulator of RAC1***

Thursday, October 9, 2025

7:30 AM – 8:30 AM	**Breakfast** **Ballroom DEF**
8:30 AM – 3:30 PM	**Registration/Information** **Front Foyer**
Session XIV	**Dense-Core Vesical Fusion and Neuronal Endosome-Lysosome** Cedric Asensio, University of Denver, USA, Chair
8:30 AM – 8:55 AM	Bettina Winckler, University of Virginia, USA ***Endosomes and Lysosomes in Neurons: What Happens When They Break?***
8:55 AM – 9:20 AM	Cedric Asensio, University of Denver, USA ***Sorting of Synaptotagmins to the Insulin Secretory Granule***
9:20 AM – 9:45 AM	Julie Brill, Hospital for Sick Children, Canada ***Making a Granule: How PI4KII and OSBP Regulate Secretory Granule Maturation***
9:45 AM – 10:00 AM	Quanfeng Zhang, Peking University, China* ***Tuning the Size of Large Dense-Core Vesicles and Quantal Neurotransmitter Release via Secretogranin II Liquid–Liquid Phase Separation***
10:00 AM – 10:15 AM	Madhurima Chattopadhyay, Weill Cornell Medicine, USA* ***Finally Visualizing How MUNC13 Opens Syntaxin-1***
10:15 AM – 10:45 AM	**Coffee Break** **Front Foyer**
Session XV	**Cryo-EM and -ET Of Synapses, Clathrin, Dynamin, and Ferrosome** John R. Jimah, Princeton University, USA, Chair

10:45 AM – 11:10 AM	John R. Jimah, Princeton University, USA ***Structural Basis of Organelle Division in Malaria Parasites***	
11:10 AM – 11:35 AM	Axel Brunger, Stanford University, USA ***Architecture of Synapses Revealed by Cryo-Electron Tomography***	
11:35 AM – 12:00 PM	Qiangjun Zhou, Vanderbilt University, USA ***Molecular Mechanism for Ferrosome Formation***	
12:00 PM – 1:30 PM	**Lunch**	**Ballroom DEF**

Session XVI	**Synaptic Vesicle Exocytosis: Lipid Regulation and Suspended Bilayer Platform** Sathish Ramakrishnan, Yale School of Medicine, USA, Chair	
1:30 PM – 1:55 PM	Sathish Ramakrishnan, Yale School of Medicine, USA ***Single-Molecule Decoding of Membrane Fusion with Suspended Bilayers***	
1:55 PM – 2:20 PM	Min Ju Shon, Pohang University of Science and Technology, South Korea ***Probing SNARE-Mediated Vesicle Fusion on Freestanding Membranes***	
2:20 PM – 2:45 PM	Yongli Zhang, Yale University, USA ***Mechanical Forces in Membrane Fusion and Bulk Lipid Transfer***	
2:45 PM – 3:00 PM	**Break**	
3:00 PM – 3:50 PM	Reinhard Jahn, University of Göttingen, Germany ***SNARE-Mediated Membrane Fusion - Where Are the Energy Barriers?***	
3:50 PM – 4:10 PM	**Closing Remarks and *Biophysical Journal* Poster Awards** Arun Anantharam, University of Toledo, USA	
4:10 PM – 6:00 PM	**Free Time**	
6:00 PM – 8:00 PM	**Banquet Dinner**	**Ballroom DEF**

**Contributed talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

DIVERSITY OF SHORT-TERM PLASTICITY: A SYNAPTIC MECHANISM FOR SCULPTING NEURONAL ACTIVITY

David DiGregorio[1]
[1]University of Colorado School of Medicine, Aurora, CO, USA

No Abstract

B-CELL MEMBRANE TRAFFICKING: DYNAMIN-REGULATED AUTOLYSOSOME FISSION

Xuelin Lou[1]
[1]Medical College of Wisconsin, Milwaukee, WI, USA

No Abstract

HOW THE PRESYNAPSE FORMS AND FUNCTIONS

Volker Haucke[1]
[1]Leibniz-FMP, Berlin, Germany

No Abstract

SYNAPTIC VESICLE ENDOCYTOSIS: ROLES OF AMPHIPATHIC MOTIFS

Jihong Bai[1]
[1]Fred Hutch Cancer Center, Seattle, WA, USA

No Abstract

DISTINCT AND OVERLAPPING FUNCTIONS OF DROSPHILA DYNAMIN PRD ISOFORMS

Avital Rodal[1]
[1]Brandeis University, Waltham, MA, USA

No Abstract

ASYMMETRIC BRANCHED ACTIN ASSEMBLY DRIVEN BY N-WASP AROUND A MEMBRANE NECK LEADS TO NECK BREAKAGE

Simli Dey[1]; Ann-Sophie Mace[1]; Luka Mesarec[2]; Aurelie Bertin[1]; Nir Gov[3]; John Manzi[1]; Patricia Bassereau[1]; Ales Iglic[2]; Christophe Le Clainche [4]; Julien Berro[5]; Julien Heuvingh[6]; **Feng-Ching Tsai**[1]
[1]Curie Institute, Physics of Cells and Cancer (PCC), PARIS, France; [2]University of Ljubljana, Faculty of Electrical Engineering, Ljubljana, Sloveni; [3]Weizmann Institute of Science, Rehovot, Israel; [4]Université Paris-Saclay, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France; [5]Yale University, epartment of Molecular Biophysics & Biochemistry, Department of Cell Biology, New Haven, CT, USA; [6]Université Paris Diderot, Laboratoire PMMH, Paris, France

Actin assembly plays a critical role in various membrane remodelling processes, including intracellular trafficking. In this process, the membranes often form vesicular buds connected to the plasma membrane by saddle-shaped necks. However, the molecular mechanisms regulating actin assembly at these membrane necks remain elusive. Moreover, it is still unclear how this dense actin network produces force to facilitate membrane fission at the necks. To address this fundamental question, in this work, we developed a reconstituted system featuring saddle-shaped membrane necks, generated from wrapping the lipid bilayers around beads grafted onto a glass substrate. The formation of these curved membrane necks at the base of the beads was confirmed by the enrichment of the curvature sensing BAR protein SNX9. Our results show that upon introducing actin nucleation-promoting factor N-WASP, together with Arp2/3 complex and actin monomers, branched actin network assembles asymmetrically around the membrane necks. This asymmetric actin assembly exert force on the necks, leading to their displacement, as evidenced by the lateral and vertical displacement of the beads. This suggests that the neck structure was disrupted. In contrast, when using a truncated formed of N-WASP (pWA), which still activates Arp2/3, the actin network grows symmetrically around the necks while no bead displacement was observed. These findings align with recent reports showing that in mammalian cells, actin assembly at stalled clathrin-mediated endocytosis sites is asymmetric, acting like a “bottle opener” to pull off endocytic buds. Together, our findings provide new insights into how actin nucleation-promoting factors spatially regulate actin assembly at complex membrane geometries, advancing our understanding of processes such as endocytosis.

MOLECULAR MECHANISMS UNDERLYING DISEASE-CAUSING MUTATIONS IN SNAP-25

Jacqueline Burré[1]
[1]Weill Cornell Medicine, Brain and Mind Research Institute, New York, NY, USA

Heterozygous mutations in SNAP-25 cause epileptic encephalopathies. These devastating diseases start early in childhood, usually around the first year of life, and are characterized primarily by unremitting epileptic activity combined with progressive cerebral dysfunction and cognitive, sensory and/or motor deterioration. Seizures respond well to antiepileptics in only 50% of patients; the other symptoms remain refractory to treatment. Thus, the prognosis is very poor. Animal studies suggest that the presence of SNAP-25 is required for synaptic maturation and neuronal function. Yet, it is not well-understood how mutations in SNAP-25 affect its function. In addition, it is unclear how mutations in a single gene lead to differences in clinical presentations, necessitating a mechanistic study. Knockout of SNAP-25 in mice is embryonic lethal. Hemizygous SNAP-25 mice are viable but exhibit seizures, mild hyperactivity, and some cognitive impairments. Yet, no mouse model of human disease-causing SNAP-25 mutations exists, and it remains therefore unclear if patient phenotypes are due to haploinsufficiency or a gain of toxic activity. Using in vitro and cellular studies in wild-type and SNAP-25 heterozygous mouse neurons, we find that 18 disease-causing mutations in SNAP-25 lead to neuronal deficits due to altered interactions of SNAP-25 with its cognate SNARE proteins and SNARE regulatory proteins. We find deficits in binding of SNAP-25 interactors to be mutation-specific, suggesting that mutant variants affect synaptic vesicle release at distinct steps. Interestingly, cellular deficits in heterozygous neurons are more pronounced in presence of mutants, suggesting a dominant negative activity of mutant SNAP-25. Based on these findings, we propose that therapeutic strategies need to focus on removing the mutant allele while at the same time stabilizing wild-type SNAP-25.

MUNC18 MODULATES SYNTAXIN PHASE SEPARATION FOR EXOCYTOSIS

Jiajie Diao[1]
[1]University of Cincinnati College of Medicine, Cancer Biology, Cincinnati, OH, USA

The soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein syntaxin mediates neuronal exocytosis and self-assembles into large clusters in the plasma membrane. The formation and function of these clusters, and whether they promote or inhibit synaptic vesicle fusion remain unclear. Here, using optogenetic control of syntaxin clustering in vitro and in vivo, as a light-inducible gain-of-function assay, we show that light-enhanced clustering reduces both spontaneous and triggered vesicle fusion, and this impairs mouse hunting behavior. Cluster formation is induced by liquid-liquid phase separation (LLPS) of the SNARE domain of syntaxin. For the regulatory mechanism, Munc18, which is known to alter syntaxin conformation, acts to reduce LLPS for cluster formation, thereby promoting active syntaxin. These results suggest exocytosis regulation involves LLPS-induced syntaxin clusters that serve as a syntaxin reservoir, while Munc18 releases syntaxin monomers to form syntaxin/Munc18 complex setting the stage for efficient fusion.

INSIGHTS INTO THE LIPID-PROTEIN AND PROTEIN-PROTEIN INTERACTIONS GUIDING SNARE MEDIATED MEMBRANE FUSION

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

Many intracellular membrane fusion events are catalyzed by members of the SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) protein family in combination with Synaptotagmins (Syt) acting as Ca^{2+} sensors. SNARE proteins form heterotrimers or tetramers that bridge separate membranes and assemble a coiled helical bundle of four 60-70 residue "SNARE motif" domains to induce fusion. The particularly highly Ca^{2+}-regulated fusion events between neuronal synaptic vesicles and the plasma membrane are catalyzed by a SNARE complex composed of Syntaxin-1a and SNAP25 on the plasma membrane and Synaptobrevin-2 on the vesicle membrane. Bottom-up approach experiments utilizing supported membranes in combination with single vesicle fusion, fluorescence interference contrast (FLIC) microscopy, and fluorescence resonance energy transfer (FRET) assays reveal details of the diverse lipid-protein and protein-protein interactions that contribute to the delicate regulation of the fusion process. We present data demonstrating how Ca^{2+} mediated membrane binding of Syt1's C2 domains change the physical properties of the lipid bilayer and how correlating changes in the conformation of membrane anchored SNARE proteins modulate the fusion probability. A detailed analysis of single fusion events between purified PC12 dense core vesicles and supported membranes reveals how interactions between Syt1's C2B domain and the lipid bilayer control fusion pore expansion. Unlike its synaptic SNARE partners and most other cellular SNAREs, SNAP25 contains two SNARE motif domains connected by a flexible linker and does not contain a transmembrane domain, instead associates to the plasma membrane through posttranslational lipidation of the unstructured linker domain that separates the two SNARE motifs. We have generated membrane-associated single SNARE domain constructs of SNAP25 that have allowed us to dissect the structural and functional contributions of each domain. FRET-based measurements and single vesicle fusion experiments show that each domain forms a fusion-capable SNARE complex with its cognate SNAREs, though with different efficiency and kinetics.

BUILDING THE FUSION PORE AT THE NANOSCALE: A SISYPHEAN VIEW

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In this talk, I will present some of the work we have done on the nanoscale organization of the exocytic machinery in live cells using single-molecule microscopy. Not surprisingly, most of the exocytic machinery located on the plasma membrane is organized in nanoclusters. What was surprising however, is that these nanoclusters were only transient molecular traps, constitutively appearing and disappearing at seemingly random locations across the plasma membrane of PC12 cells. To explore these transient nanoscale platforms further, we developed an in-house spatiotemporal clustering algorithm from which we derived several key metrics that define their behavior. We have tracked and analyzed several of the key exocytic components such as Syntaxin1A, Munc18, Munc13, RIM, SV2A, Doc2a and Doc2b. The transient nature of these events likely reflects the ability of the plasma membrane to assemble and disassemble docking sites from which fusion pores may (or may not) form. In a Sisyphean view, the fusion pore can only form when the correct combination of molecular effectors are recruited and remain immobilized in space and time at the interface between the vesicle and the plasma membrane.

CARBONYL REACTIVITY OF SYNAPTOTAGMINS AND RELATED SECRETORY PATHWAY PROTEINS

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C2 domains are membrane binding protein motifs that play key roles in exocytosis including secretory vesicle trafficking, docking, and membrane fusion. C2 domains from synaptotagmins and related proteins bind selectively to the signaling lipid phosphatidylinositol-(4,5)-biphosphate (PIP_2) via a conserved positively charged surface centered on a cluster of lysine residues. Lysine sidechains are among the few that can react covalently with carbonyl compounds generated during oxidative stress, such as malondialdehyde and 4-hydroxy-2-nonenal (4HNE). This process, termed protein carbonylation, irreversibly modifies protein structure and can alter function. We are investigating the reactivity of C2 domains from synaptotagmins and synaptotagmin-like proteins and their impact on protein structure and function using chemical, proteomic, and protein biophysics approaches along with cell-based measurements of secretion. We observe strong reactivity of lysine clusters from phosphoinositide-binding C2 domains towards a range of carbonyl compounds, suggesting it is likely a conserved feature with broad relevance. Carbonyl modification weakens C2 domain membrane binding affinity and alters the folding stability of some proteins. We even observe novel intra-molecular crosslinks that elude identification via typical proteomics analysis tools, suggesting that this lysine cluster reactivity may have been overlooked in previous studies of post-translational modifications. Treatment of insulin-secreting cells with carbonyl compounds associated with inflammation leads to abundant protein carbonylation and inhibition of insulin secretion within minutes, a timescale consistent with direct protein modification rather than changes in gene expression. The observed pattern of protein carbonylation in 4HNE-treated cells is similar to that of pre-diabetic NOD mouse islets. Because protein carbonylation due to reactive aldehydes is known to accompany oxidative and inflammatory stresses, our results suggest a possible novel mechanism for cellular regulation of secretion in response to oxidative stress and inflammation.

DIFFERENTIAL TUNING OF COMPLEXIN CLAMPING MECHANISM ACROSS SPECIES

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No Abstract

SYNAPTOTAGMIN IN HEALTH AND DISEASE

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No Abstract

A PRYSYNAPTIC FORM OF HOMEOSTATIC PLASTICITY

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No Abstract

SYNAPTIC VESICLE RECYCLING: A METABOLIC VULNERABILITY ASSOCIATED WITH NEURODEGENERATION

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No Abstract

AGE-DEPENDENT MOLECULAR CHANGES IN THE SYNAPTIC VESICLE

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No Abstract

GLUTAMATE RELEASE AT RIBBON SYNAPSES OF ROD PHOTORECEPTOR CELLS

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Humans are capable of perceiving light flashes consisting of only a few photons scattered across the retina. This is initiated by the capture of single photons by individual rod photoreceptor cells. For visual perception, the small rod response generated by a single photon must be reliably transmitted to second-order rod bipolar cells but this response causes only a brief pause in the ongoing release of synaptic vesicles in darkness. How is a small light-evoked pause in release distinguished from a random pause? One proposed solution requires a dark release rate of >100 vesicles/s while another is that release may occur at regular intervals. We are using a combination of approaches to analyze rates and patterns of release from mouse rods. These include whole cell recordings from rods to detect anion currents activated presynaptically by glutamate binding to EAAT5 glutamate transporters, membrane capacitance measurements of exocytosis and optical measurements using a fluorescent glutamate sensor, iGluSnFr3, selectively expressed in rods. We complement physiological measurements with computational studies using anatomically realistic reconstructions from serial block face electron micrographs and the Monte Carlo modeling program MCell4. Our results suggest that release from rods occurs in darkness at relatively slow rates of <40 v/s with components of regularity that can improve post-synaptic detection. These results highlight the importance of the geometric tortuosity imparted by the unique anatomy of the invaginating rod synapse as well as unusual properties of the exocytotic Ca^{2+} sensor synaptotagmin 1 employed at this synapse.

ULTRAFAST ENDOCYTOSIS AT AN INHIBITORY SYNAPSE

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Stable and efficient microcircuits in the brain require well balanced excitatory and inhibitory synapses to convey small signals over noisy backgrounds and large signals without saturation of synaptic gain. Inhibitory synapses help maintain well balanced circuits in the CNS by displaying robust and enduring transmitter release via powerful inhibitory GABAergic and glycinergic synapses in fast spiking inhibitory interneurons. However, the kinetics of synaptic vesicle endocytosis remains largely unexplored at inhibitory synapses. The mammalian retina contains many different types of inhibitory amacrine cells that release GABA and glycine unto excitatory neurons. Our Objective was to study the kinetics and mechanisms of synaptic vesicle endocytosis. Methods: Here we will show membrane capacitance measurements from live presynaptic terminals of glycinergic amacrine cells embedded in adult rat retina. Results: After exocytosis, these inhibitory synapses displayed ultrafast endocytosis with a bi-linear decay kinetics. Longer depolarizing pulses, with more Ca^{2+} influx, produced progressively steeper decay slopes. This occurs even in the presence of a strong but slow Ca^{2+} buffer (10 mM EGTA). However, faster Ca^{2+} buffering with 1 mM BAPTA fully blocked endocytosis, while only reducing exocytosis by 30%. Ultrafast endocytosis was thus Ca^{2+} dependent. It was also dependent on GTP, dynamin, actin and calmodulin, but surprisingly unlike excitatory synapses, it was blocked by Pitstop2, a clathrin-disrupting drug. Conclusions: Our findings suggest ultrafast endocytosis is clathrin-dependent and we suggest that hotspots of membrane retrieval are positioned further from Ca^{2+} channels than sites of fast exocytosis, because the Ca^{2+}-dependent triggering of ultrafast endocytosis is EGTA-insensitive, but highly BAPTA-sensitive.

SYNAPTOTAGMIN-3 AND -7 DYNAMICALLY REGULATE THE READILY RELEASABLE POOL

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Presynaptic terminals rely on a sophisticated protein machinery to coordinate the release of synaptic vesicles from the readily releasable pool (RRP). However, the proportion of vesicles that constitute the RRP and how it adapts during ongoing activity remain incompletely understood. One long-standing hypothesis posits that short-term facilitation is achieved via the dynamic recruitment of vesicles to transiently expand the RRP. Testing this hypothesis has been challenging, however, as existing methods to estimate RRP size perform poorly at facilitating synapses. Here, we developed a novel, biophysically informed RRP estimation algorithm based on kinetic models of vesicle pools, validated for both depressing and facilitating synapses. We then combined this approach with whole-cell electrophysiology in acute brain slices from single and double knockout (DKO) mice lacking Synaptotagmin-3 (SYT3), Synaptotagmin-7 (SYT7), or both. We recorded evoked EPSCs from cerebellar, hippocampal, and medial prefrontal cortex synapses, observing that paired-pulse facilitation was consistently accompanied by a frequency-dependent increase in RRP size. Deletion of either SYT3 or SYT7 reduced both facilitation and RRP dynamics, while DKO abolished them entirely. These findings establish SYT3 and SYT7 as cooperative regulators of dynamic vesicle docking and RRP expansion during repetitive activity. Their distinct calcium-binding properties confer temporally layered control of synaptic strength, identifying dynamic docking as a key biophysical mechanism of short-term plasticity and synaptic computation.

ATP DEPENDENT REGULATION OF SPONTANEOUS NEUROTRANSMISSION

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The human body is heavily dependent upon adenosine triphosphate (ATP) production through glycolysis and oxidative phosphorylation. Despite the brain accounting for 2% of our body's mass, it consumes close to 20% of all ATP produced. The majority of ATP is consumed in the maintenance of resting membrane potentials, reversing presynaptic calcium entry, and maintaining synaptic transmission. During evoked neurotransmission, ATP production is increased through activation of the oxidative phosphorylation (OXPHOS) pathway and is responsible for maintaining synaptic transmission. Thus, the location and availability of mitochondria is critical for neurons to sustain synaptic transmission. To maintain neurotransmission, ATP produced through OXPHOS is needed, and the bulk of that energy is consumed maintaining vesicle recycling. While the energy demands of evoked neurotransmission have been extensively studied, the means by which ATP is utilized in the regulation of spontaneous neurotransmission is largely unknown. We utilized primary hippocampal neurons and patch clamp electrophysiology to examine the ATP dependence of spontaneous neurotransmission. Following rapid ATP depletion (confirmed using newly developed iATPSnFR2), there was a robust increase in miniature excitatory postsynaptic current (mEPSC) frequencies, and we found that this increase in mEPSC frequency is partially calcium independent. Our findings show that ATP is utilized to regulate spontaneous neurotransmission, and ATP depletion leads to unclamping of spontaneous neurotransmission in a partially calcium independent manner. These findings reveal that proper ATP production is critical for the regulation of spontaneous neurotransmission.

PUSHING THE ENVELOPE OF SUPERRESOLUTION MICROSCOPY TO STUDY ENVELOPED VIRUS BUDDING

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No Abstract

AMYLOID BETA 42 MECHANOSENSITIVE SIEVE MODELS OF FUSION PORES

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Alzheimer's Disease (AD) is associated with an over-abundance of Amyloid beta (A?) peptides. A?42 is the only variant that forms transmembrane channels in neurons. Synaptic transmission is affected by A?42 even in early stages of AD. Our group has been developing structural models of transmembrane channels and transporters for ~ 50 years and has modeled A?42 assemblies for over 30 years. These A?42 models include concentric ?-barrel models of soluble oligomers, annular protofibrils, transmembrane oligomers (TMOs), and ion channels. Our models are consistent with numerous experimental findings, protein modeling methods and criteria, ?-barrel theory, and are energetically sound. The number of peptides per assembly vary enormously, from four to over 200. GM1 gangliosides increase A?42 toxicity and facilitate formation of large assemblies that fuse membranes. We are now modeling A?42/GM1 hexagonal lattices. A?42 competes with VAMP2 for binding to synaptophysin. The VAMP2/Synaptophysin transmembrane complex has been proposed to reside in the center of the closed fusion pore assembly when the vesicle membrane first contacts the plasma membrane. It has 6-fold radial symmetry about a putative central transmembrane pore and is surrounded by a 6-fold symmetric SNARE complex that supplies the energy to open the fusion pore. In our simplest model, an A?42 hexagonal lattice is located between the SNARE assembly and the central VAMP2/synaptophysin complex. Fusion pore opening is a multistep process. Initially 48 impermeant A?42 hexamer TMOs comprise the lattice. In response to membrane tension, the hexamers merge to form 24 dodecamers with small pores, these then merge to form twelve 24mers with large pores, which then merge to form six 48mers with very large pores, which may then morph into a single gigantic fusion pore. Our hypothetical models can be tested experimentally in numerous ways and suggest how some structures can be stabilized for structural studies.

ROLE OF GPCRS IN REGULATION OF HORMONE AND NEUROTRANSMITTER RELEASE

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No Abstract

THE READILY RETRIEVABLE POOL OF SYNAPTIC VESICLES

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No Abstract

STRUCTURAL INSIGHTS INTO CURVATURE-SORTED DYNAMIN PLECKSTRIN-HOMOLOGY DOMAIN (PHD)-PHOSPHOLIPID INTERACTIONS DURING ENDOCYTIC VESICLE SCISSION

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No Abstract

HARNESSING ACTIN ASSEMBLY FORCES FOR ENDOCYTIC VESICLE FORMATION

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No Abstract

LIPID LOGIC: HOW CARDIOLIPIN CONVERSION ENABLES SELECTIVE MITOCHONDRIAL FUSION

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Mitochondria are the power house of eukaryotic cells, responding and adapting to various stressors and metabolic demands through their dynamic nature. Mitochondrial dynamics rely on the coordinated regulation of proteins and phospholipids to control the processed of fission, fusion and transport. Cardiolipin, a unique phospholipid enriched in the inner mitochondrial membrane, promotes inner membrane fusion but facilitates outer membrane fission when externalized upon mitochondrial stress. Interestingly, cardiolipin can be hydrolyzed by phospholipase D 6 (PLD6) into phosphatidic acid (PA), promoting mitochondrial fusion; however, the exact mechanism by which cardiolipin conversion drives mitochondrial fusion remain unclear. We discover nucleotide diphosphate kinase NME3 as a key player in PLD6-mediated mitochondrial fusion. NME3 directly binds to PA via its N-terminal amphipathic helix and is enriched at the contact sites between closely positioned mitochondria in a PLD6-dependent manner. The ability of NME3 to bind PA and form hexamers enables its mitochondrial tethering activity. Notably, we observe that nutrient starvation enhances cardiolipin externalization and NME3 enrichment at mitochondrial contact sites, and the ability to promote fusion is critical for mitochondrial fusion and cell survival under starvation. Our findings reveal a mechanism by which cardiolipin conversion selectively drives mitochondrial fusion and contributes to mitochondrial quality control.

BEYOND EXOCYTOSIS: SNARE-MEDIATED HIJACKING AT THE MITOCHONDRIA

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By 2045, projections show that ~ 783 million will be living with type 2 diabetes (T2D), an increase of 46%. The transformational anti-obesity drugs address a substantial facet of the obese population with T2D and insulin resistance (IR). However, their use is limited with certain comorbidities, especially in the T2D-susceptible aging population. Further, some populations have a high incidence of (pre)T2D in lean, non-obese individuals. Declines in exocytosis events in insulin-producing pancreatic islet β-cells and in glucose-absorbing skeletal muscle (skm) cells disrupt whole-body glucose homeostasis resulting in (pre)T2D. The exocytosis protein Syntaxin 4 (STX4) is a promising target for T2D. Recent breakthroughs point to an additional requirement for STX4 in mitochondrial function in these cells. Understanding the molecular mechanisms underlying these subcellular roles for STX4 will help meet the unmet need for (pre)T2D therapies. Evidence suggests that skm mitochondrial dysfunction begins at prediabetes and exacerbates enroute to T2D as skm IR worsens. Our provocative data reveals that skm-STX4 enrichment in vivo reverses diet-induced IR,and is associated with increased healthspan and longevity; these positive effects were previously ascribed to STX4's required role at the PM in exocytosis events. Notably, we revealed that mitochondrial-localized-STX4 exists in both skm and islet beta cells and interacts with mitochondrial proteins. These interactions and emergent mechanisms include removal/repair of damaged mitochondria, potentially involving mitochondrial turnover by mitophagy, and regulation of mitochondrial dynamics via Drp1. STX4 also regulates mitochondrial dynamics via Drp1. SNARE hijacking as a mechanism yielding improved mitochondrial morphology and function will be discussed, as well as additional unconventional mechanisms involving STX4 that provide new and tantalizing opportunities for drug discovery.

CARDIOLIPIN DYNAMICS PROMOTE MITOCHONDRIAL MEMBRANE REMODELING

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Cardiolipin (CL) is a mitochondria-specific phospholipid that forms heterotypic interactions with membrane-shaping proteins and regulates the dynamic remodeling and function of mitochondria. However, the precise mechanisms through which CL influences mitochondrial morphology are not well understood. In this study, employing molecular dynamics (MD) simulations, we determined that CL molecules extensively engage with the paddle domain (PD) of mitochondrial fusion protein Optic Atrophy 1 (OPA1), which controls membrane-shaping mechanisms. Structure-function analysis confirmed the interactions between CL and two conserved motifs of OPA1 at the membrane-binding sites. We further developed a bromine-labeled CL probe to enhance cryoEM contrast and characterized the structure of OPA1 assemblies bound to the CL-brominated lipid bilayers. Our images provide direct evidence of CL enrichment within the OPA1-binding leaflet. Last, we observed a decrease in membrane remodeling activity for OPA1 in lipid compositions with increasing concentrations of monolyso-cardiolipin (MLCL). This suggests that the partial replacement of CL by MLCL, as observed in Barth syndrome-associated mutations of the tafazzin phospholipid transacylase, alters the malleability of the membrane and compromises proper remodeling. Together, these data provide insights into how biological membranes regulate the mechanisms governing mitochondrial homeostasis.

THE BRIDGE-LIKE LIPID TRANSFER PROTEIN VPS13D CONTROLS MEMBRANE DYNAMICS AT THE TRANS GOLGI/TGN

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VPS13 family proteins were recently proposed to function as bridges that couple lipid synthesis in the ER to their transfer to other membranes at contacts of the ER with other organelles. Of the four mammalian paralogues, VPS13D is the only one essential for life. Previous studies based on exogenous VPS13D showed that VPS13D resides in the Golgi complex area, as well as ER contacts with mitochondria and peroxisomes. Here we confirm, based on endogenous tagging, that a major pool of VPS13D resides at the Golgi complex. Moreover, using exogenous VPS13D to obtain a more robust fluorescent signal, we found that VPS13D localizes at the trans Golgi/TGN. We then used a combined KO/knockdown approach to investigate defects in the Golgi of HeLa cells when VPS13D is nearly depleted (total VPS13D loss results in cell death). In these cells, swelling of trans Golgi cisternae and dispersion of TGN46 was observed. Using the RUSH assay, we observed slowed exit of surface-directed cargo from the Golgi, including lysozyme C (secreted cargo), GPI-AP (ordered membrane domain cargo), and VSVG (disordered membrane domain cargo). Defects in the maturation of transferrin receptor's glycosylation (as detected by a shift in western blotting) were additionally observed. In contrast, RUSH revealed no traffic delay for lysosomal-directed proteins examined by imaging. Finally, we observed changes in the plasma membrane (PM) bilayer, including altered segregation properties of ordered and disordered domains in GPMVs, which may reflect abnormal lipid composition of TGN-to-PM vesicles in VPS13D depleted cells. Consistent with this hypothesis, we observe primarily a shift to shorter chain sphingolipid species by lipidomic analysis of the PM. Collectively, our results reveal that VPS13D, most likely via its lipid transport properties, plays an important role in controlling the lipid environment of the trans Golgi complex where vesicular carriers destined to the PM are generated.

GOLGI STACK IS ORGANIZED BY A PRECISE TETRAPLEX OF RIM GOLGIN PROTEINS

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Golgin protein have long been suspected to be organizers of the Golgi stack. Using 4Pi-SMS super-resolution microscopy, we comprehensively localize the human golgin family at the outer surface of the Golgi apparatus at 10-20 nm resolution in situ. Unexpectedly, we find that the golgins are precisely organized into four discrete layers, each containing a specific set of rim golgins, with the sheet golgins covering the cis and trans surface. Golgin-160 uniquely localizes to both sheet region and edge of internal rim, and it forms microvilli across the Golgi stack. Biochemically characterizing most of the golgins as isolated proteins, we find that they form anti-parallel dimers and further self-assemble into bands of multi-micron-long filaments. Based on our findings, we propose an "outside-in" physical model, the Golgin Organizer Hypothesis, in which the Golgi stack of cisternae directly result from adhesion of rim golgin filaments onto a membrane surface. As a validation to this model, we have observed that removing the golgins of a specific location significantly disrupts that particular cisterna without affecting any other layers.

LIPIDS FACILITATE PROTEIN ASSEMBLY AT MEMBRANES THROUGH PREWETTING

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Proteins that assemble into condensates in solution can also condense into surface phase domains when a subset of condensate components are tethered to or interact with fluid membranes. These domains are dynamic, effectively two-dimensional, and considered "prewet" because they occur well outside of the coexistence region of the condensate system alone. We show that proximity to the membrane phase transition dramatically potentiates biopolymer prewetting through a combination of theory, simulation, and experiments in well-defined reconstituted systems. When taken into cells, we show that protein prewetting at the plasma membrane inner leaflet is sensitive to perturbations of plasma membrane composition and structure. More generally, we speculate that prewetting at membranes represents a general protein assembly mechanism involved in a broad array of cell processes and sensitive to both membrane and protein properties.

DESIGNER NANODISCS TO PROBE AND REPROGRAM MEMBRANE FUSION AND PORE FORMATION

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Nanodisc has emerged as a powerful tool for the characterization of membrane proteins. However, the limited size range and capacity of current nanodiscs necessitate further improvement. On this front, we first created a simple, one-step approach to ease the construction of circularized nanodiscs (cNDs) using the SpyCatcher-SpyTag technology. This new method increases the yield of cNDs by over 10-fold and is able to rapidly generate cNDs with diameters ranging from 11 to over 100 nm, which allows for the capture of protein-lipid interactions and membrane fusion intermediates that are not possible for small nanodiscs. Building upon the success of the SpyCatcher-SpyTag cNDs, we employed split GFP to circularize nanodiscs into a robust fluorescent probe for reporting membrane binding and remodeling reactions. Biophysical dissection of several of these membrane-disrupting proteins inspired us to develop novel scaffolds to enable detergent-free reconstitution of membrane proteins with native lipids in nanodiscs. With these powerful tools in hand, we provided novel insights into the molecular mechanism of membrane fusion and pore formation in exocytosis and bacterial infection. In addition, we generated fusogenic NDs to readily reprogram the cell surface through the delivery of synthetic ion channels and signaling lipids. Together, our designer nanodiscs are biochemical reagents useful in the research and therapeutic applications of membrane biology, opening doors to the biochemical space unattainable in previous studies.

ROLES OF SYNTAXIN3 AT RETINAL RIBBON-STYLE SYNAPSES

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Syntaxin3B (Stx3B), a retinal specific transcript of the Stx3 gene, has been suggested to have both an essential role and a regulatory role in neurotransmitter release at photoreceptor and bipolar cell ribbon-style synapses. To test these hypotheses, we monitored neurotransmitter release in retinal slices or from isolated neurons using high-resolution, single-cell electrophysiological approaches. Stx3B function was inhibited by the introduction of a peptide that blocks the formation of new Stx3B-containing SNARE complexes or via inactivation of the Stx3 gene. In the presence of the peptide, vesicles in both the rapidly releasing pool (RRP) and the release pool (RP) retained the ability to undergo stimulus-evoked exocytosis, indicating that vesicles in these pools had already formed the SNARE complexes required for membrane fusion, while recruitment of new vesicles to the fusion-competent state was inhibited. At the single vesicle level, Stx3B was found to be required for multi-vesicular release in photoreceptors and bipolar cells, while parallel experiments performed in peritoneal mast cells showed that Stx3 was required for a specialized mode of multi-vesicular release called compound exocytosis. Phosphorylation of Stx3B at T14 has been suggested to enhance its ability to participate in SNARE complex formation. In a mouse line wherein Stx3B-T14 is highly phosphorylated in rod bipolar cells, we observed little difference in the magnitude of either the RRP or the RP. However, following RRP depletion, there was a striking increase in the rate at which new vesicles entered the RRP relative to control. Together, these findings suggest that Stx3B has an essential role in neurotransmitter release, and in particular, multi-vesicular release. In addition, they suggest that new SNARE complex formation and the rate at which new vesicles enter the fusion competent state is modulated by phosphorylation of Stx3B at T14.

MUNC18 AND MEMBRANE LIPIDS COOPERATE TO REGULATE THE LATERAL DISTRIBUTION OF SYNTAXIN-1A IN THE PLASMA MEMBRANE

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Neurotransmitter release in synapses occurs through SNARE-mediated fusion of secretory vesicles with the presynaptic plasma membrane in response to neuronal stimulation. Prior to fusion, secretory vesicles are stably docked at the plasma membrane, where a variety of regulatory proteins orchestrate the SNARE proteins' interactions and configurations that will prime the vesicles to rapidly fuse upon calcium influx into the cell. One of the key elements that contributes to the efficient docking of secretory vesicles at the plasma membrane is the formation of nanoscale clusters of the plasma membrane SNARE protein Syntaxin-1a. This cluster formation has been shown to depend on Syntaxin's interaction with both Munc18 and phosphatidylinositol phosphates such as $PI(4,5)P_2$ and $PI(3,4,5,)P_3$. While there have been numerous studies exploring the role of the lipid environment on Syntaxin's distribution in the membrane, and Munc18's role in Syntaxin's conformation and SNARE complex assembly, the interplay of how these two factors cooperate to facilitate vesicle docking is still unclear. To explore this regulatory connectivity, we reconstituted Syntaxin in proteoliposomes and planar supported membranes of defined lipid compositions. We examined Munc18's binding and its effects on the clustering and conformation of Syntaxin on the membrane in the presence and absence of PIP_2s. We show that the lipid environment modulates Syntaxin's lateral distribution and ability to bind Munc18, which in turn modulates Syntaxin's conformation, ability to bind its partnering t-SNARE SNAP25, and engage with and fuse secretory vesicles.

REGULATION OF ATRIAL NATRIURETIC PEPTIDE SECRETION BY THE RAB3 CYCLE

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No Abstract

REGULATION OF ATRIAL NATRIURETIC PEPTIDE SECRETION BY THE RAB3 CYCLE

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No Abstract

KV2-MEDIATED PRESYNAPTIC ER/PM JUNCTIONS ORGANIZE CALCIUM SIGNALING AND REGULATE VESICLE FUSION

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Synaptic transmission demands coordination between electrical changes in membrane potential and intracellular Ca^{2+} signaling to fuse synaptic vesicles and sustain the release of neurotransmitter. The endoplasmic reticulum (ER) is the largest and most extensive Ca^{2+} storage organelle, occupying more than 10% of a neuron's volume and extending into both dendritic spines and axonal boutons. However, within the highly buffered cytosol of excitable cells, Ca^{2+} signaling is severely restricted spatially. ER junctions with the plasma membrane (PM) are critical hubs that coordinate Ca^{2+} signaling with membrane depolarization to activate several key biological processes including: myosin activation in muscle cell contraction; immediate early gene transcription in neurons; and CaMKII activation in dendritic spines. Here we provide evidence that ER/PM junctions organized by voltage-gated potassium channels (Kv2) and the ER-protein, VAP, play a key role in organizing vesicle fusion in presynaptic terminals. Here we provide evidence that Kv2-mediated ER/PM junctions organize a secondary calcium signaling pathway in the axon. This unique calcium signaling pathway strongly modulates synaptic strength and vesicle fusion by controlling vesicle participation in recycling and reserve pools of vesicles in synaptic boutons.

PIKFYVE PROMOTES CELL MIGRATION VIA CONTROL OF A NEGATIVE REGULATOR OF RAC1

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PIKfyve, a PI3P 5-kinase, provides the sole source of the signaling lipid PI(3,5)P2, as well as most PI5P. PIKfyve is known for its essential roles in lysosomal function and autophagy. However, cells with impaired PIKfyve activity have pleiotropic defects suggesting that PIKfyve regulates additional pathways from distinct locations. To uncover additional PIKfyve functions, we investigated how PIKfyve regulates cell migration. Indeed, we found that PIKfyve contributes to cell migration in part via recycling integrins from VPS35-containing endosomes to the plasma membrane, and that PIKfyve colocalizes with VPS35-containing endosomes. Here we show that PIKfyve also regulates actin dynamics in lamellipodia. Within 5 minutes of PIKfyve inhibition, there is a decrease in lamellipodia dynamics and a concomitant loss of F-actin. Furthermore, we show that following acute inhibition of PIKfyve there is a loss of active RAC1 that occurs within the same time-course as the decrease in lamellipodia dynamics. These findings suggest that PIKfyve regulates actin dynamics via regulation of RAC1. To gain further insights into how PIKfyve regulates RAC1, we performed proximity labeling. Among the top hits, we obtained a RAC1 GAP and importantly found that PIKfyve regulation of cell migration and lamellipodia dynamics requires the Rac1 GAP. In live cell imaging using TIRF microscopy, we observe dynamic VPS35-containing PIKfyve puncta within the TIRF field, which suggests that PIKfyve activity on these puncta may inhibit the RAC1 GAP, and in turn contribute to RAC1 activation. Together, these studies reveal that PIKfyve regulates cell migration via at least two pathways 1) the regulation of integrin recycling from VPS35 endosomes and 2) inhibition of a RAC1 GAP and the regulation of actin dynamics. That PIKfyve is required for at least two pathways connected to cell migration may explain in part why PIKfyve inhibition is currently in clinical trials for multiple cancers.

ENDOSOMES AND LYSOSOMES IN NEURONS: WHAT HAPPENS WHEN THEY BREAK?

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No Abstract

SORTING OF SYNAPTOTAGMINS TO THE INSULIN SECRETORY GRANULE

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No Abstract

MAKING A GRANULE: HOW PI4KII AND OSBP REGULATE SECRETORY GRANULE MATURATION

Julie Brill[1]
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No Abstract

TUNING THE SIZE OF LARGE DENSE-CORE VESICLES AND QUANTAL NEUROTRANSMITTER RELEASE VIA SECRETOGRANIN II LIQUID–LIQUID PHASE SEPARATION

Zhaohan Lin[1]; Yinglin Li[1]; Zhe Zhang[1]; **Quanfeng Zhang**[1]; Zhuan Zhou[1]
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Large dense-core vesicles (LDCVs) are larger in volume than synaptic vesicles, and are filled with multiple neuropeptides, hormones, and neurotransmitters that participate in various physiological processes. However, little is known about the mechanism determining the size of LDCVs. Here, it is reported that secretogranin II (SgII), a vesicle matrix protein, contributes to LDCV size regulation through its liquid–liquid phase separation in neuroendocrine cells. First, SgII undergoes pH-dependent polymerization and the polymerized SgII forms phase droplets with Ca^{2+} in vitro and in vivo. Further, the Ca^{2+}-induced SgII droplets recruit reconstituted bio-lipids, mimicking the LDCVs biogenesis. In addition, SgII knockdown leads to significant decrease of the quantal neurotransmitter release by affecting LDCV size, which is differently rescued by SgII truncations with different degrees of phase separation. In conclusion, it is shown that SgII is a unique intravesicular matrix protein undergoing liquid–liquid phase separation, and present novel insights into how SgII determines LDCV size and the quantal neurotransmitter release.

FINALLY VISUALIZING HOW MUNC13 OPENS SYNTAXIN-1

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Neuronal communication relies on the fusion of synaptic vesicles at nerve terminals, which results in release of neurotransmitters with strict temporal and quantal precision. The active zone protein Munc13-1 plays a central role in regulating synaptic vesicle docking and priming by catalyzing the opening of the 'closed' conformation of the SNARE protein syntaxin-1 bound to Munc18-1, and by bridging the vesicle to the plasma membrane. Syntaxin-1 opening is a central event for neurotransmission, but the underlying molecular mechanism has remained unsolved for decades due to the weak nature of the Munc13-1-syntaxin-1 interactions. Our research addresses this question by tricking AlphaFold into the prediction of the open conformation of Syntaxin-1 bound to Munc13 and experimentally validating the model by highly sensitive paramagnetic NMR spectroscopy. In the closed conformation of Syntaxin-1, its SNARE motif interacts extensively with the H_{abc} domain. As soon as we cut half of the SNARE motif to eliminate many of the interactions, AlphaFold predicted an exciting model of the open conformation of Syntaxin-1 bound to the MUN domain of Munc13-1. In support of the model, a DOTA-Gd^{3+} tag attached to cysteines placed at positions near the predicted interacting region (residues 182, 184, 60 of syntaxin-1) caused strong broadening of multiple methyl HMQC cross-peaks of the Munc13-1 MUN domain. We assigned two peaks near the 'NF' pocket of MUN domain expected to bind to syntaxin-1 by mutagenesis, and both are strongly broadened in the presence of Syntaxin-1 labeled with Gd^{3+}. These results suggest a new model of the transition from the closed to the open conformation of Syntaxin-1 whereby Syntaxin-1 residues 182-185 interact with the NF pocket of MUN. Our results revealing the Syntaxin-MUN interaction sites can be useful in designing molecules that specifically stimulate or inhibit the binding of Syntaxin to Munc13-1, thereby controlling neurotransmitter release.

STRUCTURAL BASIS OF ORGANELLE DIVISION IN MALARIA PARASITES

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No Abstract

ARCHITECTURE OF SYNAPSES REVEALED BY CRYO-ELECTRON TOMOGRAPHY

Axel Brunger[1]
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No Abstract

MOLECULAR MECHANISM FOR FERROSOME FORMATION

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Clostridioides difficile is a Gram-positive bacterium and the leading cause of nosocomial and antibiotic-associated infections in the United States. To thrive within the host, C. difficile employs specialized strategies for iron uptake, storage, and detoxification to maintain iron homeostasis. Our previous work demonstrated that C. difficile stores iron in membrane-bound ferrosome organelles, which help the bacterium evade nutritional immunity and support colonization and survival during infection. However, the molecular mechanisms underlying ferrosome biogenesis remain largely unknown. In this study, we successfully co-expressed FezA and FezB in Escherichia coli, and cryo-electron tomography revealed the formation of abundant, uniformly sized ferrosome-like vesicles. Structured illumination microscopy (SIM) further confirmed the colocalization of FezA and FezB. These findings offer important insights into the molecular basis of ferrosome formation.

SINGLE-MOLECULE DECODING OF MEMBRANE FUSION WITH SUSPENDED BILAYERS

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No Abstract

PROBING SNARE-MEDIATED VESICLE FUSION ON FREESTANDING MEMBRANES

Min Ju Shon[1]
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No Abstract

MECHANICAL FORCES IN MEMBRANE FUSION AND BULK LIPID TRANSFER

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The assembly of three synaptic SNARE proteins into a four-helix bundle drives membrane fusion by bringing vesicular and plasma membranes into close proximity. The role of SNARE transmembrane domains (TMDs) in this process remains controversial. Using high-resolution optical tweezers, we characterized the reversible folding and assembly of a single full-length SNARE complex anchored in a model membrane. Unlike the zippered TMDs observed in detergent-based crystal structures, we find that TMDs destabilize the SNARE complex. PIP2 slows SNARE zippering, whereas complexin-1, a critical SNARE-binding protein, significantly accelerates it, enhancing neurotransmitter release. In contrast to SNARE-mediated quantal lipid trafficking, bridge lipid transfer proteins (BLTPs) facilitate bulk lipid transport as continuous lipid conduits, supporting lipid homeostasis and organelle dynamics. We propose lipid osmosis as a novel biophysical mechanism generating membrane tension to drive bulk lipid flow, potentially regulating membrane expansion during vesicle and autophagosome formation.

SNARE-MEDIATED MEMBRANE FUSION - WHERE ARE THE ENERGY BARRIERS?

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No Abstract

POSTER ABSTRACTS

Monday, October 6
POSTER SESSION I
2:45 PM – 4:15 PM

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. Presenting authors with odd-numbered poster boards should present from 2:45 – 3:30 PM and those with even-numbered poster boards should present from 3:30 PM – 4:15 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 2:45 – 3:30 PM | Even-Numbered Boards 3:30 – 4:15 PM

An, Dong	**1-POS**	**Board 1**
Cha, Minkwon	**2-POS**	**Board 2**
Dutta, Mandira	**5-POS**	**Board 5**
Eitel, Anna	**6-POS**	**Board 6**
Jones-Nelson, Seraya	**9-POS**	**Board 9**
Kumar, Avinash	**10-POS**	**Board 10**
O'Shaughnessy, Ellen	**13-POS**	**Board 13**
Paton, Cameron	**14-POS**	**Board 14**
Riccio, Amanda	**17-POS**	**Board 17**
Rowan, Fiona	**18-POS**	**Board 18**
Vacha, Robert	**21-POS**	**Board 21**
Wehman, Ann	**22-POS**	**Board 22**

Posters should be set up on the morning of Monday, October 6 and removed by 4:30 PM on Tuesday, October 7. All uncollected posters will be discarded.

1-POS Board 1

REGULATION OF SNARE-MEDIATED FUSION BY SYNAPTOTAGMIN AND COMPLEXIN ISOFORMS REVEALED BY MARTINI SIMULATIONS

Dong An[1]; Satyan Sharma[2]; Manfred Lindau[1]
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Neurotransmitter release via synaptic vesicle–plasma membrane fusion is driven by SNARE proteins (Synaptobrevin-2 (Syb2), Syntaxin (Stx), and SNAP-25) and regulated by accessory proteins including Synaptotagmin (Syt), Complexin (Cpx), Munc13, and Munc18. Despite extensive experimental study, the molecular mechanisms and dynamics underlying SNARE-mediated fusion remain incompletely understood due to nanometer and millisecond resolution limits. Here, we used coarse-grained molecular dynamics (CGMD) simulations to investigate how Syt1 and Cpx isoforms regulate SNARE-mediated membrane fusion, particularly in response to Ca^{2+} binding. To mimic Ca^{2+}-bound Syt1 in the MARTINI2 force field, we introduced charge-flip mutations (D303K, D309K, D363K, D365K, D371K) into the Syt1 C2B domain, exaggerating electrostatic effects to emulate $Ca2^{+}$ accumulation at PIP2-rich membranes. In fusion pore (FP) simulations using a 10-nm nanodisc and a plasma membrane bridged by four SNARE complexes, Syt1 C2B consistently delayed FP opening at both primary and tripartite interfaces. Notably, Ca^{2+}-dependent modulation of FP formation was observed only at the tripartite interface with Cpx1 accessory helix (AH, residues 27–47) and central helix (CH residues 48–70). In contrast, Cpx2 AH and CH failed to modulate FP formation in a Ca^{2+}-dependent manner. Backbone contact analysis revealed that only Cpx1 exhibited calcium-dependent changes in AH–Syb2 and AH–Stx1A interactions, likely due to greater AH flexibility supported by atomistic simulations. The increases of AH–Syb2 and AH–Stx1A contact distances also resulted from lipid binding by Cpx1 AH in the Ca^{2+}-free C2B state, which disappeared in the Ca^{2+}-bound mimic. No calcium-dependent differences were observed in CH–SNARE contacts for either isoform. These findings highlight isoform-specific roles of Syt1 C2B and Cpx1 in modulating SNARE conformational dynamics and fusion pore behavior, offering new insights into Ca^{2+}-sensitive neurotransmitter release. Cpx2 may play alternative roles beyond membrane fusion. Supported by NIH grant R35GM139608.

2-POS Board 2

CONTROL OF SNARE-DRIVEN VESICLE FUSION BY SYNAPSIN CONDENSATES ON FREESTANDING MEMBRANES

Minkwon Cha[1,2]; Jaehyeon Shin[1]; Hyun-Ro Lee[3]; Taehyun Yang[1]; Eunji Cho[4]; Sunghoe Chang[4]; Seung Soo Oh[3,5]; Min Ju Shon[1,5,6]
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[5]Yonsei University, Institute for Convergence Research and Education in Advanced Technology (I-CREATE), Seoul, South Korea; [6]Pohang University of Science and Technology (POSTECH), School of Interdisciplinary Bioscience and Bioengineering, Pohang, South Korea

Single-molecule observations of membrane protein dynamics have long been challenging due to the limitations of traditional model membrane systems. Here, we developed a simple method to prepare freestanding lipid bilayers (FLBs) for studying protein diffusion and clustering. An array of large-area, protein-embedded membrane patches were stably formed on an electron microscopy grid for high-resolution fluorescence imaging. Applying this technique to SNARE-driven vesicle fusion, the core mechanism for synaptic vesicle release, we dissected vesicle docking and membrane fusion activities through diffusion-based analysis at the single-particle level, revealing distinct intermediate species on membranes. Upon adding complexin and synapsin, well-established regulators of the synaptic vesicle cycle, we observed their unique effects on the membrane-bound populations. Notably, synapsin condensates not only clustered VAMP2-containing vesicles but also engaged closely with the target membrane, releasing the vesicles for immediate fusion upon dispersal. This suggests a more direct role for synapsin in vesicle release than previously assumed. The FLB platform enables the study of diverse protein–membrane activities, with future applications in membrane deformation and tension.

5-POS Board 5

LIPID-REGULATED CONFORMATIONAL SWITCHING OF SARS-COV-2 M PROTEIN CONTROLS VIRAL ASSEMBLY

Mandira Dutta[1]; Kimberly A Dolan; Souad Amiar; Elijah J Bass; Rokaia Sultana; Gregory A Voth[1]; Stephen G Brohawn; Robert V Stahelin
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The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had profound consequences for global health and the global economy, prompting unprecedented worldwide research efforts. The SARS-CoV-2 virion comprises four primary structural proteins: Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N). Among these, the M protein is the most abundant and plays a critical role in virion assembly and budding. M adopts two conformations: M-short and M-long, and a regulated transition between these states is believed to coordinate the viral assembly process. However, the molecular mechanisms underlying this conformational switch remain poorly understood. Experimental studies have shown that M directly binds to Golgi-localized anionic lipids, particularly ceramide-1-phosphate (C1P), to regulate its structural dynamics. Using explicit atomistic molecular dynamics simulations, we demonstrate that C1P binding promotes the transition from M-long to M-short. To characterize the thermodynamics of this process, we employed Metadynamics to explore the free energy landscape. Our results reveal that M-short corresponds to a lower free energy state than M-long, independent of lipid composition. Notably, in the presence of C1P, we observe direct binding of C1P to M, which further stabilizes the M-short conformation by deepening its free energy minimum. These findings suggest that the M-long to M-short transition follows an induced-fit mechanism facilitated by C1P. Supporting this, cryo-EM structures show that C1P engages a conserved binding pocket that bridges the transmembrane and cytoplasmic regions of M in its M-short conformation. To further investigate the large-scale organization and dynamics of M within membranes, we developed a bottom-up coarse-grained model of the M protein-lipid system, enabling the study of their collective behavior and functional roles in viral assembly.

6-POS Board 6

MOLECULAR MECHANISM OF GBETA/GAMMA-SNARE MEDIATED INHIBITION OF SYNAPTIC VESICLE FUSION

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G-protein βγ heterodimers (Gβγ) liberated upon activation of presynaptic inhibitory G-protein Coupled Receptors ($G_{i/o}$ GPCRs) inhibit neurotransmission downstream of Ca^{2+} influx through direct interactions with the ternary N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. The neuronal ternary SNARE complex is composed of synaptosomal-associated protein, 25kDa (SNAP-25), syntaxin-1A, and synaptobrevin-2. Previous work from the Hamm lab identified regions of both Gβγ and tSNARE that are critical for the interaction, but a sufficient understanding of the molecular mechanism remains elusive due to lack of structural data for the complex. To address this, we have expressed and purified a pre-fusion ternary SNARE mimetic containing a C-terminal truncation of synaptobrevin-2 which prevents full zippering of the SNARE complex. This partially zippered SNARE construct has a higher affinity for Gβ1γ2 than the fully zippered version as determined by microscale thermophoresis (MST).

We hypothesize that Gβγ disrupts incorporation of the C-terminal region of full length synaptobrevin into the fully zippered SNARE complex, leading to a block of exocytosis. To investigate the interaction further, we stabilized the Gβ1γ2-pre-fusion SNARE complex using crosslinking and structurally characterized this complex using single particle cryo-EM. Our preliminary cryo-EM density map suggests that the N-terminal coiled-coil of Gβγ interacts at the C-terminus of the SNARE complex. We used Chai-1 and Rosetta docking to predict the structure of the Gβ1γ2 N-coiled-coil domain bound to the C-terminus of tSNARE. This structural prediction suggests that the N-termini of both Gβ1 and Gγ2 form an interface with the C-terminal helix of SNAP-25, and that Gγ2 inserts into the C-terminal SNARE helical bundle. Our combined results suggest a molecular mechanism in which Gβγ disrupts SNARE complex zippering by steric hindrance of synaptobrevin binding, thereby inhibiting vesicle fusion. These data provide a roadmap for future experiments to further elucidate the molecular details of the interface.

9-POS Board 9

STRUCTURAL BASIS OF DYNAMIN REGULATION BY AMPHIPHYSIN DURING ENDOCYTOSIS

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The extra- and intracellular eukaryotic membranes undergo constant membrane remodeling, including concerted cycles of fission and fusion events, essential for critical biological processes such as intracellular trafficking and endocytosis. Endocytosis is important for nutrient uptake, receptor recycling, and cell signaling - all fundamental for eukaryotic life. The membrane fission protein, dynamin, catalyzes the scission of endocytic vesicles from plasma membranes. While work over the last decades has elucidated the structure-mechanism of dynamin, the structural basis of dynamin regulation by other endocytic proteins remains elusive. Amphiphysin is an endocytic protein with a Bin/amphiphysin/Rvs (BAR) domain that mediates the invagination and fission steps of vesicles by sensing or facilitating membrane curvature and stimulating the GTPase activity of dynamin. Amphiphysin also plays a role in recruiting dynamin to endocytic sites during fission and in regulating dynamin's GTPase activity. Despite this, the structural mechanism underlying their interaction and the regulation of dynamin's GTPase activity by amphiphysin is poorly understood. Towards understanding the structural basis of amphiphysin interaction with and regulation of dynamin, I am using a cryo-EM approach to determine the structure of the complex on lipid tubules, mimicking their organization on the neck of budding endocytic vesicles. I have determined that both are functional with amphiphysin binding lipids and dynamin having GTPase activity, binding lipids and tubulating liposomes. I have determined that both proteins interact directly and currently optimizing formation of dynamin-decorated lipid tubules, which will be used for complex formation with amphiphysin, for cryo-EM studies. The cryo-EM structure of dynamin and amphiphysin assembled on lipid membranes will provide critical insights into the molecular mechanism of amphiphysin/dynamin-mediated fission, a key step in endocytosis.

10-POS Board 10

MEMBRANE-MEDIATED MODULATION OF SNARE COMPLEX ASSEMBLY AND DISASSEMBLY DYNAMICS

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Membrane fusion is a fundamental cellular process driven by the assembly of SNARE proteins into a stable four-helix bundle. While the structure and behavior of the soluble SNARE complex have been extensively characterized, the specific contributions of the transmembrane domains (TMDs) of Syntaxin and VAMP2 remain less well understood. In this study, we investigate how the TMDs influence SNARE complex assembly and disassembly at the membrane interface. Utilizing high-resolution optical tweezers, we manipulated individual SNARE complexes reconstituted on small unilamellar vesicles (SUVs), enabling us to resolve unfolding intermediates and quantify disassembly kinetics under applied force. Surprisingly, we found that the presence of the lipid membrane destabilizes the SNARE complex, an effect not observed when the complex is pulled in the absence of membrane anchoring. Our data also show that mutations in the linker domains (LDs) of SNARE proteins significantly reduce the stability of the C-terminal domain (CTD), suggesting a functional coupling between the LD and CTD. Furthermore, we observed a significant decrease in SNARE assembly kinetics in the presence of PIP2, indicating that the LDs interact strongly with membrane lipids. Overall, these findings provide new insights into how membrane interactions regulate the mechanical behavior of SNARE complexes and contribute to the control of membrane fusion.

13-POS Board 13

TOWARD BUILDING A BIOPHYSICAL MODEL OF MEMBRANE EXPANSION DURING NEURONAL MORPHOGENESIS

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During development neurons progress through a series of morphological stages, increasing dramatically in both surface area and complexity. The rapid expansion of surface area requires the delivery of large amounts of membrane that we hypothesize is achieved through exocytosis. Through direct visualization of exocytosis in developing cortical neurons we find high rates of exocytosis in the soma, and low rates in the tips of growing neurites. This finding presents an interesting conundrum: how is membrane delivered to the tips of growing neurites where it is needed when the majority of this material is supplied in the soma? To address this fundamental question in neuronal development we seek to build a biophysical model of the plasma membrane and its remodeling through direct visualization of dynamics, composition, and tension. To measure membrane dynamics we have developed markers for the inner leaflet (Lyn11-Halo and Halo-CAAX), the outer leaflet (SNAP-GPI) and an extracellular transmembrane probe (Halo-PDGFRtm). Using these tools we perform photoactivated localization microscopy and single particle tracking (SPT-PALM) to quantify the movement (diffusion and/or flow) of the membrane over developmental time. Our preliminary results indicate that movement of the membrane is subdiffusive and faster on the inner leaflet compared with a PM-spanning probe. To measure membrane tension throughout development we have employed the FLIM-based sensor, FlipperTR. Our preliminary data indicate that PM tension is higher in neurites than the soma and potentially at neurite branch points relative to the soma. These empirical data will inform a predictive computational model of morphogenesis with the ultimate goal of building a biophysical model of how the membrane is remodeled during neuronal development.

14-POS Board 14

ER-RESIDENT TETHERING PROTEIN, VAP, REGULATES PRESYNAPTIC ORGANELLE CALCIUM HANDLING AND SYNAPTIC TRANSMISSION.

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Membrane contact sites (MCS) are a common structural feature in cell biology with both physiological and pathological implications. They exist in even the most polarized subcellular compartments, including the presynaptic terminal of neurons, where they play critical roles in intracellular communication. We recently demonstrated that dynamic uptake of calcium (Ca^{2+}) into the presynaptic endoplasmic reticulum (ER) during electrical activity relies on MCS between the ER and plasma membrane formed by voltage-gated potassium channel, Kv2.1. Interestingly, this uptake is not a result of potassium conduction. Rather, it occurs through a noncanonical FFAT motif contained within Kv2.1's C-terminus. Loss of the Kv2.1 C-terminus impairs neurotransmission and ER Ca^{2+} uptake by more than 50%. Previous studies in heterologous cells suggest that Kv2.1 forms ER/PM MCSs through interaction with VAMP-associated proteins (VAPs) embedded in the ER membrane. To further investigate VAP's molecular function in the axon, we developed a CRISPR-based knockdown (KD) approach to acutely deplete both isoforms of VAP (VAPA and VAPB) in primary hippocampal neurons. We combined this knockdown approach with noninvasive optical indicators of synaptic vesicle exocytosis and subcellular Ca^{2+} in cultured primary rat hippocampal neurons. We demonstrate that loss of VAPA/B decreases vesicle fusion (>50%), disrupts the formation of Kv2.1 clusters, and impairs Ca^{2+} influx during electrical activity within both mitochondria (>75%) and the endoplasmic reticulum (>50%). Impaired synaptic transmission by loss of VAP is caused by change in vesicle distribution between the recycling and reserve pools of synaptic vesicles suggesting a new presynaptic role for MCS mediated by VAP in synaptic transmission.

17-POS Board 17

A MALARIA PARASITE PROTEIN FORMS FILAMENTOUS SPIRALS THAT CATALYZE THE DIVISION OF MULTIPLE-MEMBRANE BOUND ORGANELLES

Amanda Riccio[1]; Abdur Rahman [1]; MacKenzie Cassity [1]; Erik Zupa[1]; Jonathan Bouvette [1]; Venu Gopal Vandavasi[2]; Philip Mwendwa[1]; John R Jimah[1]
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Plasmodium falciparum, the parasite responsible for over 200 million malaria infections and approximately 600,000 deaths annually, relies on rapid and highly coordinated cell division for its propagation and infectivity. Division of the parasite relies on the division of the parasite's single-copy endosymbiotic organelles, the mitochondrion and apicoplast, and their distribution to daughter parasites. While the division of the apicoplast, a unique, quadruple-membrane bound plastid organelle is essential for Plasmodium replication and infection, the molecular mechanism(s) governing apicoplast division remain poorly understood. Here, we employ single-particle cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET) to characterize structural states of a key fission protein involved in apicoplast division. This protein contains a distinctive putative intrinsically disordered region with lipid-binding capacity, facilitating its interaction with organellar membranes, assembles as filamentous spiral scaffold on membranes and catalyzes membrane fission. Our structural analyses provide insight into the dynamic cellular environment of a replicating parasite. In addition to identifying conformational states in vitro, we investigated the in vivo significance of these structures, revealing functional impairments in protein-lipid interactions and overall parasite segmentation and fitness in parasites expressing proteins that had key residues mutated. These findings contribute to our understanding of the molecular machinery underpinning apicoplast division.

18-POS Board 18

INVESTIGATING DETERMINANTS OF MYOMAKER FUNCTION IN CELL-CELL FUSION

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Skeletal myofibers acquire nuclei from the fusion of mononucleated muscle progenitors during development and regeneration. The identification of myomaker as a muscle-specific fusion factor necessary for mediating the mixing of apposing lipid bilayers (hemifusion) significantly advanced knowledge of how skeletal muscle undergoes myonuclear accrual. However, a detailed mechanistic understanding regarding how myomaker functions to mediate lipid mixing remains limited with understanding particularly confounded by its structural divergence from canonical fusion proteins. Myomaker structure consists of seven-pass transmembrane domains and a short ectodomain which suggests that it cannot mechanically mediate lipid mixing of trans bilayers and leads us to hypothesize that it may utilize its cis lipid environment to initiate fusion. For this, we have previously sought to identify structural features necessary for myomaker function and found that C-terminal palmitoylation, a modification often associated with membrane affinity and localization, is required for fusion. In this study, we aimed to further investigate how palmitoylation state impacts functionality particularly through how it may alter the local lipid environment around myomaker. To address this question, we utilized mass spectrometry to compare lipids derived from viral particles pseudotyped with fusogenic myomaker and a non-fusogenic palmitoylation variant and found neutral lipid enrichment surrounding fusogenic myomaker. Additionally, we found that increasing synthesis of neutral lipids enhances fusion mediated by the muscle fusogens without altering fusogen levels. These data suggest that neutral lipids may have a critical role in how myomaker mediates lipid mixing and overall provide insight into a novel means by which myomaker may harness lipids within its environment to catalyze muscle fusion through a unique pathway.

21-POS Board 21

NANOPARTICLE INDUCED FUSION OF LIPID MEMBRANES

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Membrane fusion is crucial for infection of enveloped viruses, cellular transport, and drug delivery via liposomes. Nanoparticles can serve as fusogenic agents facilitating such membrane fusion for direct transmembrane transport. However, the underlying mechanisms of nanoparticle-induced fusion and the ideal properties of such nanoparticles remain largely unknown. We used molecular dynamics simulations to investigate the efficacy of spheroidal nanoparticles with different size, prolateness, and ligand interaction strengths to enhance fusion between vesicles. By systematically varying nanoparticle properties, we identified how each parameter affects the fusion process and determined the optimal parameter range that promotes fusion. These findings provide valuable insights for the design and optimization of fusogenic nanoparticles with potential biotechnological and biomedical applications.

22-POS Board 22

LIPID ASYMMETRY IS PROGRESSIVELY LOST DURING SPERMATID BUDDING

Katherine Maniates[2]; Saai Suryanarayanan[2]; Alissa Rumin[3]; Andrew Singson[2]; **Ann M. Wehman**[1]
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During fertilization, sperm and egg membranes signal and fuse to form a zygote and begin embryonic development. We investigated the role of lipid asymmetry in gametogenesis, fertilization, and embryogenesis. We find that phosphatidylethanolamine asymmetry is lost during meiosis as spermatids bud from the residual body in C. elegans and that phosphatidylethanolamine exposure occurs prior to phosphatidylserine exposure. We show that TAT-5, the P4-ATPase that maintains phosphatidylethanolamine asymmetry, is required for both oocyte formation and sperm activation, albeit at different levels of lipid flippase activity. Loss of TAT-5 significantly decreases fertility in both males and hermaphrodites and decreases sperm activation. TAT-5 localizes to the plasma membrane of primary spermatocytes but is sorted away from maturing spermatids during meiosis. Our findings demonstrate that phosphatidylethanolamine asymmetry plays key roles during gametogenesis and sperm activation, expanding the roles of lipid dynamics during meiotic cell division and cell fusion.

Tuesday, October 7
POSTER SESSION II
2:35 PM – 4:05 PM

Posters are available for viewing only during their scheduled date of presentation. Below are the formal presentation times. Presenting authors with odd-numbered poster boards should present from 2:35 – 3:20 PM and those with even-numbered poster boards should present from 3:20 – 4:05 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 2:35 – 3:20 PM | Even-Numbered Boards 3:20 – 4:05 PM

Cortez, Monica	**3-POS**	**Board 3**
Dey, Himani	**4-POS**	**Board 4**
Gordon, Lila	**7-POS**	**Board 7**
Heldwein, Ekaterina	**8-POS**	**Board 8**
Li, Andy Yu Zhi	**11-POS**	**Board 11**
Malik, Sheeba	**12-POS**	**Board 12**
Planitzer, Steven	**15-POS**	**Board 15**
Rahman, Abdur	**16-POS**	**Board 16**
Ryken, Samantha	**19-POS**	**Board 19**
Tankard, Amari	**20-POS**	**Board 20**
Wherley, Tanner	**23-POS**	**Board 23**
Zupa, Erik	**24-POS**	**Board 24**

Posters should be set up on the morning of Monday, October 6 and removed by 4:30 PM on Tuesday, October 7. All uncollected posters will be discarded.

3-POS Board 3

ENDOSOMAL FUSION OF SARS-COV-2 MEDIATED BY TMPRSS2-CLEAVED SPIKE GLYCOPROTEIN

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SARS-CoV-2 entry depends on binding of its Spike (S) glycoprotein to host-cell receptor, angiotensin converting enzyme 2 (ACE2). A subsequent cleavage event at the site denoted S2' mediated by cell surface Transmembrane Serine Protease 2 (TMPRSS2) is crucial for rendering S fusion competent. In the absence of TMPRSS2, S cleavage is mediated by endosomal cathepsins (Cat L/B) that drive virus entry through an endosomal pathway. Here, we sought to characterize the role of TMPRSS2 in regulating SARS-CoV-2 entry site preference. We hypothesize that the expression of TMPRSS2 and the competing rate of virus uptake are the main determinants of the site of SARS-CoV-2 entry. A bulk virus-cell fusion assay showed that TMPRSS2 expression accelerates SARS-CoV-2 fusion, and as expected, TMPRSS2 inhibitors blocked virus fusion. By contrast, a pan-cathepsin inhibitor E64d was without effect on viral fusion. To assess whether expression of TMPRSS2 leads to direct fusion with the plasma membrane, we tracked entry of single SARS-CoV-2 pseudoviruses co-labeled with fluorescent markers of viral core, a releasable marker, and lipophilic membrane dye. Single virus particle fusion is manifested by release of a viral content marker, while the redistribution of the viral membrane marker allows pinpointing the sites of viral fusion. Retention of the lipophilic dye corresponds to fusion with the endosomal membrane, whereas loss indicates viral fusion with the plasma membrane. Surprisingly, we found that a large fraction of SARS-CoV-2 fusion (38%) occurred in endosomes of ACE2 and TMPRSS2 expressing A549 cells, with comparable kinetics between plasma membrane fusion and virus endocytosis. Future studies will investigate whether the competing rates of viral fusion and endocytosis modulate SARS-CoV-2 sensitivity to neutralizing antibodies and explore entry of variants of concern. This work was supported by the NIH R37 AI150453 grant to GBM.

4-POS Board 4

CALCIUM-BINDING PROTEIN 5 (CABP5) MODULATES NEUROTRANSMITTER RELEASE IN ROD BIPOLAR CELLS.

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CaBP5, a calmodulin-like Ca^{2+} binding protein, is expressed in rod bipolar cells (RBCS), where it has been proposed to regulate neurotransmitter release. To test this hypothesis, we compared spontaneous and stimulus-evoked release in retinal slices from RBC terminals in wild-type (WT) and CaBP5$^{-/-}$ mice. Spontaneous release events, recorded in postsynaptic AII amacrine cells, were detected in both WT and CaBP5$^{-/-}$ mice. The amplitude distribution of spontaneous events in WT mice revealed a major peak at ≈7pA, while in CaBP5$^{-/-}$ there was a rightward shift in the amplitude distribution suggestive of increased multivesicular release. By contrast, there was no difference in the amplitude of a flash-evoked EPSC. In isolated RBCs, exocytosis was evoked by a train of depolarizing stimuli that depleted and tracked the recovery of the RRP and was monitored using membrane capacitance measurements. No difference was observed in the response to the first pulse in the train, indicating that the magnitude of the rapidly-releasable vesicle pool (RRP) was not impacted by CaBP5 loss. However, the capacitance response as a function of pulse number showed a steeper rise in the CaBP5$^{-/-}$ RBCs, indicative of faster RRP replenishment. Given that RBC SNARE complexes contain Syntaxin3B and that the formation of Syntaxin3B-containing SNARE complexes and SNARE-mediated fusion is facilitated by the Ca^{2+}-dependent phosphorylation of Syntaxin3B-T14, we used an immunocytochemical approach to determine whether the absence of CaBP5 alters Syntaxin3B-T14 phosphorylation. Results revealed enhanced Syntaxin3B-T14 phosphorylation in CaBP5$^{-/-}$ RBC synaptic terminals relative to WT. Together, our results show that CaBP5 modulates vesicle fusogenicity and neurotransmitter release at RBC synaptic terminals. Furthermore, results suggest that CaBP5 regulates the transition of vesicles to the fusion-competent state, at least in part, via its ability to modulate Syntaxin3B-T14 phosphorylation.

7-POS Board 7

DYNAMIC ANALYSIS OF RAB GTPASES AND EFFECTORS IN DENSE CORE VESICLE DOCKING

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Cells such as neurons, neuroendocrine, and immune cells require expeditious intercellular communication in order to convey information about their internal and external environment to downstream neighbors.Within these cells, vesicles envelop relevant cues and then undergo calcium-triggered exocytosis, which is fast, ubiquitous, and reliant on a conserved protein machinery. Before exocytosis, or fusion of the vesicle with the plasma membrane occurs, vesicles undergo multiple states of maturation including arrival at the membrane and docking. While many proteins involved in docking have been identified via biochemical methods and fixed-cell imaging, the timing of the association and its effects on docking is unknown. Understanding the dynamics of protein machinery involved in vesicle docking is key to this project's exploration, so I used TIRF microscopy to visualize vesicles within 200nm of the membrane. TIRF microscopy is a highly resolved imaging technique that is compatible with live-cells.The present work investigates the role that interactions between Rab GTPases and effectors play in stereotyped vesicle docking. I've chosen to study two Rab GTPases and three effectors because understanding the timing and functional differences as they occur within protein classes can elucidate the role these proteins have in vesicle docking overall. Exocytosis via dense core vesicles is a critical means of cellular communication and understanding the proteins involved gives insight into how it occurs normally, and how it can be remedied in disease-states. Further, the present work includes the development of dynamic analysis pipeline for two-color TIRF movies, which can be applied not only to other proteins, but also to other vesicular behaviors.

8-POS Board 8

ER CHLORIDE CHANNEL CLCC1 PROMOTES NUCLEAR ENVELOPE FUSION IN HERPESVIRUSES AND THEIR HOSTS.

Bing Dai[1]; Adrian W Sperl[1]; Lucas Polack[1]; Isabel Mejia[2]; Haley Dame[1]; Tien Huynh[1]; Chloe Deveney[1]; Nathalie Lavoie[1]; Chanyoung Lee[1]; John G Doench[3]; Matthew D Daugherty[2]; **Ekaterina Heldwein**[1]
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Herpesvirales are a diverse and ancient viral order capable of lifelong infection across a broad range of hosts, from mollusks to humans. All known members share a unique nuclear egress pathway in which capsids bud through the inner nuclear membrane (INM) and subsequently fuse with the outer nuclear membrane (ONM) to reach the cytoplasm. This study aimed to identify host factors involved in the membrane fusion step of herpes simplex virus 1 (HSV-1) nuclear egress and to explore their evolutionary and functional relevance across the Herpesvirales. We performed a whole-genome CRISPR-Cas9 knockout screen in HSV-1-infected human cells to identify host genes that facilitate nuclear egress. Functional studies in infected and uninfected cells assessed candidate gene roles. Comparative genomic analyses traced the evolutionary origin of identified genes, and structural modeling was used to predict molecular mechanisms. Results CLCC1, previously proposed to encode an ER chloride channel, emerged as the top hit. We show that CLCC1 is important for the fusion step of HSV-1 nuclear egress and is also required for efficient replication of closely related HSV-2 and pseudorabies virus, suggesting a broader role. In uninfected cells, CLCC1 loss caused perinuclear blebbing, indicating a role in nuclear envelope fusion or nuclear pore complex insertion. Homologs of CLCC1 were identified in several Herpesvirales genomes, acquired independently via horizontal gene transfer from metazoan hosts. Structural modeling suggests CLCC1 oligomerizes into membrane-associated rings that promote membrane remodeling and fusion. CLCC1 is a critical host factor for HSV-1 nuclear egress and likely participates in a conserved membrane fusion pathway. Its independent acquisition by multiple viral lineages supports the model that Herpesvirales co-opted an ancient host fusion mechanism for replication. We propose that CLCC1 ring oligomers mediate nuclear membrane fusion in both cellular and viral contexts.

11-POS Board 11

BIOALCHEMY!

Andy Yu Zhi Li; Jack W Szostak[1,2]
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How exactly did life begin in the Universe? Is it possible to repeat that origin-of-life process in the lab? Experimentally demonstrating the transition from non-life to life, bioalchemy, is one of the ultimate goals of my research. I study oleic acid protocells which can grow, divide, fuse, and perform endocytosis. Although these protocells are not yet alive, they provide a good model system for studying cellular biophysics. Characterizing these protocells and combining them with genomic material will propel us closer to the elusive goal of understanding how life first arose.

12-POS Board 12

SIMULATION AND GENERALIZED LANGEVIN EQUATION STUDY OF LIPID SUBDIFFUSION IN BIOMEMBRANE PHASES

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In biomembranes, lipid mobility exhibits deviations from the classical diffusive behavior of Brownian particles namely, anomalous diffusion. A key question is how this anomalous behavior varies across gel, ripple, and fluid biomembrane phases. In this study, we perform all-atom molecular dynamics (MD) simulations of dimyristoylphosphatidylcholine (DMPC) bilayers in these three phases and analyze the results using the Generalized Langevin Equation (GLE) framework. This approach emphasizes sub-diffusive behavior on short, picosecond-to-nanosecond timescales, capturing local molecular constraints and transient caging effects during the crossover from vibrational to incipient anharmonic motion. The ripple and gel phases are found to exhibit strong transient caging and prolonged memory effects, resulting in distinct sub-diffusive behavior. We also examine the role of hydrogen bonding in lipid confinement, demonstrating its influence on phase-dependent molecular ordering and short-time diffusional constraints. These findings highlight the utility of the GLE framework in characterizing molecular transport and lipid dynamics, with implications for understanding longer-timescale membrane behavior.

15-POS Board 15

INFLUENZA A NEURAMINIDASE ACTIVITY ALTERS MEMBRANE FUSION IN A RECEPTOR-DEPENDENT MANNER

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Influenza A virus' cell entry pathway begins with engagement of sialic acid receptors on the cell by viral surface protein hemagglutinin (HA) leading to endocytosis. In the endosome, a cluster of HA trimers mediates membrane fusion by a series of conformational changes. In contrast, another viral surface protein, neuraminidase (NA), destroys sialic acid receptors, permitting viral mobility on the cell surface during entry and later efficient release of newly assembled virions. How the functional balance between receptor binding by HA and receptor destruction by NA regulates fusion in the endosome has not been defined. To investigate viral membrane fusion, we employ a novel flow-cytometry assay using cells and synthetic liposomes. Large numbers of virus-target pairs in suspension are sampled individually in rapid succession, allowing robust measurement of fusion at much higher throughput than is possible in imaging-based single-particle fusion assays. We observe that decreasing receptor density on liposomes below a threshold value strongly inhibits fusion of bound virions. On erythrocyte ghosts, receptor reduction attenuates fusion, and this attenuation is partially rescued by inhibition of viral NA. We also observe a dose-dependent promotion of fusion by the NA-inhibitor oseltamivir on synthetic liposomes whereas fusion to the surface of mammalian epithelial cells is promoted by NA-inhibition before but not after desialylation. The importance of HA/NA functional balance for membrane fusion may thus depend strongly on the receptor density or complexity of a given biological context. Ongoing work investigates the effect of HA/NA functional balance on fusion within the endosomes of cultured cells. A deeper understanding of the contributions of receptor-binding and -destroying activities to endosomal membrane fusion will not only provide a more complete understanding of the mechanisms of action of modern anti-influenza therapeutics but will also inform future antiviral strategies.

16-POS Board 16

INVESTIGATING THE MOLECULAR MECHANISMS OF ORGANELLE FISSION IN MALARIA PARASITES

Abdur Rahman[1]; Amanda A Riccio[1]; MacKenzie Cassity[1]; John Jimah[1];
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Malaria, caused by Plasmodium parasites, remains a significant global health problem. Disease severity is linked to the ability of the parasite to replicate within host cells. Central to this process is the division of key organelles, including the apicoplast and mitochondria. However, the structure-function-mechanisms of proteins involved in the membrane remodeling remain largely unknown. To investigate membrane remodeling and organelle division, we combined structural and cellular approaches by performing cryo-EM alongside engineering transgenic malaria parasites for in vivo studies. In this study, we present a high-resolution single-particle cryo-EM structure of a novel membrane division protein that assembles into a linear homooligomeric. We also developed a conditionally expressed GFP-tagged proteins in the Plasmodium parasite strains using CRISPR-Cas9 technology to provide insight into membrane/organelle targets and the role of the protein within the parasite. Overall, our studies begin to identify Plasmodium proteins and their molecular mechanisms of membrane fission.

19-POS Board 19

CALCIUM REGULATED PLASMA MEMBRANE EXPANSION AND REMODELING DURING NEURONAL MORPHOGENESIS

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The plasma membrane (PM) of a developing neuron undergoes dramatic expansion and remodeling to establish the morphology necessary for neural network connectivity and function. New membrane material is inserted via SNARE-mediated exocytosis, which is required for neuronal morphogenesis. Two SNARE proteins, Vesicle-associated membrane protein (VAMP) 2 and VAMP7, are enriched in the embryonic brain and associated with distinct vesicle populations prior to synapse formation. While knockout studies in mice have shown that both VAMP2 and VAMP7-mediated vesicle fusion are required for proper neuronal morphogenesis, the mechanisms regulating the trafficking and fusion of these vesicles during development is not known. Here we utilize TIRF microscopy to image components of PM remodeling in developing neurons. Automated image analysis allows for the unbiased analysis of spatial and temporal parameters of PM remodeling to reveal their role in neuronal development. We show that VAMP2 and VAMP7 mediate non-synaptic exocytic events that cluster in different areas of the developing neuron, suggesting distinct regulatory pathways dictating their distribution and fusion. Additionally, my preliminary data suggest that VAMP2-mediated exocytosis is sensitive to Ca^{2+} chelation, whereas VAMP7-mediated exocytosis is not, mirroring the differential Ca^{2+} sensitivity of VAMP2 and VAMP7 vesicle pools observed at the synapse of mature neurons. This suggests a potential role for Ca^{2+} signaling in the regulation of VAMP2 and VAMP7-mediated exocytosis during development. Endoplasmic reticulum (ER)-PM membrane contact sites, key regulators of Ca^{2+} dynamics, may play a role in the regulation of exocytosis through Ca^{2+} signaling and provide membrane material through non-vesicular lipid transfer. We show that ER-PM contact sites in developing neurons are dynamic and form in both the soma and the growth cone. Ongoing studies investigate the relationship between Ca^{2+} signaling, SNARE-mediated exocytosis, and ER-PM contact sites and how they contribute to neuronal shape change during development.

20-POS Board 20

INVESTIGATING THE MOLECULAR MECHANISM OF A MEMBRANE FISSION GTPASE KEY TO SECRETORY ORGANELLE BIOGENESIS IN TOXOPLASMA PARASITES

Amari Tankard; Jennifer Nwokeji[1]; John R Jimah[1]
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Membrane remodeling is indispensable across eukaryotic cells, from singular celled parasites to human cells, for diverse cellular processes including exocytosis and endocytosis, membrane fission and fusion, and membrane repair. Central to the pathogenicity of the single-cell parasite Toxoplasma gondii, responsible for the toxoplasmosis infections of one-third of the global population, are specialized secretory organelles—micronemes and rhoptries— critical for host-cell invasion and propagation. A parasite protein predicted to be a GTPase and related to dynamin superfamily proteins has been implicated in secretory organelle biogenesis, but the molecular mechanism and structural basis of its function is largely unknown. Here, we determined the cryo-EM structure of this parasite GTPase, elucidating the protein architecture and revealing its role in membrane dynamics. Our biochemical and biophysical characterization reveal that it is a bona fide GTPase, engages specific membrane lipids, and is sufficient for membrane fission. Ultimately, this work advances our understanding of the molecular mechanism of a membrane fission protein that is key for Toxoplasma's secretory organelle biogenesis, host infection, and may unveil new targets for future drug discovery.

23-POS Board 23

MYOMAKER AND ETHER LIPIDS COOPERATE TO PROMOTE FUSION-COMPETENT MEMBRANE STATES

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Cell-cell fusion is a fundamental process critical for skeletal muscle development and regeneration. Myomaker and Myomerger are the muscle-specific fusogens and both are essential for membrane fusion in muscle cells and together can induce fusion of cells that are normally fusion-incompetent. However, a mechanistic understanding for Myomaker activity remains poorly understood. Here, we identify ether-linked phospholipids as modulators of Myomaker activity. While Myomaker exhibits homology to ceramidases, we observe no ability for the protein to regulate ceramides. Myocytes treated with the ceramide synthase inhibitor Fumonisin B1 increased fusion and lipidomic analysis revealed an increase in the cellular content of ether lipids. Using a lentiviral pseudotyping system to isolate fusogenic Myomaker-containing membranes, we found ether lipids were enriched in Myomaker viral particles. In non-myogenic cells that are fusion-competent due to forced Myomaker expression, enrichment of ether lipids induced complete fusion, even in the absence of Myomerger. By elevating ether lipids, levels of plasma membrane Myomaker increased and correlated with cell surface exposure of phosphatidylethanolamine and phosphatidylserine, indicating a role for Myomaker in remodeling lipid distribution within the plasma membrane. These findings reveal a lipid-dependent mechanism by which ether lipids enhance aspects of Myomaker function, establishing a new link between membrane composition, lipid asymmetry, and the molecular machinery responsible for cell fusion in skeletal muscle.

24-POS Board 24

PIPELINE FOR TARGETING MEMBRANE REMODELING PROCESSES WITHIN CELLS FOR IN-SITU CRYOET

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In-situ cryo-electron tomography remains the only method for structural studies of molecular machines in close to native environments, within cells, at high-resolution. While this method offers a huge opportunity to investigate the molecular mechanisms of various biological processes, its main limitation is how to effectively target specific features of interest or biological processes for imaging. To study membrane remodeling processes in our lab using cryoET, we apply cryo-confocal imaging to identify and target cellular features and processes of interest. Here we present pipeline for studying membrane remodeling processes using mitochondrial fission as an example. Membrane remodeling processes, including fusion or fission, occur at specific time and space and requires optimizing our cellular systems and workflows for successful in-situ cryoET studies. First, we developed a transgenic cell line expressing fluorescently labeled mutant membrane remodeling protein, that acts slowly to stall the membrane remodeling processes for visualization by cryoET. The organelle targets of the membrane remodeling process are also fluorescently labelled, enabling the identification of regions where membrane remodeling is occurring. These cells are applied on cryoEM grids, either in suspension or seeded on gold grids in case of adherent cell lines, then frozen in liquid ethane. The samples are imaged by the cryo-confocal microscope, and the imaged locations of interest are transferred to the cryo-FIB/SEM microscope to guide lamella preparation at cellular sites of interest. Overall, this strategy results in a higher rate of lamellae that contain regions of interest and enables our structural studies of membrane remodeling within cells.