Quantitative Aspects of Membrane Fusion and Fission

Padova, Italy | May 6–10, 2019
Organizing Committee

Sebastian Barg, Uppsala University, Sweden
Jenny Hinshaw, NIH, USA
Dinah Loerke, University of Denver, USA
Morten Gram Pedersen, University of Padova, Italy
Jakob B. Sørensen, University of Copenhagen, Denmark
Thank You to Our Sponsors
Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting on *Quantitative Aspects of Membrane Fusion and Fission*.

Quantitative understanding of the biophysical mechanisms causing membrane fusion or fission requires analysis of dynamical and physiologically relevant localized changes and interactions of proteins, lipids, and messenger molecules. This interdisciplinary meeting will address the growing need for collaboration between experimentalists and theorists to fully take advantage of the quantitative nature of the experimental observations in this field and to improve the quantitative descriptions of membrane events.

The scientific program offers 44 talks and 64 posters covering a wide range of cell types and membrane structures, and methodologies spanning from mathematical simulations via image and statistical analysis to different cutting-edge experimental techniques.

We hope that the meeting will not only provide a venue for sharing recent and exciting progress, but also promote discussions and foster future collaborations in the area of membrane fusion and fission. With participants from five continents and with highly diverse backgrounds, there should be excellent opportunities for interacting with new acquaintances during coffee breaks, the banquet, and the organized tours of the University Botanical Gardens and the historical University of Padova. We also hope you will enjoy the cultural and historical attractions of Padova.

Thank you all for joining our Thematic Meeting. We look forward to seeing you in Padova!

Best regards,

The Organizing Committee

*Sebastian Barg*
*Jenny Hinshaw*
*Dinah Loerke*
*Morten Gram Pedersen*
*Jakob B. Sørensen*
Biophysical Society Code of Conduct Anti-Harassment Policy

Adopted by BPS Council November 2015

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 a working environment that is free of inappropriate behavior and harassment by or toward all attendees of Society meetings and Society-sponsored activities, including scientists, students, guests, exhibitors, staff, vendors, and other suppliers.

This global policy applies to all locations and situations where BPS business is conducted and to all BPS-sponsored activities and events. This policy does not replace the specific staff policies for situations in which only staff are involved.

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.

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.

Investigative Process

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 remediating the problem or if complainant does not feel comfortable with such an approach, he/she should contact BPS's Executive Director or the Society President, or any BPS Officer. All complaints will be promptly and thoroughly investigated.

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.

No retaliation will be taken against any employee, member, volunteer, exhibitor, or supplier because he or she reports a problem concerning possible acts of harassment. Employees, members, volunteers, exhibitors, or suppliers can raise concerns and make reports without fear of reprisal.

Investigative Procedure

Once a complaint of harassment or sexual harassment is received, BPS will begin a prompt and thorough investigation.

- An impartial investigative committee, consisting of the Past-President, current President, and President-Elect will be established.
- 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.

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 verbal warning to ejection from the meeting or activity in question without refund of registration fees and the reporting of their behavior to their employer. Repeat offenders may be subject to further disciplinary action, such as being banned from participating in future Society meetings or Society-sponsored activities. In the event that the individual is dissatisfied with the results of the investigation, he or she 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.

BPS Management Responsibility

Every officer, director, supervisor, and manager is responsible for ensuring that BPS provides an environment free of harassment and inappropriate behavior and that complaints are handled promptly and effectively. The BPS Society Office and Officers must inform the Society membership and all vendors and suppliers about this policy, promptly investigate allegations of harassment, take appropriate disciplinary action, and take steps to assure retaliation is prohibited.
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<tr>
<td>Poster Sessions</td>
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GENERAL INFORMATION

Registration Hours/Information Location and Hours
On Monday, registration will be held at the University Botanical Gardens of Padova located at Via Orto Botanico, 15, 35123 Padova, Italy. Registration will be located in the Foyer at the University Botanical Gardens of Padova. Registration hours are as follows:

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Location</th>
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<tbody>
<tr>
<td>Monday, May 6</td>
<td>16:30 – 18:30</td>
<td>Foyer, Botanical Gardens</td>
</tr>
<tr>
<td>Tuesday, May 7</td>
<td>08:00 – 18:00</td>
<td>Foyer, Botanical Gardens</td>
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<tr>
<td>Wednesday, May 8</td>
<td>12:30 – 19:00</td>
<td>Foyer, Botanical Gardens</td>
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<tr>
<td>Thursday, May 9</td>
<td>08:00 – 19:00</td>
<td>Foyer, Botanical Gardens</td>
</tr>
<tr>
<td>Friday, May 10</td>
<td>08:00 – 12:30</td>
<td>Foyer, Botanical Gardens</td>
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</tbody>
</table>

Instructions for Presentations
(1) Presentation Facilities:
A data projector will be available in the Auditorium. Speakers are required to bring their own laptops and adaptors. It is recommended to have a backup of the presentation on a USB drive in case of any unforeseen circumstances. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Sessions:
1) All poster sessions will be held in the Sala delle Colonne of the University Botanical Gardens of Padova.

2) A display board measuring 2 meters high (6.5 ft) x 1 meter wide (3.2 ft) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as listed in the e-book.

3) Posters should be set up in the evening of May 6 and removed by noon on May 10. All posters are available for viewing during all poster sessions; however, there will be formal poster presentations at the following times:

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<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Location</th>
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<tbody>
<tr>
<td>Tuesday, May 7</td>
<td>14:30 – 15:15</td>
<td>Odd-numbered poster boards</td>
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<tr>
<td>Tuesday, May 7</td>
<td>15:15 – 16:00</td>
<td>Even-numbered poster boards</td>
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<tr>
<td>Wednesday, May 8</td>
<td>14:50 – 15:35</td>
<td>Odd-numbered poster boards</td>
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<tr>
<td>Wednesday, May 8</td>
<td>15:35 – 16:20</td>
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<tr>
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<td>15:15 – 16:00</td>
<td>Even-numbered poster boards</td>
</tr>
</tbody>
</table>

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

5) All posters left uncollected at the end of the meeting will be disposed.
**Meals and Coffee Breaks**
There will be a two hour Welcome Reception on Monday evening from 17:30 – 19:30. This reception will be held at the University Botanical Gardens of Padova.

Coffee Breaks (Tuesday – Friday) and Lunches (Tuesday and Thursday) will be served in the Sala delle Colonne.

On Thursday, there will be a Banquet starting at 19:00. Walking directions to Pedrocchi Café will be provided at onsite registration.

Advanced sign-up was required for the Botanical Gardens Tour, Historical University Tour, and the Banquet. Tickets are required for admittance to these functions and will be provided at onsite registration.

**Smoking**
Please be advised that smoking is not permitted at the University Botanical Gardens of Padova.

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

**Internet**
Wifi will be provided at the venue. Attendees will receive the account number and password at registration.

**Contact**
If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from May 6-10 during registration hours.

In case of emergency, you may contact the following:

Sara Borgato  
Email: [eventi@ortobotanicopd.it](mailto:eventi@ortobotanicopd.it)

Dorothy Chaconas  
Email: [dchaconas@biophysics.org](mailto:dchaconas@biophysics.org)

Ally Levine  
Email: [alevine@biophysics.org](mailto:alevine@biophysics.org)
Quantitative Aspects of Membrane Fusion and Fission
Padova, Italy
May 6-10, 2019

PROGRAM

**Monday, May 6, 2019**

<table>
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<tbody>
<tr>
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**Tuesday, May 7, 2019**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>8:00 – 18:00</td>
<td>Registration/Information</td>
</tr>
<tr>
<td>8:30 – 8:40</td>
<td>Morten Gram Pedersen, University of Padova, Italy</td>
</tr>
<tr>
<td></td>
<td>Opening Remarks</td>
</tr>
<tr>
<td>8:40 – 9:10</td>
<td>Arun Anantharam, University of Michigan, USA</td>
</tr>
<tr>
<td></td>
<td>Synaptotagmin-7 Endows a Population of Chromaffin Granules with Distinct Calcium Sensing and Fusion Properties</td>
</tr>
<tr>
<td>9:10 – 9:40</td>
<td>Ben O'Shaughnessy, Columbia University, USA</td>
</tr>
<tr>
<td></td>
<td>Coarse-Grained Mathematical Modeling of Neurotransmitter Release</td>
</tr>
<tr>
<td>9:40 – 10:00</td>
<td>Yongli Zhang, Yale University, USA*</td>
</tr>
<tr>
<td></td>
<td>Sec1/Munc18 Proteins Catalyze SNARE Assembly by Templating SNARE Folding and Association</td>
</tr>
<tr>
<td>10:00 – 10:20</td>
<td>Alex Kreutzberger, University of Virginia, USA*</td>
</tr>
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<td>Reconstitution of Regulated Exocytosis of Different Secretory Vesicle Types</td>
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<td>10:20 – 10:50</td>
<td>Coffee Break</td>
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**Session II**

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<tr>
<td>10:50 – 11:20</td>
<td>Jens Rettig, Saarland University, Germany</td>
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<td></td>
<td>Molecular Insights Into Exo- and Endocytosis of Cytotoxic Granules</td>
</tr>
<tr>
<td>11:20 – 11:50</td>
<td>Tomas Kirchhausen, Harvard University, USA</td>
</tr>
<tr>
<td></td>
<td>Imaging Subcellular Dynamics from Molecules to Multicellular Organisms</td>
</tr>
<tr>
<td>11:50 – 12:10</td>
<td>Belinda Akpa, North Carolina State University, USA*</td>
</tr>
<tr>
<td></td>
<td>Multiscale Modeling of Plant Vacuole Fusion in Guard Cells: Positioning HOPS as the Key Regulator of Stoma Morphology</td>
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<tr>
<td>Time</td>
<td>Speaker and Affiliation</td>
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<tr>
<td>13:10 – 13:40</td>
<td>Patrik Rorsman, OCDEM, United Kingdom</td>
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<tr>
<td>13:40 – 14:10</td>
<td>Uri Ashery, Tel-Aviv University, Ramat Aviv, Israel</td>
</tr>
<tr>
<td>14:10 – 14:30</td>
<td>Agata Witkowska, Max Planck Institute for Biophysical Chemistry, Germany*</td>
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<tr>
<td>14:30 – 16:00</td>
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<tr>
<td>16:00 – 16:30</td>
<td>Michelle Knowles, University of Denver, USA</td>
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<tr>
<td>16:30 – 17:00</td>
<td>Ravi Radhakrishnan, University of Pennsylvania, USA</td>
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<tr>
<td>17:00 – 17:30</td>
<td>Mohsen Sadeghi, Freie Universität, Berlin, Germany</td>
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<tr>
<td>17:30 – 17:50</td>
<td>Comert Kural, The Ohio State University, USA*</td>
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<tr>
<td>18:00 – 19:30</td>
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**Wednesday, May 8, 2019**

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<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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<tbody>
<tr>
<td>8:45 – 11:00</td>
<td>Historical University Tour (lunch on own)</td>
<td>Foyer (Directions provided onsite) Palazzo Bo Ticket Office</td>
</tr>
<tr>
<td>12:30 – 19:00</td>
<td>Registration/Information</td>
<td>Foyer</td>
</tr>
<tr>
<td>13:00 – 13:30</td>
<td>Thomas Pucadyil, Indian Institute of Science Education and Research, India</td>
<td><strong>ATP-Dependent Membrane Remodeling Links EHD1 Functions to Endocytic Recycling</strong></td>
</tr>
<tr>
<td>13:30 – 14:00</td>
<td>Patricia Bassereau, Institut Curie, France</td>
<td><strong>ESCR-T-III Filaments Have Opposite Curvature-Related Orientations on Membranes</strong></td>
</tr>
<tr>
<td>14:00 – 14:30</td>
<td>Jeanne Stachowiak, University of Texas at Austin, USA</td>
<td><strong>Intrinsically Disordered Proteins as Physical Drivers of Membrane Fission</strong></td>
</tr>
<tr>
<td>Time</td>
<td>Speaker</td>
<td>Title</td>
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<tr>
<td>14:30 – 14:50</td>
<td>Henry Nguyen, University of California, San Francisco, USA*</td>
<td><em>Cryo-EM Structures Reveal Progressive Membrane Constriction by the ESCRT-III Proteins IST1 and CHMP1B</em></td>
</tr>
<tr>
<td>14:50 – 16:20</td>
<td>Coffee Break/Poster Session II</td>
<td>Sala delle Colonne</td>
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**Session VI**

**Synapses**

Morten Gram Pedersen, University of Padova, Italy, Chair

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<tr>
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<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:30 – 17:00</td>
<td>Takeshi Sakaba, Doshisha University, Japan</td>
<td><em>Comparison of the Transmitter Release Properties Between the Calyx of Held Synapse and Mossy Fiber-CA3 Synapse</em></td>
</tr>
<tr>
<td>17:00 – 17:30</td>
<td>Alexander Walter, Leibniz-Institut für Molekulare Pharmakologie (FMP), Germany</td>
<td><em>Release Site Recruitment and Activation as Mechanisms of Presynaptic Plasticity</em></td>
</tr>
<tr>
<td>17:30 – 18:00</td>
<td>Stephanie Gupton, University of North Carolina at Chapel Hill, USA</td>
<td><em>An Unbiased Classification Approach Reveals Multiple Fusion Categories of VAMP2-Mediated Exocytosis</em></td>
</tr>
<tr>
<td>18:00 – 18:15</td>
<td>Break</td>
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</table>

**Session VII**

**Superresolution**

Dinah Loerke, University of Denver, USA, Chair

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:15 – 18:45</td>
<td>Susan Cox, King’s College London, United Kingdom</td>
<td><em>Faster and Better: Taking Localization Microscopy into Live Cells</em></td>
</tr>
<tr>
<td>18:45 – 19:15</td>
<td>Katharina Gaus, University of New South Wales, Australia</td>
<td><em>Single Molecule Localization Microscopy of Receptor Signaling</em></td>
</tr>
<tr>
<td>19:00 – 20:00</td>
<td>Dinner on own</td>
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**Thursday, May 9, 2019**

<table>
<thead>
<tr>
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<th>Registration/Information</th>
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<td>8:00 – 19:00</td>
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**Session VIII**

**Fusion Pore II**

Sebastian Barg, Uppsala University, Sweden, Chair

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30 – 9:00</td>
<td>Ling-Gang Wu, NINDS, NIH, USA</td>
<td><em>Visualizing Membrane Structural Remodeling During Fusion and Fission in Live Cells</em></td>
</tr>
<tr>
<td>9:00 – 9:30</td>
<td>Manfred Lindau, Cornell University, USA</td>
<td><em>The Structure of the Fusion Pore</em></td>
</tr>
<tr>
<td>9:30 – 9:50</td>
<td>Raya Sorkin, Vrije Univit Amsterdam, The Netherlands*</td>
<td><em>Membrane Binding, Bending, and Remodeling by Synaptotagmin-1 and Doc2b</em></td>
</tr>
<tr>
<td>9:50 – 10:10</td>
<td>Rafael Lira, Max Planck Institute, Germany*</td>
<td><em>How and How Much Has It Fused? Detecting Fusion Intermediates and Quantifying Fusion Efficiency</em></td>
</tr>
<tr>
<td>10:10 – 10:40</td>
<td>Coffee Break</td>
<td>Sala delle Colonne</td>
</tr>
</tbody>
</table>
Session IX

Exocytosis II
Jakob B. Sørensen, University of Copenhagen, Denmark, Chair

10:40 – 11:10
Frederic Meunier, Queensland Brain Institute, Australia
Need for Speed: Dynamic Nanoclustering and Unclustering of Munc18/Syntaxin-1 During Exocytosis

11:10 – 11:40
Giuliana Cortese, University of Padova, Italy
Advanced Statistics for Spatio-Temporal Events of Exocytosis

11:40 – 12:00
Nikhil Gandasi, Uppsala University, Sweden*
Birth of a Nanodomain: Vesicle Docking is Initiated by Rab3 Positive Vesicles Identifying Rim Sites to Tether at the Plasma Membrane

12:00 – 13:00
Lunch Sala delle Colonne

Session X

Fusion Proteins
Morten Gram Pedersen, University of Padova, Italy, Chair

13:00 – 13:30
Laura Endter, University of Göttingen, Germany
Molecular Simulations of Protein-Mediated Membrane Remodeling

13:30 – 13:50
Pavel Jungwirth, IOCB Prague, Czech Academy of Sciences, Czech Republic*
Cell Penetration and Membrane Fusion: Two Sides of the Same Coin

13:50 – 14:10
Mahmoud Moradi, University of Arkansas, USA*
Influenza Hemagglutinin-Mediated Membrane Fusion: An All-Atom Molecular Dynamics Study

14:10 – 14:30
James Munro, Tufts University, USA*
Conformational Dynamics Related to Membrane Fusion Observed in Single Viral Envelope Glycoproteins

14:30 – 16:00
Coffee Break/Poster Session III Sala delle Colonne

Session XI

Neuronal Endocytosis
Sebastian Barg, Uppsala University, Sweden, Chair

16:00 – 16:30
Jürgen Klingauf, University of Münster, Germany
Visualizing Compensatory Endocytosis Dynamics in ‘Xenapses,’ TIRFM-Amenable Synapses

16:30 – 17:00
Ira Milosevic, European Neuroscience Institute Göttingen, Germany
Novel Functions of Endophilins-A in Exocytosis and Membrane Trafficking

17:00 – 17:15
Break

Keynote Lecture
Jakob B. Sørensen, University of Copenhagen, Denmark, Chair

17:15 – 18:00
Axel Brunger, Stanford University, USA, Keynote Lecturer
Molecular Mechanisms of Neuronal Exocytosis

19:00
Banquet Pedrocchi Café
Friday, May 10, 2019

8:00 – 12:30  Registration/Information  Foyer

Session XII  Mitochondrial Fusion and Fission
Jenny Hinshaw, NIH, USA, Chair

8:30 – 9:00  Karin Busch, University of Münster, Germany
Mitochondrial Membrane Dynamics Versus Steady Compartmentalization: A Contradiction? What Superresolution Imaging Can Tell Us

9:00 – 9:30  Luca Scorrano, University of Padova, Italy
Consequences of Mitochondrial Fusion Changes

9:30 – 9:50  Dora Mahecic, EPFL Institute of Physics, Switzerland*
Membrane Bending Energy and Tension Govern Mitochondrial Division

9:50 – 10:10  Katja Faelber, Max Delbrück Center, Germany*
Structural Insights into Mitochondrial Inner Membrane Remodeling

10:10 – 10:40  Coffee Break  Sala delle Colonne

Session XIII  Exocytosis III
Jakob B. Sørensen, University of Copenhagen, Denmark, Chair

10:40 – 11:10  Fernando Marengo, University of Buenos Aires, Argentina
Endocytosis and Vesicle Replenishment after the Exocytosis of the Immediately Releasable Pool in Mouse Chromaffin Cells

11:10 – 11:40  Aleksandra Radenovic, École Polytechnique Fédérale de Lausanne, Switzerland
The Power of Correlative Superresolution Imaging

11:40 – 12:00  Francesco Montefusco, University of Padova, Italy*
How is Granule Release Affected By Location and Number of Different Types of Ca\(^{2+}\) Channels? Markov Chain Models Provide Analytic Results

12:00 – 12:15  Morten Gram Pedersen, University of Padova, Italy
Closing Remarks and Biophysical Journal Poster Awards

*Short talks selected from among submitted abstracts
SPEAKER ABSTRACTS
SYNAPTOTAGMIN-7 ENDOWS A POPULATION OF CHROMAFFIN GRANULES WITH DISTINCT CALCIUM SENSING AND FUSION PROPERTIES

Mounir Bendahmane¹; Alex J Kreutzberger²; Alina Chapman-Morales¹; Noah A Schenk¹; Volker Kiessling²; J D Castle²; Lukas K Tamm²; David R Giovannucci³; Arun Anantharam¹;
¹University of Michigan, Ann Arbor, Michigan, USA
²University of Virginia, Charlottesville, Virginia, USA
³University of Toledo, Toledo, Ohio, USA

Synaptotagmin-7 (Syt7) is one of two major calcium sensors for regulated exocytosis in adrenal chromaffin cells. Its high sensitivity allows tunable secretory responses to a range of stimuli that result in graded increases in intracellular calcium. Despite the undoubted importance of Syt7, questions remain as to whether the protein operates from the granule or plasma membrane and to what degree the functions of chromaffin cell Syts are redundant. Here, these issues were examined using two distinct experimental preparations – primary mouse chromaffin cells lacking endogenous Syt7 and a reconstitution assay employing cell-derived granules expressing either Syt7 or Syt1. First, we find that mouse Syt7 is punctate in appearance, consistent with its sorting to organelles. Antibody-based staining reveals it to be co-localized with plasminogen activator inhibitor 1 (PAI1) – a protein of the granule dense core – but rarely with Syt1. Functionally, chromaffin cells lacking Syt7 (knockout, KO) exhibit properties that readily distinguish them from WT cells. For example, lumenal cargo proteins are released at faster rates from cells only expressing Syt1 than WT cells expressing both Syts. KO cells also exhibit deficits in fusion efficacy, both in response to elevated KCl depolarization and cholinergic stimulation. To further distinguish between the roles of Syt7 and Syt1 in fusion, purified dense core granules expressing only one of the two proteins were triggered to fuse on reconstituted planar supported bilayers bearing t-SNAREs. These studies demonstrate that Syt7 confers substantially greater calcium sensitivity to granule fusion than Syt1 and slows that rate at which cargos are released, just as in primary cells employing overexpressed Syts. By virtue of its sorting and biochemistry, Syt7 serves unique roles in the biology of the chromaffin cell secretory response in ways that distinguish it from Syt1.
COARSE-GRAINED MATHEMATICAL MODELING OF NEUROTRANSMITTER RELEASE

Ben O'Shaughnessy¹;
¹Columbia University, Chemical Engineering, New York, New York, USA

Tightly synchronized release of neurotransmitters (NTs) from synaptic vesicles at neuronal synapses is accomplished by a machinery that senses Ca when an action potential arrives, fuses the vesicular and plasma membranes, and releases NTs through a fusion pore. Many components of the machinery are now identified, and structural information is emerging. However, it remains a major challenge to understand how the components cooperate as a machine that accomplishes membrane fusion on sub millisecond timescales. Mathematical modeling is needed to help this quest, but describing a machinery comprising tens of proteins on millisecond timescales is currently beyond all-atom or moderately coarse-grained computational approaches. Radical coarse-graining is required. Our approach is molecularly explicit representation based on systematic coarse-graining procedures, sufficiently coarse-grained to access collective behavior on the long timescales of physiological fusion. The framework allows different hypothesized mechanisms to be tested quantitatively. I will describe results that incorporate the calcium sensor Synaptotagmin (Syt) and the SNARE proteins which constitute the core of the fusion machinery. We are testing the hypothesis that ring-like oligomers of Syt clamp fusion by spacing the membranes, until fusion is triggered by calcium-mediated dissociation of the Syt rings (Wang et al., 2014). Simulations describe a 3-stage process: first, calcium-triggered unclamping of the SNAREs as the Syt ring disassembles; second, self-assembly of SNARE complexes into a ring at the fusion site; third, SNARE-mediated membrane fusion. Stages 2 and 3 are driven by entropic forces among the SNAREs and membranes (Mostafavi et al., 2017; McDargh et al., 2018). Computed release rates versus calcium concentration are compared with experimental electrophysiological measurements.
SECI/MUNC18 PROTEINS CATALYZE SNARE ASSEMBLY BY TEMPLATING SNARE FOLDING AND ASSOCIATION

Yongli Zhang¹; Junyi Jiao¹; Mengze He¹; Sarah A Port²; Richard W Baker²; Yonggang Xu¹; Hong Qu¹; Yujian Xiong¹; Yukun Wang¹; Huaizhou Jin¹; Travis J Eisemann²; Frederick M Hughson²;
¹Yale School of Medicine, Department of Cell Biology, New Haven, Connecticut, USA
²Princeton University, Department of Molecular Biology, Princeton, New Jersey, USA

Sec1/Munc18-family (SM) proteins are required for SNARE-mediated membrane fusion, but their mechanism(s) of action remain controversial. Using single-molecule force spectroscopy, we found that the SM protein Munc18-1 catalyzes step-wise zippering of three synaptic SNAREs (syntaxin, VAMP2, and SNAP-25) into a four-helix bundle. Catalysis requires formation of an intermediate template complex in which Munc18-1 juxtaposes the N-terminal regions of the SNARE motifs of syntaxin and VAMP2, while keeping their C-terminal regions separated. The template complex is relatively weak, with an unfolding energy of 3.1 kcal/mol, and is stabilized by the N-terminal regulatory domain (NRD) of syntaxin. SNAP-25 binds the templated SNAREs to induce full SNARE zippering. Munc18-1 and SNARE mutations modulate the stability of the template complex in a manner consistent with their effects on membrane fusion, indicating that chaperoned SNARE assembly is essential for exocytosis. Two other SM proteins, Munc18-3 and Vps33, similarly chaperone SNARE assembly via a template complex, suggesting that SM protein mechanism is conserved.
RECONSTITUTION OF REGULATED EXOCYTOSIS OF DIFFERENT SECRETORY VESICLE TYPES

Alex J.B. Kreutzberger\textsuperscript{1,2}; Volker Kiessling\textsuperscript{1,2}; J David Castle\textsuperscript{1,3}; Reinhard Jahn\textsuperscript{4}; Lukas K Tamm\textsuperscript{1,2};
\textsuperscript{1}University of Virginia, Center for Membrane and Cell Physiology, Charlottesville, Virginia, USA
\textsuperscript{2}University of Virginia, Department of Molecular Physiology and Biological Physics, Charlottesville, Virginia, USA
\textsuperscript{3}University of Virginia, Cell Biology, Charlottesville, Virginia, USA
\textsuperscript{4}Max Planck Institute for Biophysical Chemistry, Neurobiology, Göttingen, Niedersachsen, Germany

Dense core vesicles have previously been purified from an immortalized rat chromaffin cell line (PC12 cells) and incorporated into a fusion assay with planar supported bilayers reconstituted with the complete required target membrane fusion machinery (Kreutzberger et al. Sci Adv. 2017). This assay has been extended to be used with synaptic vesicles purified from rat brains and insulin vesicles purified from an immortalized rat beta cell line (INS-1 cells). Docking and fusion of all three secretory vesicles can be observed using different content labeling strategies. All three secretory vesicles dock in an arrested state when the planar supported bilayers contain syntaxin-1a and SNAP-25 incubated with Munc18 and complexin-1. Perfusion of calcium stimulates these vesicles to fuse. Three major differences were observed for the different secretory vesicle types. First, a differential requirement for a recombinant fragment of Munc13 was observed, with it being necessary for a robust calcium response of synaptic vesicles but not required for dense core vesicles. Second, a large disparity in fusion rates after calcium arrival was observed with synaptic vesicles being the fastest, dense core vesicles intermediate, and insulin vesicles being the slowest. Finally, there was a different affinity for calcium for each secretory vesicle type due to the different synaptotagmin isoforms present on the vesicles. Calcium responses of fusion depended strongly on the concentration of PI(4,5)P\textsubscript{2} present in the planar supported bilayer.
Ca\(^{2+}\)-dependent exocytosis of signalling substances is one of the most important tasks of any cell in our body. The most heavily studied exocytic event takes place at synapses between neurons where neurotransmitters are released from synaptic vesicles. However, the molecular mechanism of neurotransmitter release is difficult to study due to technical reasons like synapse size and speed. Cytotoxic T lymphocytes (CTLs) are part of the adaptive immune system and kill target cells by formation of an immunological synapse (IS) followed by the directed release of toxic substances from cytotoxic granules (CGs). Interestingly, a number of proteins like Munc13, Munc18 or syntaxin, which have been shown to be involved in neurotransmitter release, are instrumental for CG release as well. We have investigated the molecular mechanism of IS formation and function in primary CTLs from mouse and human. Knockout/knockdown approaches have been combined with high-resolution fluorescence microscopy, electron microscopy and functional assays to elucidate the contribution of several key proteins. In addition, molecular states preceding LG fusion could be resolved by total internal reflection fluorescence microscopy (TIRFM) in combination with whole-cell patch-clamp recordings. Furthermore, we could demonstrate that membrane components of freshly fused CGs undergo fast endocytosis and significantly contribute to the killing of further target cells. I will present the latest findings from our lab which identify parts of the molecular machinery that is required for sequential fusion events occurring at the IS.
IMAGING SUBCELLULAR DYNAMICS FROM MOLECULES TO MULTICELLULAR ORGANISMS

Tomas Kirchhausen;
1Harvard Medical School, Cell Biology, Boston, Massachusetts, USA

Frontier optical-imaging modalities exemplified by the lattice light-sheet microscope invented by Eric Betzig sets new visualization standards for analyzing and understanding sub-cellular processes in the complex and dynamic three-dimensional environment of living-cells in isolation and within tissues of an organism. By using ultra-thin sheets of light to rapidly illuminate biological samples with extremely low photon doses, 3D experiments previously limited to seconds or minutes by photo-bleaching or by photo- toxicity, can now be done at diffraction limited resolution and high-temporal precision with unprecedented duration of minutes or hours. We believe this ability to image with minimal perturbations is ideally suited to support hypothesis-generating research geared towards new discoveries. The talk will illustrate our use of lattice light-sheet microscopy to ‘see’ in three dimensions processes that mediate and regulate the biogenesis of organelles in living cells maintained in tissue culture conditions and will also describe our most recent efforts using lattice light sheet microscopy with adaptive optics to investigate with subcellular precision process in cells within tissues of a living zebrafish embryo.
MULTISCALE MODELING OF PLANT VACUOLE FUSION IN GUARD CELLS: POSITIONING HOPS AS THE KEY REGULATOR OF STOMA MORPHOLOGY

Belinda S. Akpa¹; David Flaherty¹; Natalie Clark²; Aniket Antad³; Rosangela Sozzani²; Marcela Rojas-Pierce²;
¹North Carolina State University, Molecular Biomedical Sciences, Raleigh, North Carolina, USA
²North Carolina State University, Plant and Microbial Biology, Raleigh, North Carolina, USA
³North Carolina State University, Electrical & Computer Engineering, Raleigh, North Carolina, USA

Stomata are the pores on a leaf surface that regulate gas exchange. Each stoma is made of 2 guard cells whose movements regulate pore opening and thereby control CO2 fixation and water loss. Guard cell movements depend critically on the remodeling of cell vacuoles. These organelles have been observed to change morphology from a highly fragmented state to a fused state during stomata opening. The evolution of vacuole morphology requires a membrane fusion mechanism that responds rapidly to environmental signals, allowing plants to respond to diurnal cues or environmental stresses such as drought. With guard cells being both large and responsive to external signals, stomata represent a unique system in which to delineate mechanisms of membrane fusion and fission. Objective: To resolve a counter-intuitive observation regarding the role of HOPS in regulating vacuole morphology, we derived a quantitative model of vacuole fusion dynamics and used it to generate testable predictions about the dynamics of HOPS-SNARE interactions. Method: We derived our model from limited – and, initially, qualitative – data by integrating statistical inference and machine learning with quantitative fluorescence imaging and mechanistic modeling. The dynamic model predicted the evolution of vacuole morphology as it arises from intracellular signaling events that include: cytosol-to-membrane recruitment, chaperoned protein complexation, and complex disassembly. Results: We made specific predictions about the state of the biomolecular agents of fusion (e.g. HOPS, SNARE) prior to and during stoma opening. By constraining the model parameters to yield the emergent outcomes observed for stoma opening (as induced by two distinct signals), we proposed a dual role for HOPS and identified a stalled form of the SNARE complex that differs from phenomena reported in yeast. Conclusions: We predicted that HOPS has apparently contradictory actions at different points in the fusion signaling pathway, promoting the formation of SNARE complexes, but limiting their activity.
INSULIN EXOCYTOSIS: NORMAL PHYSIOLOGY AND DISRUPTION IN TYPE-2 DIABETES

Patrik Rorsman\textsuperscript{1,2};
\textsuperscript{1}OCDEM, Radcliffe Department of Medicine, Churchill Hospital, University of Oxford, Oxford, United Kingdom
\textsuperscript{2}Institute of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden

Insulin is the body’s only blood glucose-lowering hormone. Insufficient release of insulin leads to diabetes, a disease that affects at least 5% of the population. Insulin is released by the beta-cells of the pancreatic islets (the endocrine part of the pancreas). The beta-cells are electrically excitable and glucose (as well as other insulin secretagogues) initiates Ca\textsuperscript{2+}-dependent action potential firing and the associated increase in intracellular Ca\textsuperscript{2+} triggers exocytosis of insulin-containing secretory granules. Electrical activity in the beta-cell is controlled by ATP-regulated potassium channels that close in response to a glucose-induced increase in the cytoplasmic ATP/ADP ratio. Exocytosis in beta-cells proceeds at very high rates despite the a Ca\textsuperscript{2+} channel density being only 5-10% of that found in other neuroendocrine cells. This is because the Ca\textsuperscript{2+} channels physically associate with release-competent secretory granules, allowing economical use of Ca\textsuperscript{2+} entering the beta-cells. Disruption of this arrangement selectively interferes with rapid depolarization-evoked exocytosis but does not affect ‘asynchronous’ release. Experimental conditions emulating diabetes (such as chronic exposure to non-esterified fatty acids) results in the disassembly of the Ca\textsuperscript{2+} channel/secretory granule complexes and reduces glucose-induced insulin secretion in a way resembling that seen in clinical diabetes. Experiments using fluorescently tagged Ca\textsuperscript{2+} channels confirm that they normally cluster close to insulin granules undergoing exocytosis and but that the formation of such clusters is prevented following exposure to NEFA and not seen in beta-cells from donors diagnosed with type-2 diabetes. We propose that tight coupling of Ca\textsuperscript{2+} entry to the release machinery provides the insulin-secreting beta-cells with the means of high-capacity exocytosis at minimal expenditure of metabolic energy to buffer Ca\textsuperscript{2+}. This prevents reactivation of the ATP-sensitive potassium channels and inhibition of electrical activity/insulin secretion that would otherwise occur.
FUSION PORE CONSTRICTION CONTROLS THE DYNAMICS OF VESICULAR CONTENT RELEASE

Uri Ashery
Tel-Aviv University, Ramat Aviv, Israel

No Abstract

INSIGHTS ON SNARE-MEDIATED FUSION LEARNED FROM EARLY MEMBRANE FUSION INTERMEDIATES

Agata Witkowska1; Susann Spindler2,3; Vahid Sandoghdar2,3; Reinhard Jahn1;
1Max Planck Institute for Biophysical Chemistry, Department of Neurobiology, Göttingen, Niedersachsen, Germany
2Max Planck Institute for the Science of Light, Nano-Optics Division, Erlangen, Bayern, Germany
3Friedrich Alexander University Erlangen-Nuremberg, Department of Physics, Erlangen, Bayern, Germany

SNARE proteins are the main catalysts for membrane fusion in the secretory pathway of eukaryotic cells. SNARE-mediated membrane fusion is induced by sequential N- to C-terminal assembly of four SNARE motifs coming from proteins anchored to different membranes that results in pulling the membranes towards each other. Despite many years of research, the exact mechanism of how SNARE proteins overcome the repulsion energy of two fusing membranes is still debated. During neurotransmission, tight control over timing and extreme efficiency are needed for synaptic vesicle exocytosis. This means that neuronal fusion machinery has to be highly specialized for overcoming membrane repulsion energy. We have previously established an efficient protocol for preparation of giant unilamellar vesicles (GUVs) containing SNARE proteins (Witkowska et al., Sci Rep, 2018) and a novel platform for monitoring SNARE-mediated docking and fusion on a single vesicle level in vitro between GUVs and smaller liposomes (Witkowska & Jahn, Biophys J, 2017). In this system, 100 nm liposomes as well as purified secretory vesicles fuse with GUVs with only few milliseconds delay between docking and fusion, a rate close to fast neuronal exocytosis. Here, we utilize this GUV-liposome system in combination with interferometric scattering microscopy (iSCAT), cryo-electron microscopy, and mathematical modelling, in order to characterize recently described by us (Yavuz et al., J Biol Chem, 2018) arrested early membrane fusion intermediates, namely loosely and tightly docked vesicles. With this system, we were able to characterize diffusional properties of vesicles in different fusion stages and gain insights into energy landscape of fusion intermediates.
PROTEIN REGULATION OF EXOSOME SECRETION

Michelle K. Knowles¹,²;
¹University of Denver, Chemistry and Biochemistry, Denver, Colorado, USA
²University of Denver, Molecular and Cellular Biophysics Program, Denver, Colorado, USA

Exosomes are small vesicles (diameter < 200 nm) that are secreted by most types of cells for intercellular communication. Exosome biogenesis is initiated in multivesicular bodies (MVBs) and their secretion into the extracellular fluid is driven by the fusion of MVBs with the plasma membrane. Once in the extracellular fluid, exosomes can be taken up by surrounding cells, and can, thus, be used to transfer important biomolecules such as nucleic acids, lipids and proteins between cells. This mode of communication has recently gained interest because it is exploited by diseased cells. For instance, it is well documented that exosome secretion is upregulated in cancer cells where exosomes carry nucleic acids with oncogenic mutations that have the potential to alter gene expression in recipient cells, leading to the progression of disease. Despite the recent growth of interest in exosomes and their use in early screening assays, little is known about the protein regulators of fusion. Past work suggests a role for VAMP7, SNAP23, and actin in the process. In this work, we have investigated the role of SNAREs and SNARE interacting proteins in the regulation of exosome fusion. Cells (A549, PC12) expressed a pHluorin or pHuji labeled CD63 on exosomes as a marker of MVB fusion events and the presence of proteins in another color channel was concurrently measured using TIRF microscopy methods. The timing of protein arrival and loss from exosome fusion sites was measured for a variety of proteins involved with the membrane fusion process with the goal of identifying targets for therapeutic intervention in the future.
BIOPHYSICS OF MEMBRANE CURVATURE REMODELING AT MOLECULAR AND MESOSCOPIC LENGTH SCALES

Ravi Radhakrishnan¹;
¹University of Pennsylvania, Bioengineering, Philadelphia, Pennsylvania, USA

At the micron scale, cell organelles display an amazing complexity in their shape and organization. The physical properties of a biological membrane can be understood using continuum models subject to thermal undulations. Yet, the chief orchestrators of these complex and intriguing shapes are a specialized class of membrane associating often peripheral proteins called curvature remodeling proteins (CRPs) that operate at the molecular level through protein-lipid interactions. We discuss multiscale methodologies to model these systems at the molecular and the cellular scales, and present an energy landscape perspective of membrane remodeling through the organization and assembly of CRPs. We discuss the morphological space of nearly planar to highly curved membranes, methods to include thermal fluctuations, and review studies that model such proteins as curvature fields to describe the emergent curved morphologies. We also discuss several mesoscale models applied to a variety of cellular processes, where the phenomenological parameters are determined using molecular simulations. Much insight can be gained from the calculation of free energies of membranes states with protein fields, which enable accurate mapping of the state and parameter values at which the membrane undergoes morphological transformations such as vesiculation or tubulation. By tuning the strength, anisotropy, and spatial organization of the curvature-field, one can generate a rich array of membrane morphologies that are highly relevant to shapes of several cellular organelles. We review describe of these models to budding of vesicles commonly seen in cellular signaling and trafficking processes such as clathrin mediated endocytosis, sorting by the ESCRT protein complexes, and cellular exocytosis regulated by the exocyst complex. We discuss future prospects where such models can be combined with other models for cytoskeletal assembly, and discuss their role in understanding the effects of cell membrane tension and the mechanics of the extracellular microenvironment on cellular processes.
PARAMETRIC PARTICLE-BASED MODEL FOR LARGE-SCALE SIMULATIONS OF MEMBRANE DYNAMICS

Mohsen Sadeghi¹; Frank Noé¹;
¹Freie Universität Berlin, Department of Mathematics and Computer Science, Berlin, Berlin, Germany

We have developed a computationally efficient coarse-grained membrane model, to be used in concert with particle-based reaction-diffusion simulations [1]. The model is purely based on nearest-neighbor interactions between particles that each represent a coarse patch of a lipid monolayer. Interaction potentials are parameterized so as to reproduce the local membrane mechanics, whereas the in-plane fluidity is implemented with Monte Carlo bond-flipping moves. To tackle different kinetics of in-plane and out-of-plane degrees of freedom, and to achieve a realistic model of membranes suspended in solvents, we have also developed an anisotropic stochastic dynamics scheme, based on exact solutions of Stokes equations. While drastically increasing the available sampling range, this approach also allows for modeling hydrodynamic interactions mediated by the solvent. Different aspects of the model are put to the test through studying equilibrium thermal undulations, isothermal area compressibility, diffusion and viscosity, dispersion relations, and interaction of the bilayer membrane with curvature-inducing agents. We expect this model to be of high practical usability in the context of ultra coarse-grained interacting particle reaction-dynamics (iPRD) simulations of cellular dynamics [2].

CURVATURE GENERATION BY ENDOCYTIC CLATHRIN COATS

Comert Kural;
1Ohio State University, Physics, Columbus, Ohio, USA

Clathrin coat assembly at the plasma membrane constitutes the major cellular internalization route for receptors and their ligands. Sculpturing a flat patch of membrane into an endocytic vesicle requires curvature formation on the cell surface, which is the primary function of endocytic protein complexes. The mechanism through which membrane curvature is imposed during formation of endocytic vesicles is still a subject of confusion and controversy. Using super-resolved live cell fluorescence imaging, we demonstrate that curvature generation by clathrin-coated pits can be detected in real time within cultured cells and tissues of metazoan organisms. Curvature is already generated when clathrin-coated pits reach the maximum projected area. These findings rule out the possibility of a previously proposed flat-to-curved transition during late stages of clathrin-coated pit formation. Therefore, curvature generation by clathrin-coated pits does not necessitate a dynamically unstable clathrin lattice.
ATP-DEPENDENT MEMBRANE REMODELING LINKS EHD1 FUNCTIONS TO ENDOCYTIC RECYCLING

**Thomas J. Pucadyil**\(^1\); Raunaq Deo\(^1\); Manish Kushwah\(^1\); Nagesh Kadam\(^2\); Kavita Babu\(^2\); Anand Srivastava\(^3\);

\(^1\)IISER Pune, Pune, Maharashtra, India
\(^2\)IISER Mohali, Mohali, Punjab, India
\(^3\)Indian Institute of Science, Bengaluru, Karnataka, India

Endocytic and recycling pathways generate cargo-laden transport carriers by membrane fission. Classical dynamins, which generate transport carriers during endocytosis, are known to constrict the membrane and cause fission in response to GTP hydrolysis. Relatively, less is known about the ATP-binding Eps15-homology domain-containing protein1 (EHD1), a dynamin family member that localizes to the endocytic-recycling compartment (ERC). Using cross complementation assays in C. elegans, we find that EHD1’s membrane binding and ATP hydrolysis activities are necessary for endocytic recycling. In reconstitution assays, a preference for high positive membrane curvature directs EHD1 to bind and organize into membrane-active scaffolds that bulge the tube. Reactions with ATP promote self-assembly of the scaffold, which propagates the bulge along the length of the tube causing intermediate regions to thin down. On tubes below 25 nm in radius, such thinning leads to scission. Coarse-grained molecular dynamics simulations corroborate this pathway to fission. N-terminal residues in EHD1 are important since their absence renders membrane bulges to catastrophically disappear in presence of ATP leading to defects in stable scaffolding, scission and endocytic recycling. Due to its significantly lower ATPase activity, the closely related ortholog EHD2 is dramatically less effective in membrane remodeling. Thus, ATP hydrolysis-dependent membrane remodeling links EHD1 functions to endocytic recycling.
ESCRT-III FILAMENTS HAVE OPPOSITE CURVATURE-RELATED ORIENTATIONS ON MEMBRANES

Nicola de Franceschi*1,2; Aurélie Bertin*1; Sourav Maity3; Maryam Alqabandi1; Nolwen Miguet2; Wouter Roos3; Winfried Weissenhorn2; Stéphanie Mangenot1; Patricia M. Bassereau1;
1Institut Curie, PhysicoChimie Curie, Paris, Paris, France
2University Grenoble Alpes, Institut de Biologie Structurale, Grenoble, Isère, France
3Rijksuniversiteit Groningen, Moleculaire Biofysica, Groningen, The Netherlands

The multi-proteins ESCRT-III complexes are involved in membrane scission in many different cellular processes. In contrast to dynamin polymers that assemble outside budding vesicle/tubule necks, they assemble inside the bud necks where the membrane has a curvature Gaussian negative. The organization of the proteins of this complex and even more the mechanism of membrane scission remain highly debated. By combining membrane nanotube pulling experiments, CryoEM and high speed AFM on a minimal set of human ESCRT proteins, we have obtained very unexpected results. We have shown that the CHMP proteins assemble in filaments with contrasted affinities for curved membranes. a) CHMP4 form spiral filaments that do not tubulate membranes and rather flatten them. b) CHMP2A with CHMP3 have affinity for positively curved membranes since they form helical structures on the external side of tubules only. c) Together CHMP4, CHMP2A and CHMP3 deform liposomes into helical tubes (with a corkscrew shape) when incubated with liposomes with orthogonal orientations of the filaments; moreover they are not recruited inside tubes pulled from giant vesicles, but only inside their neck, as observed in cells. The peculiar shape of the membrane reveals the mechanical stresses imposed by these ESCRT-III assemblies to the membranes and might provide new insight on the mechanism of scission in the presence of the ATPase Vps4.
INTRINSICALLY DISORDERED PROTEINS AS PHYSICAL DRIVERS OF MEMBRANE FISSION

Jeanne C. Stachowiak;
1University of Texas at Austin, Austin, Texas, USA

Membrane traffic, an essential cellular process that plays a role in many human diseases, requires key biophysical steps including formation of membrane buds, loading of these buds with specific molecular cargo, and separation from the parent membrane through the process of membrane fission. The prevailing view has been that structured protein motifs such as wedge-like amphipathic helices, crescent-shaped BAR domains, curved coats, and constricting dynamin rings drive these processes. However, many proteins that contain these structural motifs also contain large intrinsically disordered protein (IDP) domains of 300-1500 amino acids, including most clathrin and COPII coat components. While these IDP domains have been regarded primarily as flexible biochemical scaffolds, we have recently discovered that IDPs are highly efficient physical drivers of membrane budding fission. How can molecules without a defined structure drive membrane remodeling? Our results support the idea that disordered domains generate entropic pressure at membrane surfaces, which is critical to overcoming key biophysical barriers to membrane traffic. IDPs are particularly efficient generators of entropic pressure owing to their very large hydrodynamic radii, potential for electrostatic repulsion owing to high net charge, and the substantial entropic cost of extending them. More broadly our findings suggest that any protein, regardless of structure, can contribute to membrane remodeling by increasing entropic pressure, and paradoxically, that proteins that lack a defined secondary structure, IDPs, may be among the most potent drivers of membrane fission.
CRYO-EM STRUCTURES REVEAL PROGRESSIVE MEMBRANE CONSTRICITION BY THE ESCRT-III PROTEINS IST1 AND CHMP1B

Henry C Nguyen¹; Nathaniel Talledge²; John McCullough²; Wesley I Sundquist²; Adam Frost¹;  
¹University of California, San Francisco, Biochemistry and Biophysics, San Francisco, California, USA  
²University of Utah, Biochemistry, Salt Lake City, Utah, USA

The Endosomal Sorting Complexes Required for Transport (ESCRT) mediate critical membrane remodeling events throughout the mammalian cell cycle, including, but not limited to, HIV budding, cytokinetic abscission, and sealing of the nuclear envelope. ESCRT-III proteins polymerize into membrane-binding filaments to catalyze these reactions, but the structures and functions of these assemblies remain poorly understood. Our collaborative team recently determined the first atomic-resolution structure of an ESCRT-III filament – a hetero-polymer consisting of IST1 and CHMP1B. Our previous structure demonstrated how one of these subunits, CHMP1B, transitions from a “closed” to an “open” state to form an interlocked and domain-swapped filament. Moreover, we and others have shown that the IST1-CHMP1B copolymer participates in non-canonical, positive-curvature membrane fission pathways. Very recent work on other ESCRT-III proteins indicated that the mechanisms of opening and assembly we reported are conserved, but also raised questions regarding membrane binding and remodeling activities. To address these gaps in our understanding, we have determined the high-resolution structure of a membrane-bound IST1-CHMP1B assembly by cryo electron microscopy (cryoEM). We find that CHMP1B induces a high degree of curvature alone, and that deposition of the IST1 strand further constricts the membrane by more than 2-fold – almost to the fission point. Notably, the distance between outer leaflet lipid headgroups is ~10 nm and the distance between inner leaflet lipid headgroups is reduced to only ~4 nm. Conserved residues along helix a1 of CHMP1B serve as the major membrane binding surface and exploit both electrostatic as well as hydrophobic interactions with the convex leaflet of the membrane tubule. Our atomic-resolution cryoEM study reveals the structural mechanisms governing ESCRT-III assembly, membrane-binding, and positive-curvature membrane deforming activities.
COMPARISON OF THE TRANSMITTER RELEASE PROPERTIES BETWEEN THE CALYX OF HELD SYNAPSE AND MOSSY FIBER-CA3 SYNAPSE

Takeshi Sakaba¹;
¹Doshisha University, Kyotanabe, Kyoto, Japan

Calyx of Held synapses are known as one of the model synapses in the mammalian CNS, and exhibit short-term depression during repetitive stimulation. In contrast, hippocampal mossy fiber synapses show pronounced facilitation during repetitive stimulation and exhibit various forms of long-term synaptic plasticity. Biophysical analysis of this synapse-type remains to be carried out. To investigate the mechanism of transmitter release and presynaptic modulation at the mossy fiber synapse, we used presynaptic capacitance measurements and postsynaptic recording to measure the transmitter release kinetics. In addition, total internal reflection fluorescence (TIRF) microscopy was used to visualize dynamics of single synaptic vesicles near the plasma membrane. Readily releasable synaptic vesicles are mediated by fusion of already-tethered vesicles within the TIRF field. Vesicle replenishment had fast and slow phases. The fast phase of vesicle replenishment likely depends on synaptic vesicle priming from already-tethered vesicles rather than synaptic vesicle tethering toward the release sites. cAMP, a molecule crucial for short and long-term plasticity, mainly increases the vesicular release probability without changing the number of readily-releasable synaptic vesicles, cAMP possibly increased the release probability by changing the (spatial) coupling between Ca²⁺ channels and synaptic vesicles. We compared these findings, such as basic release properties and their modulation by second messengers, with those obtained at the calyx of Held synapse, and discussed similarities and differences between two synapse types. Supported by JSPS/MEXT KAKENHI grants (18H02530/17H05753) and the JSPS Core-to-Core program A. Advanced Research Networks.
RELEASE SITE RECRUITMENT AND ACTIVATION AS MECHANISMS OF PRESYNAPTIC PLASTICITY

Alexander M. Walter;
1Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany
2Institute for Biology/Genetics, Freie Universität Berlin, Berlin, Germany
3University of Southern California, Department of Neurobiology, Los Angeles, California, USA
4Institut für Neurophysiologie, Charité Universitätsmedizin, Berlin, Germany
5Max Planck Institute for Biophysical Chemistry, Department of Nanobiophotonics, Gottingen, Baden-Württemberg, Germany
6Buck Institute for Research on Aging, Novato, California, USA

Neuronal communication across synapses relies on neurotransmitter release from presynaptic active zones (AZs) followed by postsynaptic transmitter detection. Synaptic plasticity homeostatically maintains functionality during perturbations and enables memory formation. Postsynaptic plasticity targets neurotransmitter receptors, but presynaptic mechanisms regulating the neurotransmitter release apparatus remain largely enigmatic. By studying Drosophila neuromuscular junctions (NMJs) we show that AZs consist of nano-modular release sites and identify a molecular sequence that adds modules within minutes of inducing homeostatic plasticity. This requires cognate transport machinery and specific AZ-scaffolding proteins. Structural remodeling is not required for immediate potentiation of neurotransmitter release, but necessary to sustain potentiation over longer timescales. Finally, mutations in Unc13 disrupting homeostatic plasticity at the NMJ also impair short-term memory when central neurons are targeted, suggesting that both plasticity mechanisms utilize Unc13. Together, while immediate synaptic potentiation capitalizes on available material, it triggers the coincident incorporation of modular release sites to consolidate synaptic potentiation.
AN UNBIASED CLASSIFICATION APPROACH REVEALS MULTIPLE FUSION CATEGORIES OF VAMP2-MEDIATED EXOCYTOSIS

Fabio Urbina¹; Stephanie L Gupton¹;
¹University of North Carolina, Cell Biology and Physiology, Chapel Hill, North Carolina, USA

Exocytosis is a fundamental process that secretes cargo into the extracellular space and potentially inserts lipids and proteins into the plasma membrane. A pH-sensitive variant of GFP (pHluorin) fused to the luminal end of a vesicle-SNARE protein, such as VAMP2, provides a fluorescent intensity readout of the fusion pore opening during exocytosis and subsequent fate of the v-SNARE. We previously reported an automated analysis platform that identifies such exocytic events, and records several parameters of their fusion, such as the frequency, half-life fluorescence decay, and spatio-temporal distribution. This led us to hypothesize there were discrete types of exocytic events based on the behavior of the v-SNARE after fusion pore opening. Here, we introduce a novel machine-learning method to automatically classify TIRF images of VAMP2-pHluorin exocytic events in developing murine cortical neurons. We used multiple classifiers to categorize exocytic events in an unsupervised way. Using a majority-rule committee of 28 indices, run with each of the classifiers individually, we determined the most likely number of classes. The committee always selected four discrete classes of fusion, with each classifier similarly grouping exocytic events. This was surprising, as classically, two modes of fusion are recognized. During full-vesicle-fusion (FVF), the fusion pore dilates after opening and the vesicle collapses into the membrane. During kiss-and-run (KNR) exocytosis a transient fusion pore closes after cargo secretion. We discuss the validation of these four distinct modes of fusion and provide examples of biological manipulations that alter the proportion of events and their distribution in neurons, and how this affects neuronal morphogenesis.
FASTER AND BETTER: TAKING LOCALIZATION MICROSCOPY INTO LIVE CELLS

Susan Cox
King’s College London, London, United Kingdom

No Abstract

SINGLE MOLECULE LOCALISATION MICROSCOPY OF RECEPTOR SIGNALLING

Katharina Gaus;
1EMBL Australia Node, Single Molecule Science, Sydney, New South Wales, Australia
2ARC Centre of Excellence in Advanced Molecular Imaging, University of New South Wales, Sydney, New South Wales, Australia

Antigen recognition by the T cell receptor (TCR) is a hallmark of the adaptive immune system. When the TCR engages a peptide bound to the restricting major histocompatibility complex molecule (pMHC), it transmits a signal via the associated CD3 complex. How the extracellular antigen recognition event leads to intracellular phosphorylation remains unclear. We develop single-molecule localization microscopy (SMLM) approaches and novel analyses to determine how spatial organization regulates signal initiation and propagation. For example, we used SMLM data to map the organization of TCR-CD3 complexes into nanoscale clusters and found that the spatial organization determines signaling efficiency. This lead us to propose a model in which antigen recognition is first translated into receptor clustering and then the density of receptor nanoclusters is translated into signaling (Pageon et al. PNAS 2016). We also developed novel FRET sensors to monitor the rate of receptor clustering (Ma et al. Nat Commun 2017) and a sensor that reports membrane charges (Ma et al. Nat Biotech 2017) to understand how biophysical properties of the plasma membrane contribute to TCR signaling. More recently, we developed an improved single molecule microscope that achieves ~2-3 nm localization precisions and thus enables direct distance measurements between membrane proteins that were difficult to measure via fluorescence resonance energy transfer (FRET). Taken together, single molecule data provides an experimental framework to better understand the molecular mechanisms of receptor signaling.
VISUALIZING MEMBRANE STRUCTURAL REMODELING DURING FUSION AND FISSION IN LIVE CELLS

Ling-Gang Wu

Fusion and fission mediate many biological processes, such as exocytosis, endocytosis, intracellular trafficking, and viral entry. Despite intense studies, membrane structural remodeling mediating fusion and fission had not been real-time observed, and is thus poorly understood. With super-resolution STED microscopy, we observed the hemi-fused ?-shaped structure for the first time in live cells, the neuroendocrine chromaffin cells and pancreatic b-cells. This structure was generated from fusion pore opening or closure (fission) at the plasma membrane. Unexpectedly, its transition to full fusion or fission was determined by competition between fusion and calcium/dynamin-dependent fission mechanisms, and was surprisingly slow (seconds to tens of seconds) in a significant fraction of the events. These results provide key missing evidence over the past three decades proving the hemi-fusion and hemi-fission hypothesis in live cells, and reveal the hemi-fused intermediate as a key structure controlling fusion/fission, as fusion and fission mechanisms compete to determine its transition to fusion or fission. Passing through hemi-fusion, vesicle fusion at the plasma membrane generates an W-shape membrane profile, which may enlarge or shrink while maintaining vesicular membrane proteins. Closure of fusion-generated ?-profiles, which produces various sizes of vesicles, is a major mechanism mediating rapid and slow endocytosis. Strong calcium influx triggers dynamin-mediated closure, whereas weak calcium influx facilitates the merging of ?-profiles with the plasma membrane via shrinking rather than full-collapse as generally believed. W-profile shrinking and merging are mediated by strong membrane tension provided by dynamic assembly of filamentous actin, involving ATP hydrolysis, N-WASP and formin. In summary, we have visualized a series of membrane structural changes that mediate vesicle fusion, merging with the plasma have membrane, fission, their crucial function in exo- and endocytosis, and a part of their underlying molecular mechanisms.
THE STRUCTURE OF THE FUSION PORE

Manfred Lindau1,2; Satyan Sharma2; Ying Zhao2; Qinghua Fang2; Joannalyn Delacruz1; Shailendra Rathore1; Meng Huang1; Nathan Zimmerberg1;
1Cornell University, School of Applied & Engineering Physics, Ithaca, New York, USA
2Max-Planck-Institute for Biophysical Chemistry, Nanoscale Cell Biology Group, Göttingen, Niedersachsen, Germany

Release of neurotransmitters begins with a narrow fusion pore with an estimated diameter of ~1-2 nm based on electrophysiological measurements. To obtain a structural model of the fusion pore, we performed coarse grained molecular dynamics simulations of fusion between a nanodisc and a planar bilayer, bridged by four partially unzipped SNARE complexes. The simulations revealed that spontaneous re-zipping of the SNARE complexes pulls on the polar C-terminal residues of the synaptobrevin2 and syntaxin1 transmembrane domains to form a hydrophilic core between the two distal leaflets, leading to fusion pore formation. The estimated conductances of the fusion pores formed in these simulations are in agreement with experimental values. Two SNARE protein mutants that inhibit fusion experimentally produced no fusion pores in simulations. When the nanodisc was replaced by a 40 nm vesicle, fusion pore formation did not occur but instead an extended hemifusion diaphragm formed while the SNARE complexes moved away from each other, indicating that restricted SNARE mobility may be required for rapid fusion pore formation. Consistent with this hypothesis, fusion pores formed rapidly in the 40-nm vesicle-planar membrane system when the mobility of the SNARE complexes was restricted by external forces keeping the different SNARE complexes close to each other. Removal of the restriction is required for fusion pore expansion. These simulations provide a real time movie of spontaneous fusion pore formation and reveal a proteo-lipidic fusion pore structure. Experimentally, we recorded structural changes in the SNARE complexes associated with fusion pore formation by FRET imaging of SNAP-25 based constructs and simultaneous electrochemical imaging of individual fusion events using electrochemical detector arrays. The experiments indicate a rapid conformational change preceding fusion pore formation that is abolished by vSNARE deletion. The number of SNAP-25 constructs changing conformation depends on the size of the fusing vesicle. Supported by ERC, NIH.
Ca\(^{2+}\) triggered neurotransmitter release by synaptic exocytosis is a highly regulated process that enables neuronal communication. Synaptotagmin-1 (Syt1) is a calcium sensor protein that regulates synchronous neurotransmitter release. Despite extensive research, it remains a challenge to dissect the contribution of membrane remodeling induced by this protein to membrane fusion. Here, we use optical tweezers in combination with confocal fluorescence microscopy to quantify the protein-induced interactions between pairs of optically-trapped beads coated with synthetic membranes. We explore membrane remodeling in the presence of two proteins: Syt1 and Doc2b, a Ca\(^{2+}\) sensor protein that triggers spontaneous neurotransmitter release. We find that Syt1 and Doc2b strongly affect the probability and strength of membrane-membrane interactions in a strictly Ca\(^{2+}\) and protein-dependent manner. When comparing symmetrical (both sides) and asymmetrical (one side) presence of protein on the membranes, Syt1 favors an asymmetrical and Doc2b a symmetrical configuration, as inferred from higher tether probabilities and break forces. Further, we quantify the probability of hemifusion in the presence of Syt1 vs Doc2b, and find that Doc2b allows both hemifusion and membrane bridging, while Syt1 only bridges membranes. We also reveal lipid composition dependence of membrane remodeling by the calcium sensor proteins. To better understand how these proteins affect membranes, we probe membrane mechanical properties using a novel AFM-based method and reveal a decrease in membrane bending moduli following protein binding. Our results suggest an active role of C\(_2\)AB domains in membrane remodeling during the fusion process: in addition to bringing the membranes into close proximity, Syt1-C\(_2\)AB and Doc2b may also contribute to fusion by directly lowering the energy barrier. Overall, our approach provides new insights into the action mechanisms of calcium sensor proteins during neurotransmitter release, and can be readily extended to explore other membrane fusion events.
HOW AND HOW MUCH HAS IT FUSED? DETECTING FUSION INTERMEDIATES AND QUANTIFYING FUSION EFFICIENCY

Rafael B. Lira1; Tom Robinson1; Karin A Riske2; Rumiana Dimova1;
1Max Planck Institute of Colloids and Interfaces, Theory, Potsdam, Brandenburg, Germany
2Federal University of Sao Paulo, Biophysics, Sao Paulo, Brazil

Membrane fusion is a ubiquitous process in the cell. It transits through a number of fusion intermediates and involves the merging of two separated membranes, forming a compartment whose area is the sum of the two fusing bilayers; increase in area inevitably accompanies fusion. Due to the high complexity, membrane fusion is commonly studied with spectroscopic techniques and reconstituted bulk liposomal systems. They bear the disadvantage that intermediates are not explicitly detected, heterogeneity is hidden, efficiency is indirectly probed and increase in area is not measurable. We studied the fusion of cationic large unilamellar vesicles (LUVs) with giant unilamellar vesicles (GUVs) with increasing negative charge. We developed a method to detect fusion intermediates, quantify the fusion efficiency and the amount of membrane area gained via fusion on a single vesicle as assessed by observation of GUV morphology, intensity and lifetime FRET, content mixing and GUV electrodeformation. At low charge, LUVs dock and hemifuse to GUVs, increasing membrane tension that causes GUV rupture. At intermediate to high charge, fusion is very fast and efficient and the transferred lipids lead to GUV area gain, assessed from vesicle deformation in AC fields. The increase in area scales with GUV charge density. Initial LUV curvature leads to the formation of outward buds/tubules on the GUVs due to increased spontaneous curvature. Both hemifusion and full fusion are leakage-free. From FRET, we were able to retrieve the GUV final composition upon fusion. All of the above parameters can be measured in real-time on the single-vesicle level (Biophys. J. 116:79, 2019). The possibility to detect fusion intermediates, quantify fusion efficiency, retrieve membrane’s final composition and to manipulate membranes on a single-vesicle level opens up many possibilities not accessible with classical fusion systems.
NEED FOR SPEED: DYNAMIC NANOCLUSTERING AND UNCLUSTERING OF MUNC18/SYNTAXIN-1 DURING EXOCYTOSIS

Frederic A. Meunier;
1The University of Queensland, Queensland Brain Institute, Brisbane, Queensland, Australia

ADVANCED STATISTICS FOR SPATIO-TEMPORAL EVENTS OF EXOCYTOSIS

Giuliana Cortese
1University of Padova, Padova, Italy

Hormones and neurotransmitters are released when secretory granules or synaptic vesicles fuse with the cell membrane, a process denoted exocytosis. Modern imaging techniques, in particular total internal reflection fluorescence microscopy, allow the investigator to monitor secretory granules at the plasma membrane, before and when they undergo exocytosis, and to observe their spatial relation. However, rigorous statistical approaches for temporal and spatial analysis of such exocytosis data are still lacking. We show that various advanced statistical methods are well suited to analyze this biological process. We apply statistical regression models based on time-to-event analysis: granules are followed over time to occurrence of exocytosis events or to the end of the experiment, and the analysis can also be corrected for granules that dock during the experiment. We quantify the rate of exocytosis, as a function of time, in response to pulses of stimuli in insulin-secreting pancreatic β-cell, and how it depends on proteins levels surrounding the granules. To study heterogeneity in granule populations, we exploit frailty models, which account for unobserved differences between granules, or between cells. Another important biological question is whether events of exocytosis are spatially correlated, and if this association depends on local levels of proteins such as syntaxin. For this scope, spatial survival models and statistical models for spatial point patterns were applied. Moreover, we extended the analysis to settings where multiple events act simultaneously (e.g., exocytosis and undocking) by using multivariate spatial point processes. Among the results, we find that the rate of exocytosis significantly increases when the local density of calcium channels is higher, it depends on syntaxin levels and exocytosis events are spatially correlated, with correlation being significant within a certain distance. This novel application of advanced statistical methods should be useful also for the study of other well-defined spatio-temporal events at the cellular level.
BIRTH OF A NANODOMAIN: VESICLE DOCKING IS INITIATED BY RAB3 POSITIVE VESICLES IDENTIFYING RIM SITES TO TETHER AT THE PLASMA MEMBRANE

Nikhil R. Gandasi; Sebastian Barg
1Uppsala University, Biomedical Centrum, Institute of Medical Cell Biology, Uppsala, Uppsala län, Sweden

Insulin is released by regulated exocytosis, which requires secretory vesicles to be docked at the plasma membrane. The number of release ready vesicles at the plasma membrane is therefore rate limiting for hormone secretion. Stable docking is preceded by a loosely tethered state, and we showed recently that this transition occurs within seconds after arrival of the vesicle by recruitment of syntaxin and munc18 to the docking site. The molecular nature of the tethered state is not known and it remains elusive whether the vesicles tether to a preexisting receptor complex in the plasma membrane or attach to random sites. To answer this we quantified GTP-binding Rab proteins and their effectors at the docking site by imaging GFP-tagged proteins using TIRF microscopy. Clusters of the Rab3 interacting protein RIM and Rabphilin existed at docking sites prior to vesicle tethering and docking. A further increase in RIM fluorescence was seen at vesicles during their maturation into the releasable pool, confirming a role of RIM in priming. Vesicles that successfully docked carried Rab3 and Rabphilin whereas those that only temporarily tethered did not. In contrast, Rab27 and its effector Granuphilin were present on both types of vesicles. These results suggest that Rab3 and Rabphilin act on the incoming granules as signal to initiate the docking process. Since RIM is thus far the only protein found to be enriched at the docking site it may act as a docking receptor for the incoming vesicle.
MOLECULAR SIMULATIONS OF PROTEIN-MEDIATED MEMBRANE REMODELING

Herre Jelger Risselada\textsuperscript{1}; Laura Endter\textsuperscript{1};
\textsuperscript{1}Georg-August University, Theoretical Physics, Goettingen, Niedersachsen, Germany

Unlocking the molecular features on how molecules either accelerate or inhibit membrane remodeling both selectively and efficiently may help in generating novel therapeutic strategies. To this aim, we are developing state-of-the-art free energy calculation methods which can directly quantify how molecules alter molecular reaction pathways in conjunction with near-atomistic coarse-grained molecular simulations. We will illustrate a few examples on how free energy barriers challenge biological membrane remodeling processes and where proteins may come into play.
CELL PENETRATION AND MEMBRANE FUSION: TWO SIDES OF THE SAME COIN

Pavel Jungwirth¹;
¹IOCB Prague, Czech Academy of Sciences, Prague, Hlavní mesto Praha, Czech Republic

Cell penetrating peptides have a unique potential for targeted drug delivery, therefore, mechanistic understanding of their membrane action has been sought since their discovery over 20 years ago. While ATP-driven endocytosis is known to play a major role in their internalization, there has been also ample evidence for the importance of passive translocation for which the direct mechanism, where the peptide is thought to directly pass through the membrane via a temporary pore, has been widely advocated. Here, we question this view and demonstrate that arginine-rich cell penetrating peptides can instead enter vesicles and cells by inducing multilamellarity and fusion, analogously to the action of calcium ions. The molecular picture of this penetration mode, which differs qualitatively from the previously proposed direct mechanism, is provided by molecular dynamics simulations. In addition, the kinetics of vesicle agglomeration and fusion by nonaarginine, nonalysine, and calcium ions are documented in real time by fluorescence techniques and the induction of multilamellar phases in vesicles and cells is revealed both via electron microscopy and fluorescence spectroscopy. We thus show that the newly identified passive cell penetration mechanism is analogous to vesicle fusion induced by calcium ions, demonstrating that the two processes are of a common mechanistic origin.

INFLUENZA HEMAGGLUTININ-MEDIATED MEMBRANE FUSION: AN ALL-ATOM MOLECULAR DYNAMICS STUDY

Mahmoud Moradi¹; Ugochi Isu¹;
¹University of Arkansas, Chemistry and Biochemistry, Fayetteville, Arkansas, USA

The influenza hemagglutinin (HA) fusion protein is a canonical example of a viral fusion protein that mediates the cell entry of a membrane-enveloped virus. Since its structure was solved about four decades ago, extensive structural studies have aimed at deciphering the relationship between HA conformational changes and its ability to fuse lipid bilayers. We have used an extensive set of all-atom molecular dynamics simulations in conjunction with statistical mechanics based enhanced sampling techniques and ensemble-based simulation methods to visualize various conformational changes of the HA2 protein involved in the fusion mechanism. Here we focus on the large-scale conformational changes of HA2 in various stages that are primarily triggered by a change in the pH. In the first stage, the unstructured B loop of HA2 protein folds into a coiled-coil motif, extending the existing coiled-coil motif of HA2. Our simulations thus provide the first physics-based model of the elusive extended intermediate of HA2, where the fusion peptide inserts into the host cell membrane. The second stage involves partial unfolding of the central helix of HA2, which can be triggered by the protonation of a single histidine residue according to our simulations. Upon the formation of a hinge region, our simulations reveal that HA2 chain of hemagglutinin can bend. The HA2 conformational changes bring the membranes in close proximity, resulting in fusion of the viral envelope and the endosomal membrane. The novel computational methodology used in this work provides a detailed description of the mechanism of HA fusion protein and elucidates that how the protonation of a few amino acids can trigger the complex conformational changes of the HA2 protein, which effectively catalyze the fusion process.
CONFORMATIONAL DYNAMICS RELATED TO MEMBRANE FUSION OBSERVED IN SINGLE VIRAL ENVELOPE GLYCOPROTEINS

James B. Munro¹; Dibyendu K Das¹; Angela Howard¹;
¹Tufts University School of Medicine, Molecular Biology and Microbiology, Boston, Massachusetts, USA

All enveloped viruses enter cells by fusing the viral membrane with a cellular membrane. This fusion event is promoted by the viral envelope glycoprotein. The current model of class-I viral membrane fusion describes a static “spring-loaded” fusion domain. Upon triggering by acidic pH or interaction with a receptor, the fusion domain undergoes a singular, irreversible conformational rearrangement. Atomic-resolution structural models of several envelope glycoproteins describe the endpoints of this dynamic event. Yet no direct visualization of these conformational dynamics has ever been made, leaving the current model of class-I-mediated membrane fusion unverified. Here, using single-molecule Förster resonance energy transfer (smFRET) imaging we directly visualized the conformational changes of individual envelope glycoprotein trimers on the surface of viral particles. Thus far, we have visualized the dynamics of influenza hemagglutinin (HA), the canonical class-I viral glycoprotein, and Ebola GP, which shares structural similarities with HA. This novel approach was facilitated by introduction of non-canonical amino acids into HA, which were subsequently labeled with fluorophores. In contrast to the current model of class-I-mediated membrane fusion, we observe spontaneous and reversible exchange between pre-fusion and intermediate conformations. Acidification of pH and interaction with receptors shift the dynamic equilibrium, favoring progression toward membrane fusion. These observations are leading to a new understanding of viral membrane fusion, and conserved and divergent features of distinct class-I viral envelope glycoproteins.
VISUALIZING COMPENSATORY ENDOCYTOSIS DYNAMICS IN ‘XENAPSES’, TIRFM-AMENABLE SYNAPSES

Jurgen Klingauf;
1University of Münster, Institute of Medical Physics and Biophysics, Münster, Nordrhein-Westfalen, Germany

Maintaining synaptic transmission requires resorting and retrieval of the fused vesicle components by compensatory endocytosis. We showed in previous studies the existence of a pre-sorted and pre-assembled readily retrievable pool (RRetP) of synaptic vesicle proteins, and identified one of them, synaptophysin 1, to be a main organizer of the RRetP by forming hetero-oligomers with the v-SNARE synaptobrevin. However, are self-assembly forces of SV components sufficient for re-clustering or do endocytic adaptor proteins and clathrin contribute? To address this, we recently developed “Xenapses”, synapses formed by cultured mouse hippocampal neurons on micropatterned host substrates functionalized with synaptogenic proteins. Xenapses show all the hallmarks of a typical synapse both structurally (SV size and cluster, distinct exocytic and endocytic zones) and physiologically (calcium exocytosis coupling, exo-endocytic coupling). The expression of XFP fusion constructs of endocytic proteins allows investigating quantitative aspects of clathrin-mediated endocytosis at room and physiological temperatures. We found that clathrin light chain (EGFP-CLC) stably decorated the RRetP patches on the membrane surface in a punctate form. A fraction of these puncta disappeared seconds after the termination of stimulation pulse followed by slower full recovery of the RRetP. The initial decay correlates with endocytosis of RRetP patches. The slower time constant for recovery suggests RRetP formation to be the rate limiting step in endocytosis. Post-hoc unroofing of these xenapses and subsequent scanning electron microscopy of platinum replicas of the inner membrane surfaces revealed EGFP-CLC to localize to the clathrin-coated structures. Together, these observations constitute the first direct observation of the RRetP dynamics in space and time. In conclusion, clathrin and its adaptors are part of the RRetP co-polymer and provide spatio-temporal control for its formation which is necessary for efficient resorting and retrieval of vesicle components.
NOVEL FUNCTIONS OF ENDOPHILINS-A IN EXOCYTOSIS AND MEMBRANE TRAFFICKING

Sindhuja Gowrisankaran\(^1\); Vicky Steubler\(^1\); Sébastien Houy\(^3\); Johanna Peña del Castillo\(^1\); Monika Gelker\(^1\); Jana Kroll\(^1\); Nuno Raimundo\(^4\); Jakob B. Sørensen\(^3\); Ira Milosevic\(^1,2\);
\(^1\)European Neuroscience Institute (ENI), Synaptic Vesicle Dynamics, Göttingen, Niedersachsen, Germany
\(^2\)University Medical Center Göttingen (UMG), Göttingen, Niedersachsen, Germany
\(^3\)University of Copenhagen, Department for Neuroscience, Copenhagen, Hovedstaden, Denmark
\(^4\)University Medical Center Göttingen (UMG), Institute for Cellular Biochemistry, Göttingen, Niedersachsen, Germany

Release of vesicular content by exocytosis governs numerous biological events, including neurotransmission and neuromodulation. Following exocytosis, endocytosis retrieves the exocytosed vesicle membrane and proteins. Two processes are tightly coordinated, but molecular mechanisms underlying such coupling are not well understood. Endophilins-A are conserved endocytic adaptors with membrane curvature-sensing and -inducing properties, known to orchestrate various steps in clathrin-dependent and clathrin-independent endocytosis. We have found that, independently of their key roles in endocytosis, endophilin-A1 and endophilin-A2 regulate exocytosis of neurosecretory vesicles. The number of neurosecretory vesicles was not altered in cells without endophilin, yet fast capacitance and amperometry measurements revealed reduced exocytosis, smaller vesicle pools and changed fusion kinetics. Both endophilin-A1 (brain-enriched) and A2 (ubiquitous) rescued exocytic defects, but endophilin-A2 was more efficient. Distribution of neurosecretory vesicles was altered in the plasma membrane proximity, but levels and distributions of main exocytic and endocytic factors were unchanged, and slow compensatory endocytosis was not robustly affected. Endophilin’s role in exocytosis is mediated through its SH3-domain and, at least in part, interaction with intersectin, a coordinator of exocytic and endocytic traffic. Altogether, we discovered that endophilins-A, key endocytic proteins linked to neurodegeneration, also regulate exocytosis by controlling vesicle recruitment, priming and fusion.
MOLECULAR MECHANISMS OF NEURONAL EXOCYTOSIS

Axel T. Brunger\textsuperscript{1};  
\textsuperscript{1}Stanford University, Molecular and Cellular Physiology, Stanford, California, USA

The central nervous system relies on electrical signals traveling along neurons at high speeds. Signals are also transmitted between two neurons, or from a neuron to a muscle fiber through synaptic junctions. Synaptic transmission relies on the release of neurotransmitter molecules into the synaptic cleft. This release in turn depends on a process called membrane fusion to ensure that the neurotransmitter molecules that are contained in synaptic vesicles are released into the synaptic cleft as quickly as possible. Membrane fusion is an important process in many areas of biology, including intracellular transport and hormone release, but it occurs much faster (< 1 millisecond) for synaptic vesicle fusion than for these other processes. Moreover, it is precisely calcium regulated. Recent structural and biophysical studies of the molecular mechanisms of neurotransmitter release will be presented.\textbf{References} Zhou, et al. \textit{Nature} 548, 420-425 (2017). Lai, et al. \textit{Neuron} 95, 591–607 (2017). Brunger, et al. \textit{Trends in Cell Biology} 28, 631-645 (2018). Choi, et al. \textit{eLife} 7, e36497 (2018). White, et al. \textit{eLife} 7, e38888 (2018).
MITOCHONDRIAL MEMBRANE DYNAMICS VERSUS STEADY COMPARTMENTALIZATION: A CONTRADICTION? WHAT SUPERRESOLUTION IMAGING CAN TELL US

Karin B. Busch\textsuperscript{1,2}; Verena Wilkens\textsuperscript{2}; Timo Appelhans\textsuperscript{1,2};
\textsuperscript{1}WWU Münster, Biology, Münster, Nordrhein-Westfalen, Germany
\textsuperscript{2}University of Osnabrück, Biology and Chemistry, Osnabrück, Niedersachsen, Germany

The interplay between mitochondrial dynamics and ultrastructure guarantees mitochondrial functionality and adaptation. How this is regulated and how responsiveness is realized is still little understood. The sub-compartmentalization of the inner membrane in cristae provides numerous distinct bioenergetic compartments but at the same time restricts the diffusion and exchange of membrane proteins as well as their assembly. Another important aspect linked to this problem is the execution of quality control: does it occur locally or mitochondria-wide? In order to understand how regulation, adaptation and quality control are realized we need to know the impact of mitochondrial fusion and fission on the distribution and localization of proteins, and the impact of the specific ultrastructure on lateral protein dynamics. By using single molecule localization and tracking microscopy, multi-color superresolution imaging and Immuno-EM, we found that mitochondria, even after several fusion and fission cycles, have a hybrid character in terms of composition—or else phrased: cristae are rather conservative. We will show that this is correlated with zones of different activity. Single mitochondria are therefore not singular units but small mosaics.
CONSEQUENCES OF MITOCHONDRIAL FUSION CHANGES

Luca Scorrano
University of Padova, Padova, Italy

No Abstract

MEMBRANE BENDING ENERGY AND TENSION GOVERN MITOCHONDRIAL DIVISION

Dora Mahecic1,4; Lina Carlini1,4; Tatjana Kleele1,4; Adai Colom2,4; Antoine Goujon3,4; Stefan Matile3,4; Aurélien Roux2,4; Suliana Manley1,4;
1Ecole Polytechnique Federale de Lausanne, Institute of Physics, Lausanne, Waadt, Switzerland
2University of Geneva, Department of Biochemistry, Geneva, Genf, Switzerland
3University of Geneva, Department of Organic Chemistry, Geneva, Genf, Switzerland
4National Centre for Competence in Research Programme Chemical Biology, Geneva, Genf, Switzerland

Mitochondria are highly dynamic organelles, whose proliferation relies on the division of existing mitochondria. Many molecular factors required for mitochondrial division have been identified. However, a physical framework – explaining how energies and forces imposed by the cytoplasmic machinery regulate mitochondrial division – is currently missing. This is in part because of the challenges involved in quantifying the relevant physical parameters in living cells. Using time-lapse super-resolution structured illumination microscopy of mitochondria in live COS7 cells, we observe that the presence and active constriction by the division machinery does not ensure that the division process will continue to completion. Instead, potential division sites accumulate molecular components and constrict to ~100 nm before either dividing, or relaxing back to an unconstricted state. Our super-resolved images allow us to measure the shape of mitochondrial constriction sites, showing that constriction sites with higher local curvatures – reflecting an increased membrane bending energy – are more likely to divide. Analyses of mitochondrial motion and shape changes demonstrate that dividing mitochondria can be under significant tension. This is corroborated by measurements using a novel fluorescent membrane tension sensor, which allows direct visualization of membrane tension distribution across mitochondria. Furthermore, we reveal that perturbations to the microtubule network can greatly diminish mitochondrial membrane tension, and concomitantly reduce the probability that constrictions divide - implicating the microtubule cytoskeleton in the generation of membrane tension during mitochondrial division. These measurements allow us to establish a physical framework, based on in situ estimates of membrane bending energy and tension, that accounts for the observed probability and timing of mitochondrial division events.
Mitochondrial Genome Maintenance Protein1 (Mgm1) is a dynamin-like GTPase in fungi that remodels the mitochondrial inner membrane (IM). Mgm1 is involved in membrane fusion and in the stabilization of the cristae architecture. Since the underlying molecular mechanisms were unknown, we solved the 3.6 Å crystal structure of Mgm1 and determined the assembly mechanism of Mgm1 on membranes by cryo electron tomography. Whereas the overall domain composition of the Mgm1 structure is similar to other dynamin superfamily members, the assembly model is remarkably different to that of dynamin. Biochemical assays and molecular dynamic simulations confirm our structural data. Based on our new structures we propose molecular models how the assembly of Mgm1 on positive or negative membrane curvature remodels the IM of mitochondria.
ENDOCYTOSIS AND VESICLE REPLENISHMENT AFTER THE EXOCYTOSIS OF THE IMMEDIATELY RELEASABLE POOL IN MOUSE CHROMAFFIN CELLS

Mauricio D Montenegro¹; Lucas Bayonés¹; Cecilia Borassi²; Luciana I Gallo¹; Fernando D Marengo¹;
¹Instituto de Fisiología, Biología Molecular y Neurociencias. Consejo Nacional de Investigaciones Científicas y Técnicas. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Departamento de Fisiología y Biología Molecular y Celular, Buenos Aires, Capital Federal, Argentina
²Fundación Instituto Leloir. Consejo Nacional de Investigaciones Científicas y Técnicas, , Buenos Aires, Capital Federal, Argentina

The immediately releasable pool (IRP) is a group of ready releasable vesicles sensitive to short depolarizations because of their proximity to voltage dependent Ca²⁺ channels. Because of these characteristics, it was proposed that IRP is important in secretion during action potentials applied at low frequencies, like action potential firing in chromaffin cell basal physiological conditions. However, previous reports showed that IRP replenishment after depletion is too slow to manage such situation. In this work we used patch-clamp measurements of membrane capacitance, confocal imaging of F-actin distribution and cytosolic Ca²⁺ measurements with Fura 2 to re-analize this question. We provide evidences that IRP replenishment has one slow (time constant between 5-10 s) and one rapid component (time constant between 0.5-1.5 s) linked to a fast, dynamin dependent, endocitosis. Both, the fast endocitosis and the rapid replenishment component were eliminated when 500 nM Ca²⁺ was added to the internal solution during patch-clamp experiments, but they became dominant and were fastened when the cytosolic Ca²⁺ buffer capacity was increased. In addition, both rapid replenishment and fast endocitosis were retarded when cortical F-actin cytoskeleton was disrupted with cytochalasin D. Finally, in permeabilized chromaffin cells stained with phalloidin rhodamin the cortical F-actine density was reduced when the Ca²⁺ concentration was increased in a range of 10–1000 nM. We conclude that low cytosolic Ca²⁺ concentrations, which favor cortical F-actin stabilization, allow the activation of a fast endocitosis mechanism and an associated rapid replenishment component of IRP exocytosis.
THE POWER OF CORRELATIVE SUPER-RESOLUTION IMAGING

Aleksandra Radenovic1,2;
1EPFL School of Engineering, Lausanne, Switzerland
2Institute of Bioengineering, Laboratory of Nanoscale Biology1015, Lausanne, Switzerland

In this talk I will review recent progress in correlative super-resolution imaging. First part of the talk will be dedicated to our efforts to build and characterize a correlated single molecule localization microscope- with an atomic force microscope (SMLM)/AFM that allows localizing specific, labelled proteins within high-resolution AFM images in a biologically relevant context. The technique has been successfully applied to the live and fixed samples. The second part of my talk will be dedicated to the discussion on the complementarity between SMLM and super-resolution optical fluctuation imaging (SOFI).

References
HOW IS GRANULE RELEASE AFFECTED BY LOCATION AND NUMBER OF DIFFERENT TYPES OF CA$^{2+}$ CHANNELS? MARKOV CHAIN MODELS PROVIDE ANALYTIC RESULTS

Francesco Montefusco$^1$; Morten G Pedersen$^{1,2,3}$;
$^1$University of Padova, Department of Information Engineering, Padova, Italy
$^2$University of Padova, Department of Mathematics "Tullio Levi-Civita", Padova, Italy
$^3$University of Padova, Padova Neuroscience Center, Padova, Italy

Most endocrine cells release hormones by calcium-regulated exocytosis: in response to a series of cellular mechanisms culminating with an increase in the intracellular Ca$^{2+}$ levels, secretory granules fuse with the cell membrane and release hormones. Here, we devise a methodology in order to characterize the local interactions between granules and Ca$^{2+}$ channels (CaVs), by developing Markov chain models that allow us to obtain analytic results for the expected exocytosis rate. First, we analyze the property of the secretory complex obtained by coupling a single granule with one CaV and, then, we extend our results to a more general case with $n$ CaVs. We show that the distance of granule from CaVs is a major factor in determining the exocytosis rate, as recently demonstrated. Moreover, we assume that the granule forms complexes with inactivating or non-inactivating CaVs. We find that increasing the number of CaVs coupled with the granule determines a much higher rise of the exocytosis rate, which in the case of inactivating CaVs is more pronounced when the granule is close to CaVs (about 10 nm), whereas for non-inactivating CaVs the highest relative increase in rate is obtained when the granule is far from CaVs (about 50 nm), suggesting that it is not necessary that the granule is very close to CaVs for triggering exocytosis. Finally, we study the relationship between calcium influx and exocytosis and find that the quantities are typically linearly related, as experimentally observed. For the case of inactivating CaVs, our analysis shows a change of the linear relation due to near-complete inactivation of CaVs: during inactivation, the effective number of CaVs reduces and hence the relation slope decreases. This confirms that a concave relation between exocytosis and calcium influx does not necessarily reflect granule pool depletion and provides a new example of such a scenario.
POSTER ABSTRACTS
Tuesday, May 7, 2019  
Poster Session I  
14:30 – 16:00  
University Botanical Gardens of Padova

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Tuesday. Presenting authors with odd-numbered poster boards should present from 14:30 – 15:15 and those with even-numbered poster boards should present from 15:15 – 16:00. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

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Posters should be set up the evening of May 6 and removed by noon on May 10.
GEOMETRIC INSTABILITY CATALYZES MITOCHONDRIAL FISSION

Ehsan Irajizad⁠¹; Rajesh Ramachandran⁠²; Ashutosh Agrawal⁠¹;  
¹University of Houston, Mechanical Engineering, Houston, Texas, USA  
²Case Western Reserve University, Department of Physiology and Biophysics, Cleveland, Ohio, USA

In eukaryotic cells, tubular mitochondria form intricate networks and undergo incessant fission and fusion. While balanced mitochondrial dynamics is believed to be essential for apoptosis, disrupted dynamics is linked to lung cancer, cardiac dysfunction and neurogenerative disorders. Pioneering experimental studies have provided insights into the molecular machinery that executes mitochondrial constriction and fission. The fission pathway is characterized by three key steps: i) the initial constriction carried out by actin polymerization and actomyosin contraction, ii) the intermediate constriction executed by Drp1, and iii) the final fission carried out by dynamin. While the membrane-squeezing proteins are recognized as the key drivers of fission, there is a growing body of evidence that strongly suggests that conical lipids play a critical role in regulating mitochondrial morphology and fission. However, the mechanisms by which proteins and lipids cooperate to execute fission have not been quantitatively investigated. Here, we computationally model tubular mitochondria to reveal a new buckling instability-based mechanism for achieving super constrictions. Employing membrane physics and differential geometry, the study reveals that buckling instabilities, triggered synergistically by cylindrical curvatures generated by proteins and spherical curvatures generated by conical lipids, can lead to extreme necking required for fission. We validate the role of conical lipids by an in vitro study in which membrane tubules with reduced concentration of conical lipids (PE) fail to undergo necking despite the presence of Drp1 proteins. Our study suggests that the buckling-induced geometric plasticity imparts significant robustness to the fission reaction by arresting the elastic tendency of the membrane to rebound during protein polymerization and depolymerization cycles. Since protein/lipid-induced curvatures are ubiquitous mechanisms for driving membrane remodeling in cells, energetic frustration due to incompatible curvatures leading to instability could be a general mechanism at play in other interfacial remodeling events in cells.
TARGETING THE HENDRA VIRUS FUSION PROTEIN TRANSMEMBRANE DOMAIN TO INHIBIT VIRAL MEMBRANE FUSION

Chelsea Barrett¹; Stacy R Webb¹; Rebecca Dutch¹;
¹University of Kentucky, Molecular and Cellular Biochemistry, Lexington, Kentucky, USA

Enveloped viruses utilize surface glycoproteins to attach to host cells and initiate fusion of the viral envelope with the host cell membrane. Hendra virus, a highly pathogenic, zoonotic, enveloped virus in the family *Paramyxoviridae*, uses two surface proteins, the attachment protein (G) and the fusion protein (F), to carry out binding and viral entry. The F protein is synthesized as a trimer in a pre-fusion meta-stable conformation that, upon receipt of a triggering signal, undergoes a large, essentially irreversible, conformational change that drives fusion of the virus and host cell membranes. Previous research has demonstrated that the transmembrane domain (TMD) of F plays a key role in the protein’s trimeric association and overall protein stability, including stability of the pre-fusion conformation. To characterize the effect of disrupting these TMD interactions on the fusion process, exogenous Hendra F TM constructs were created containing the full TM domain and limited adjacent sequences. When these TM constructs were co-expressed with the full length Hendra F protein, a significant reduction in the expression and stability of the full length protein was observed. Furthermore, the F protein had a significantly impaired ability to facilitate viral and host cell membrane fusion when the TM constructs were present. In contrast, when the Hendra TM constructs were co-expressed with Parainfluenza Virus 5 (PIV5) F, another member of *Paramyxoviridae*, no decrease in PIV5 F expression or fusion activity was observed, suggesting the protein disruption by the TM constructs occurs in a sequence specific manner. These results when taken together, demonstrate that TMD interactions can be targeted to disrupt protein function and ultimately viral entry, even after the protein has trafficked to the viral membrane.
BURSTING VERSUS SPIKING: SYSTEMATIC INVESTIGATION OF HOW PATTERNS OF ELECTRICAL ACTIVITY CONTROL LOCAL CA\(^{2+}\) AND HORMONE RELEASE

Iulia Martina Bulai\(^1\); Morten Gram Pedersen \(^{1,2}\); Joel Tabak\(^3\);
\(^1\)University of Padova, Department of Information Engineering, Padova, Italy
\(^2\) University of Padova, Department of Mathematics "Tullio Levi- Civita" Padova, Italy
\(^3\)University of Exeter, Medical School, Exeter, Devon, United Kingdom

Exocytosis is the fusion of the membranes of hormone-containing secretory granules with the cell membrane, which releases hormone molecules into the extracellular space. Endocrine pituitary cells secrete different types of hormones. These cells contain a wide range of ion channels and are electrically excitable with some cell types exhibiting spiking (repetitive action potential firing), and others exhibiting bursting (silent phases interspersed by active ones). The different patterns of electrical activity are believed to induce different amounts of hormone release from the different pituitary cell types. We study how different patterns of electrical activity control hormone secretion. We generate spiking patterns characterized by the same action potential duration but different periods of silence, and do the same for bursting. From our numerical simulations we observe that bursting generally results in larger CA\(^{2+}\) influx than spiking, since the cell is electrically active for a longer time. Secondly, we find that close to the channel (30 nm) the CA\(^{2+}\) concentration reaches tens of µM while far from the channel (500 nm) it reaches units of µM. To measure the efficiency of CA\(^{2+}\) in triggering exocytosis, we compare the cumulative number of fused granules (E) versus the integral of the measured CA\(^{2+}\) -current (Q) at different distances from the CA\(^{2+}\) channel for both spiking and bursting. We observe that close to the channel (30 nm) the E-vs-Q curves for both spiking and bursting are almost superimposed, and conclude that spiking and bursting are equally CA\(^{2+}\) efficient in triggering exocytosis. At 500 nm from the channel bursting is slightly more efficient than spiking, since the E-vs-Q bursting curves are above the curves that corresponds to spiking. In conclusion, the pattern of electrical activity is important for controlling exocytosis in subtle ways that depend also on the distance between CA\(^{2+}\) channels and granules.
NUCLEAR RECRUITMENT OF DRP6 IS MEDIATED BY CARDIOLIPIN INTERACTION AND REGULATED BY POST-TRANSLATIONAL MODIFICATION

Himani Dey¹; Usha P Kar¹; Abdur Rahaman¹;
¹National Institute of Science Education and Research, HBNI, School of Biological Sciences, Bhubaneswar, Orissa, India

Dynamins and dynamin related proteins are a large GTPases involved in various cellular processes including vesicle scission, fission and fusion of organelles, vacuolar trafficking and viral resistance. Dynamin Related Protein 6 (Drp6) in Tetrahymena localizes on the nuclear envelope (NE) and as cytoplasmic punctae, and is required for nuclear remodelling during macronucleus development. In this study, we report the mechanism of nuclear recruitment of Drp6 and its regulation. Using a lipid overlay assay with various deletion fragments of Drp6, the Lipid Binding Domain was mapped at the position equivalent to the Pleckstrin Homology (PH) domain of dynamin. Using a point mutation (I553M) in the lipid binding domain, we have demonstrated that the mutant fails to associate with nuclear envelope. Various biophysical and biochemical analysis show that the loss of nuclear recruitment is due to a defect in its interaction with cardiolipin and not in its self-assembly, ultra-structure or GTPase activity. This was further confirmed in vivo by depleting cardiolipin in cells expressing GFP-tagged Drp6. Drp6 has differential localisation at different developmental stages. To investigate if post-translational modification is involved in regulation of differential localisation, Drp6 expressed in Tetrahymena was purified and subjected to mass-spectrometric analysis. Four Serine residues (at positions 86,248,701,705) were found to be phosphorylated. Our cellular and biochemical studies show that phosphorylation at Ser248 prolongs its nuclear association, inhibits its GTPase activity and enhances its affinity for cardiolipin enriched membrane without affecting its self-assembly. These results suggest that phosphorylation at Ser248 regulates nuclear recruitment by affecting its GTPase activity and membrane interaction.
XENAPSES GROWN ON QUANTIFOIL-SUPPORTED TEM GRIDS: TOWARDS CRYO-ELECTRON TOMOGRAPHY OF EXOCYTIC AND ENDOCYTIC STRUCTURES

Junxiu Duan\textsuperscript{1,2}; Sai Krishnan\textsuperscript{1}; Yaroslav Tsytsyura\textsuperscript{1}; Nataliya Glyvuk\textsuperscript{1}; Ulrike Keller\textsuperscript{1}; Jürgen Klingauf\textsuperscript{1};
\textsuperscript{1}University of Münster, Institute of Medical Physics and Biophysics, Münster, Nordrhein-Westfalen, Germany
\textsuperscript{2}University of Münster, CiM-IMPRS Graduate Program, Münster, Nordrhein-Westfalen, Germany

For studying quantitative aspects of synaptic exo- and endocytosis at the molecular level, we have developed cultured ‘xenapses’, TIRFM-amenable purely pre-synaptic boutons. These are formed by murine hippocampal neurons cultured on microstructured glass coverslips which have been functionalized with synaptogenic proteins. In order to correlate results from fluorescence microscopy (TIRFM and dSTORM/PALM) with the endocytic ultrastructure we here developed methods for micropatterning and functionalization of Quantifoil films on transmission electron microscopy (TEM) grids. While on glass coverslips micropatterning is easily achieved by microcontact printing of the functionalized polymer, for the thin and brittle Quantifoils we developed a bottom up approach involving click chemistry and UV-photolithography. Xenapses are formed on TEM grids within a few days. Their functionality was confirmed by calcium and pHluorin imaging experiments. At xenapses expressing synaptotagmin-pHluorin we observed robust exocytosis and subsequent compensatory endocytosis upon stimulation. Xenapses, cryo-fixed by high-pressure freezing, displayed well-preserved ultrastructure, with large numbers of synaptic vesicles at or near the pre-synaptic membrane. Our results show that structure and function of xenapses on TEM grids do not differ from their counterparts on glass coverslips. Thus, xenapses grown on Quantifoil-supported TEM grids enable cryo-electron tomography of endocytic structures at different time points after stimulation.
FINGERPRINTING LABELING-INDUCED INSULIN GRANULE ALTERATIONS IN LIVING β-CELLS BY SPATIOTEMPORAL FLUCTUATION SPECTROSCOPY

Gianmarco Ferri1; Luca Digiacomo2; Zeno Lavagnino3; Margherita Occhipinti4; Marco Bugliani4; Giulio Caracciolo3; David W Piston3; Francesco Cardarelli1;
1a NEST - Scuola Normale Superiore, Istituto Nanoscienze - CNR (CNR-NANO), Pisa, Italy
2Department of Molecular Medicine, "La Sapienza" University Roma, Roma, Italy
3Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri, USA
4Department of Clinical and Experimental Medicine, Islet Cell Laboratory, University of Pisa, Pisa, Italy

The intracellular life of insulin secretory granules (ISGs) from biogenesis to secretion depends on their structural (e.g. size) and dynamic (e.g. diffusivity, mode of motion) properties. Thus, it would be useful to have rapid and robust measurements of such parameters in living β-cells. To provide such measurements, we have developed a fast spatiotemporal fluctuation spectroscopy method that lead to the calculation of an imaging-derived Mean Squared Displacement (iMSD), which simultaneously provides the size, average diffusivity, and anomalous coefficient of ISGs motion, without the need to extract individual trajectories. Clustering of the aforementioned quantities in a three dimensional parametric space defines the ISGs ‘fingerprint’ for different conditions. First, we create a reference using INS-1E cells expressing c-peptide fused to a fluorescent protein (FP) under basal culture conditions and validate our analysis by testing well-established stimuli: glucose intake, cytoskeleton disruption and cholesterol overload. After, we investigate the effect of other FP-tagged ISG protein markers on the structural and dynamic properties of the granule. While for most of the luminal markers iMSD analysis produces similar results, the transmembrane marker phogrin-FP shows a clearly altered result. Phogrin overexpression induces a substantial granule enlargement and higher mobility, together with a partial de-polymerization of the actin cytoskeleton and a reduced cell responsiveness to glucose stimulation. Our data suggest a more careful interpretation of many previous ISG-based reports in living β-cells. The presented data pave the way to high-throughput cell-based screening of ISG structure and dynamics under various physiological and pathological conditions.
REGULATION OF VESICLE ACIDIFICATION BY RABCONNECTIN-3A

**Sindhuja Gowrisankaran**¹; Andrea Raimondi²; Nicolas de Roux³; Ira Milosevic¹;
¹European Neuroscience Institute, University Medical Center Göttingen (UMG), Göttingen, Niedersachsen, Germany
²Advanced Light and Electron Microscopy BioImaging Center (ALEMBIC), Hospital San Raffaele, Milano, Italy
³Inserm U1141, Hospital Robert Debré, University Paris Diderot, Paris, France

The regulation and timing of vesicle acidification is essential for numerous cellular processes, from macro-molecule degradation in lysosomes to refilling of synaptic vesicles (SVs) at the neuronal synapse. Acidification of vesicles is achieved by vacuolar ATPases (vATPases), a family of proton pumps that controls the pH gradient across organelle membranes. Despite their critical importance at the synapse and in many intracellular trafficking routes, the regulation of vATPase activity is poorly understood. In a search for vATPase regulators, we cloned human *Dmxl2* gene encoding Rabconnectin-3a (Rbcn-3a). Rbcn-3a encodes a large 340kDa protein, whose function at the mammalian synapse remains largely unknown. An alteration in the gene dosage of *Dmxl2* in humans resulted in a complex pathology called as poly-endocrine-polyneuropathy syndrome (PEPNS). We found Rbcn-3a to be present on every organelle that acidifies; including SVs. Loss of Rbcn-3a in mice resulted in early embryonic lethality. When Rbcn-3a is eliminated from neuronal cells in culture, neurons developed normally, yet their activity was perturbed. In addition, pHluorin-based imaging revealed an alteration in SV recycling in neurons. Single vesicle analysis with a voltage sensor (Fluorovolt dye) showed that SVs from neurons without Rbcn-3a failed to acidify fully. Additionally, an ultrastructural examination of neurons without Rbcn-3a showed a reduction in the number of SVs, as well as accumulation of lysosomes-like structures and lysosomal markers. Our results suggests an unanticipated connection between the machinery for acidification, vesicle recycling and cellular homeostasis.
THE MOLECULAR MACHINERY OF MEMBRANE FUSION: SNARES VERSUS SYNAPTOTAGMIN

Cameron B Gundersen1;
1David Geffen UCLA School of Medicine, Molecular & Medical Pharmacology, Los Angeles, California, USA

Fast, synchronous exocytosis at mammalian nerve terminals takes place in <0.1 msec (Sabatini & Regehr, 1996; Nature 384:170-172). While it is clear that synaptotagmin-1 (syt-1) is the Ca2+ sensor for this process (Sudhof, 2014; Angew. Chem. Int. 53:12696-717.), the mechanism by which syts engage other proteins, including the SNAREs, munc-13, munc-18 and complexin, to catalyze membrane fusion remains unclear. This presentation will compare and contrast two models for how syts (without SNAREs) or the SNARE proteins, synaptobrevin-2 (syb-2) and syntaxin 1A/B (syx), might drive the membrane fusion step of fast, synchronous exocytosis. A key feature of these models is that specific domains of syt-1 or syb-2 and syx need to adopt a b-strand conformation. We proposed (Gundersen & Umbach, 2013; J. Theor. Biol. 332:149-60) that the presence of b-strand in the 12 residues (which include the fatty acylated cysteine residues of syt-1) following the membrane-affiliated a-helix would enable a quartet of syts to be positioned at the region of contact between a synaptic vesicle and the plasma membrane. By executing a sequence of steps (see: Gundersen & Umbach, 2013), these syts could serve as templates for Ca2+-triggered membrane fusion. Although this syt-based proposal remains hypothetical, recent experiments using a syt-1 peptide support the idea that the fatty acylated domain of syt-1 adopts b-structure in lipid bilayers. Nevertheless, further testing of this model is necessary. At the same time, it also became evident that intra-membrane b-structure could be exploited to turn syb-2 and syx into “membrane-fusion” machines (Gundersen, 2017; Int. J. Mol. Sci. 18: E1582). This model demands that the domains of syb-2 and syx currently represented as membrane-spanning a-helices temporarily adopt intra-membrane b-structure. If these b-strands are suitably arrayed at the vesicle-plasma membrane interface, a b-to-a transition (triggered by SNARE zippering) can initiate membrane fusion (see: Gundersen, 2017).
SYNAPTOTAGMIN’S CA\textsuperscript{2+} DEPENDENT ACTION ON MEMBRANE FUSION INITIATION AND FUSION PORE EXPANSION

Volker Kiessling\textsuperscript{1,2}; Alex J B Kreutzberger\textsuperscript{1,2}; Binyong Liang\textsuperscript{1,2}; Sarah B Nyenhuis\textsuperscript{1,3}; J David Castle\textsuperscript{1,4}; David S Cafiso\textsuperscript{1,3}; Lukas K Tamm\textsuperscript{1,2};
\textsuperscript{1}University of Virginia, Center for Membrane and Cell Physiology, Charlottesville, Virginia, USA
\textsuperscript{2}University of Virginia, Dept. of Molecular Physiology and Biological Physics, Charlottesville, Virginia, USA
\textsuperscript{3}University of Virginia, Dept. of Chemistry, Charlottesville, Virginia, USA
\textsuperscript{4}University of Virginia, Dept. of Cell Biology, Charlottesville, Virginia, USA

It has long been known that synaptotagmin-1 (Syt1) is the sensor that triggers fast, evoked release of neurotransmitter by fusion of synaptic vesicles to the presynaptic membrane in response to calcium. It is also well established that the SNAREs syntaxin-1a, SNAP-25, and synaptobrevin-2/VAMP-2 form the core of the membrane fusion machinery that drives calcium-triggered neuronal exocytosis. However, how synaptotagmin-1 and the fusion machinery are mechanistically coupled has been the subject of much debate. We proposed a mechanism where the lipid bilayer is intimately involved in coupling calcium sensing to fusion (Kiessling et al. 2018). Using TIRF- (total internal reflection fluorescence) and sdFLIC (site-directed fluorescence interference contrast) microscopy, we demonstrated that fusion of PC12 cell-derived dense core vesicles is strongly linked to the tilt angle of the cytoplasmic domain of the nascent SNARE complex with respect to the plane of the target membrane. As the tilt angle increases, force is exerted on the SNARE transmembrane domains to drive the merger of the two bilayers as the trans-SNARE complex completes folding into the cis-SNARE complex. The tilt angle is modulated by the order of the lipid bilayer, and the order of the bilayer is changed by Ca\textsuperscript{2+} dependent binding of the two C2 domains of Syt1, i.e. not necessarily requiring direct interactions between Syt1 and the nascent SNARE complex. In addition to fusion efficiencies and fusion kinetics, TIRF data from single vesicle fusion events contains information about how the fluorescent content is released. Of particular interest is, how fast the content is released. Here, we present data that show how different mutations in Syt1’s C2 domains, that are known to impair synaptic transmission in neurons, independently influence Ca\textsuperscript{2+} dependent fusion and fusion-pore expansion. Kiessling, V., et al. Nat.Struct.Mol.Biol. 25. 911-917 (2018).
UNRAVELLING THE MOLECULAR DYNAMICS OF ENDOCYTIC ACTORS USING “XENAPSES”, TIRFM-AMENABLE SYNAPSES

Sai Krishnan¹; Julia Lehrich¹; Junxiu Duan¹; Natalya Glyvuk¹; Yaroslav Tsytsyura¹; Ulrike Keller¹; Jürgen Klingauf¹;
¹Westfälische Wilhelms-Universität, Institute of Medical Physics and Biophysics, Münster, Nordrhein-Westfalen, Germany

In the presynapse, endocytosis takes on a specialized role due to the need for synaptic vesicles to be compensated in response to exocytosis. The fission process requires the choreography of a number of endocytic proteins which ensures formation of synaptic vesicles with high fidelity. However, despite decades of research on presynaptic vesicle recycling the precise function of the individual endocytic factors and their molecular dynamics remains hotly debated. To address this we developed “Xenapses”, TIRFM-able synapses, formed by mouse hippocampal neurons cultured on micropatterned host substrates coated with synaptogenic proteins. Xenapses show all the characteristics of a typical synapse and allow us to directly observe the behavior of proteins involved in compensatory endocytosis.

Our initial investigations followed up on our previous findings on the existence of a pre-sorted and pre-assembled readily retrievable pool (RRetP). In live xenapses, expressed EGFP-clathrin light chain (EGFP-CLC) disappeared seconds after the termination of stimulation pulse followed by slower full recovery. This response profile likely indicates the endocytosis of surface clathrin followed by the nucleation of clathrin RRetP for subsequent round of endocytosis. Xenapses also lend themselves to unroofing, revealing via SEM the EGFP-CLC to stably decorate the clathrin structures on the membrane surface. Together, these findings strengthen the evidence for the RRetP co-polymer and for clathrin to be part of it.

Extending this to other endocytic proteins revealed the fission protein dynamin 1-EGFP and the BAR-domain amphiphysin 1-EGFP to be recruited to the pre-synaptic membrane in a stimulus-dependent manner, in contrast to clathrin. EGFP-SNX9 on the other hand exhibits the same response profile as clathrin, suggesting it is part of the RRetP in the presynapse. Future work will focus on delineating with high spatial and temporal resolution the interaction between these and other endocytic proteins.
LIGAND-PROTECTED AU NANOPARTICLES DRIVE LIPOSOLE-LIPOSOLE INTERACTIONS

Enrico Lavagna\textsuperscript{1}; Sebastian Salassi\textsuperscript{1}; Giulia Rossi\textsuperscript{1};
\textsuperscript{1}University of Genoa, Physics department, Genoa, Genova, Italy

Membrane fusion is a ubiquitous and fundamental process in biological systems. The artificial synthesis of fusogenic agents can, on the one hand, shed light on the basic physical mechanisms underlying spontaneous fusion\cite{1} and, on the other hand, it can lead to new applications, such as the directed delivery of encapsulated reagents to cells or liposomes. All fusion reactions embody an elementary process that includes membrane contact, membrane merging, and opening of a fusion pore between the water compartments of the vesicles. Here we explore the potential fusogenic activity of a class of Au nanoparticles which have been previously shown to interact favorably with neutral lipid membranes\cite{2,3}. We perform molecular dynamics simulations with coarse-grained resolution and show that anionic, ligand-protected Au nanoparticles (NPs) can drive liposome-liposome interactions and cause liposome clustering, as recently shown at experimental level \cite{4}. Moreover, the NPs accumulate at liposome-liposome interfaces and can cause the formation of inter-liposome bilayer structures. These results suggest that by designing specific ligands with fusogenic activity to be covalently linked to the NP surface may be a promising route towards the development of synthetic fusogenic nanoparticles.\cite{1,2,3,4}

D’Agostino, M.; Nature, 2017\cite{1}
Simonelli, F; J. Phys. Chem. Lett., 2015\cite{2}
Salassi, S.; J. Phys. Chem. C, 2017\cite{3}
Atukorale, P. U.; Bioconjug. Chem., 2018\cite{4}
CA\textsuperscript{2+}-INDEPENDENT AND VOLTAGE-DEPENDENT EXOCYTOSIS IN MOUSE CHROMAFFIN CELLS

José Moya-Díaz\textsuperscript{1}; Lucas Bayonés\textsuperscript{1}; Mauricio Montenegro\textsuperscript{1}; Fernando D. Marengo\textsuperscript{1};
\textsuperscript{1}Instituto de Fisiología, Biología Molecular y Neurociencias. Consejo Nacional de Investigaciones Científicas y Técnicas. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Departamento de Fisiología y Biología Molecular y Celular, Buenos Aires, Capital Federal, Argentina

It is widely demonstrated that the exocytosis of synaptic and secretory vesicles is triggered by a localized Ca\textsuperscript{2+} increase associated to the activation of voltage dependent Ca\textsuperscript{2+} channels. However, in neurons and neuroendocrine cells there are evidences of a different mode of fast exocytosis, induced in response to membrane depolarization but lacking Ca\textsuperscript{2+} current and intracellular Ca\textsuperscript{2+} increase. In this work we investigated if such a mechanism can contribute to secretory vesicle exocytosis in mouse chromaffin cells. The application of brief depolarizations on cells bathed in total absence of extracellular Ca\textsuperscript{2+} induced moderate but consistent exocytosis, evaluated by patch-clamp membrane capacitance measurements and amperometry. This phenomenon was also reproduced when the release of Ca\textsuperscript{2+} from intracellular stores was pharmacologically inhibited and when high concentrations of BAPTA were introduced in the cytosol. Moreover, no increase in cytosolic Ca\textsuperscript{2+} was observed when measured with Fluo-8. This exocytosis process is dependent on the applied membrane potential, reaching the 50% of the saturating value at approximately -30 mV, and was inhibited by the neurotoxin Bont-B that specifically cleaves the SNARE protein synaptobrevin. This Ca\textsuperscript{2+}-independent and voltage-dependent exocytosis is very fast, saturating with a time constant < 10 ms, and recovers completely after depletion in less than 5 s. Due to these characteristics this mechanism is able to maintain synchronous fast exocytosis during action potential like stimuli applied at low frequencies. Finally, our data suggest that P/Q-type voltage dependent Ca\textsuperscript{2+} channels are suitable voltage sensors coupled to the activation of this mechanism of exocytosis.
ULTRASTRUCTURE OF ACTIN FUSION FOCUS IN *S. POMBE* DURING CELL-CELL FUSION

**Olivia Muriel-Lopez**¹; Laetitia Michon¹; Wanda Kukulski²; Sophie G Martin¹;
¹University of Lausanne, Fundamental Microbiology, Lausanne, Switzerland
²Laboratory of Molecular Biology, Cambridge, United Kingdom

The fission yeast *S. pombe* undergoes mating and fusion during its sexual cell cycle. The process is initiated by pheromone release and perception, and followed by a MAPK cascade that induces transcription of specific mating/fusion genes. These include Fus1, a member of the formin family of proteins, which induces actin polymerization. These actin filaments direct the transport of glucanase-containing vesicles to the shmoo tip, where they induce local cell wall digestion necessary for membrane fusion. The extremely precise spatio-temporal regulation of this process occurs thanks to the focalization of proteins involved in this process, in particular pheromone receptors and transporter, at a very specific place called actin fusion focus (AFF). We aim to understand how the actin fusion focus is structured, in particular how trafficking vesicles and actin filaments are organized, and how plasma membranes from both partner cells merge. To address this we have optimized a post-embedding correlative light and electron microscopy (CLEM) approach, developed for *S. cerevisiae*, which successfully works in *S. pombe* and in mating conditions. With the intention of obtaining all the possible details, we use tomography followed by 3D reconstruction, which allows us to identify many features, such as the two layers of membranes, endocytic events, exocytic vesicles, actin cables, etc. We are currently characterizing all these structures in WT cells. Preliminary observations show plasma membrane waviness and deformation at the site of cell-cell contact, as well as an asymmetric “attack” of the h- into the h+ cell. In order to understand these phenotypes, we are making use of fusion-deficient mutants, such as *fus1?* or *prm1?*. With this work we aim to reveal in unprecedented details the organization of the fusion structure.
PI(4,5)P2 IS NOT REQUIRED FOR SECRETORY GRANULE DOCKING

Muhmmad Omar-Hmeadi; Nikhil R Gandasi1; Sebastian Barg1;
1Uppsala University, Medical Cell Biology, Uppsala, Uppsala län, Sweden

Phosphoinositides (PIs) play important roles in exocytosis and are thought to regulate secretory granule docking by co-clustering with the SNARE protein syntaxin to form a docking receptor in the plasma membrane. Here we tested this idea by high-resolution TIRF imaging of EGFP-labeled PI markers or syntaxin at secretory granule release sites in live insulin-secreting cells. In intact cells, PI markers distributed evenly across the plasma membrane with no preference for granule docking sites. In contrast, syntaxin was found clustered in the plasma membrane, mostly beneath docked granules. We also observed rapid accumulation of syntaxin at sites where granules arrived to dock. Acute depletion of plasma membrane PI(4,5)P2 by recruitment of a 5'-phosphatase strongly inhibited Ca2+-dependent exocytosis, but had no effect on docked granules or the distribution and clustering of syntaxin. Cell permeabilization by α-toxin or formaldehyde-fixation caused PI marker to slowly cluster, in part near docked granules. In summary, our data indicate that PI(4,5)P2 accelerates granule priming, but challenge a role of PIs in secretory granule docking or clustering of syntaxin at the release site.
STRUCTURAL BASIS OF DRP1-CARDIOLIPIN INTERACTIONS IN MITOCHONDRIAL FISSION

Mukesh Mahajan¹; Bin Lu¹; Abhishek Mandal²; Nikhil Bharambe¹; Rihua Wang¹; Matthias Buck¹; Patrick van der Wel²; Xin Qi¹; Rajesh Ramachandran¹;
¹Case Western Reserve university, Physiology & Biophysics, Cleveland, Ohio, USA
²University of Pittsburgh, Structural Biology, Pittsburgh, Pennsylvania, USA

Mitochondria form tubular networks that undergo coordinated cycles of fission and fusion. Emerging evidence suggests that a direct yet unresolved interaction of the mechanoenzymatic GTPase dynamin-related protein 1 (Drp1) with mitochondrial surface-localized cardiolipin (CL) catalyzes mitochondrial fission. Here, using a comprehensive set of structural, biophysical and cell biological tools, we have uncovered a CL-binding motif (CBM) conserved between the Drp1 variable domain (VD) and the unrelated ADP/ATP carrier that intercalates into the hydrocarbon membrane core via a disorder-to-order helical structural transition to likewise effect specific CL acyl chain interactions. Yet, membrane insertion and GTP-dependent conformational rearrangements induce only transient CL nonbilayer topological forays, which favor high local membrane constriction but not fission. A conservative CBM mutation that weakens CL interactions induces ‘donut’ mitochondria formation triggered by impaired Drp1-dependent fission coupled to excessive mitofusin-enabled mitochondrial back-fusion. Our studies, for the first time, firmly establish an indispensable role for Drp1 VD-CL interactions in productive physiological mitochondrial fission.
FUSION EFFICIENCY IN MODEL SYSTEMS: A QUANTITATIVE COMPARISON

Tom Robinson; Reinhard Lipowsky¹; Rumiana Dimova¹;
¹Max Planck Institute of Colloids and Interfaces, Theory and Bio-Systems, Potsdam, Brandenburg, Germany

Biological membrane fusion is essential for cellular functions such as neurotransmission and exocytosis. The fusion of biomembranes is also involved in viral infection. Understanding the biophysical mechanisms underpinning membrane fusion is vital to unravel this fundamental process. In nature, the SNARE complex governs membrane fusion in eukaryotes. Model membrane systems are powerful tools which enable us to isolate specific factors and study their influence on fusion. Different studies have examined membrane fusion in such systems using SNAREs and SNARE-mimetics. Lipid vesicles are one of the most commonly used fusion models in the literature and in particular LUV-LUV fusion. Here, we have utilized giant and large unilamellar vesicles (GUVs and LUVs) as our model system and we show fusion via four different systems. The first being protein-free, using a pure lipid system, exhibiting high fusion efficiency [Biophys. J. 116:79, 2019]. The second is based on lipidated-DNA and shows low fusion efficiency with negligible full fusion events. The third uses lipidated-peptides (in collaboration with the Janshoff group) and while full fusion events were detected, the efficiency was low [Robinson, et al., submitted]. The final system involved reconstituted SNARE proteins (in collaboration with the Steinham lab) and displayed high fusion efficiency which proceeds to full fusion and content mixing. With all of these systems we quantitatively compare the fusion efficiency between LUVs and GUVs with results found in the literature using alternative model systems. We discuss the differences in reported fusion efficiencies, demonstrate the importance of the choice of model membrane system used to investigate fusion and highlight the pitfalls, which could lead to overestimates of fusion efficiencies. This work is performed within the MaxSynBio consortium which is jointly funded by the Federal Ministry of Education and Research of Germany and the Max Planck Society.
DIGGING INTO THE MOLECULAR DEGREES OF FREEDOM LEADING TO DYNAMIN MEDIATED MEMBRANE FISSION: INSIGHTS FROM MULTISCALE MOLECULAR MODELING ON THE "CATALYTIC" ROLE OF PLECKSTRIN HOMOLOGY DOMAIN

Kirtika Jha1; Krishnakanth Baratam1; Vikas Dubey1; Thomas J Pucadyil2; Anand Srivastava1;  
1Indian Institute of Science-Bangalore, Molecular Biophysics Unit, Bangalore, Karnataka, India  
2Indian Institute of Science Education and Research- Pune, Biological Sciences, Pune, Maharashtra, India

Classical dynamin associates with the plasma membrane localized phosphatidylinositol-4,5-bisphosphate through the centrally located pleckstrin homology domain (dyn1-PHD). Dyn1-PHD is known to be a dispensable domain as dynamin-mediated fission can take place without it as is the case with extant bacterial and mitochondrial dynamins. Interestingly however, recent reconstitution experiments show that the rate of membrane fission slows down manifold when dyn1-PHD is replaced with polyhistidine or polylysine linker in a way that doesn’t compromise the scaffold fidelity. These observations suggest that dyn1-PHD may be ‘expediting’ the fission reaction in certain ways during synaptic vesicle recycling. In this work, we have used a multiscale modeling approach by combining together atomistic molecular dynamics simulations, mixed-resolution membrane mimetic models, coarse-grained molecular simulations and free-energy advanced sampling (metadynamics) methods to explore the molecular basis of the dyn1-PHD interactions with the membrane. We report the molecular-level insights into the possible role of dyn1-PHD as ‘catalyst’ and show that: (i) dyn1-PHDs make the membrane more pliable for fission and also modulate the lipids towards conformations that favor hemi-fission states. (ii) dyn1-PHD associates with membrane in multiple orientations using variable loops as the pivoting motifs. We explain our observations against the recently published Cryo-EM data of the dynamin collar on membrane and propose an “orientation selection” design principle behind the flexibility of dyn1-PHD, a possible result of the severe demands on the reconfigurations that dynamin collar needs to undergo during the fission cycle. (iii) Lastly, we identify key residues that stabilize the inositol-PHD interactions and suggest mutations that can restrict the ability of the dyn1-PHD to associate with membrane in multiple orientations. Together, these observations provide a molecular-level understanding of the catalytic role of the PHD in dynamin-mediated membrane fission.
EXOCYTOSIS AS AN ENDOGENOUS ELECTROFUSION

Justin Teissie\textsuperscript{1}; STEFAN Weinandy\textsuperscript{1}; Valerie Réat\textsuperscript{1}; Alain Milon\textsuperscript{1}; Pascal Demange\textsuperscript{1};
\textsuperscript{1}IPBS UMR 5089 CNRS and Université P Sabatier, Toulouse, Haute-Garonne, France

It was suggested that fusion pore can be ascribed to electroporation of the membrane, triggered by the strong electric field existing at the site of exocytosis (1). Pulsed electric fields (PEF) are known to mediate a fusogenic state to plasma membranes when applied to a cell suspension. To mimic exocytosis, exocytotic vesicles are modelled by large unilamelar vesicles (LUVs). The PEF parameters are selected to affect specifically the cells leaving the vesicles unaffected. When LUVs are electrostatically brought in contact with cells by a salt bridge, their content is delivered in the cytoplasm when cells are electroporemabilized. It is the electroporemabilized state of the cell membrane that is the support of the merging between the plasma membrane and the lipid bilayer, giving an experimental support to Rosenbeck’s model (1). LUV composition should be made “fusogenic” by a proper balance between phosphatidylcholine and phosphatidylethanolamine. Mixing electroporemabilized cells with tagged LUVs results in a distribution of the fluorescent lipids in the cell plasma membrane. This is observed by confocal fluorescence microscopy with fluorescently labelled LUVs. The process is a content and “membrane” mixing, following neither a «kiss and run» mechanism nor an endocytotic pathway.

55-POS Board 55

**IN VITRO QUANTIFICATION OF THE STOICHIOMETRY OF DYNAMIN FISSION MACHINERY**

Javier Vera Lillo¹; Juan Manuel Martínez Gálvez¹; Julene Ormaetxea Gisasola¹; Anna V Shnyrova¹; Vadim A Frolov¹,²;
¹Biophysics Institute (CSIC, UPV/EHU) , Department of Biochemistry and Molecular Biology, University of the Basque Country, Leioa, Vizcaya, Spain
²IKERBASQUE, Basque Foundation for Science, Bilbao, Vizcaya, Spain

Dynamin 1 (Dyn1) is a large GTPase implicated in membrane fission during endocytosis. Dyn1 takes energy from GTP hydrolysis to severe the necks of endocytic vesicles. For that, Dyn1 oligomerizes around the necks into helical structures enabling cooperative GTP hydrolysis. The hydrolysis causes acute constriction of the helix, leading to membrane scission. The stoichiometry and pathways of self-assembly of this helical fission machinery remains a subject of debate. Here we used Dyn1-eGFP to determine the minimal number of dynamins needed to produce membrane fission in vitro. We found that eGFP addition had minor effect of on membrane-mediated self-assembly and GTPase activities of Dyn1. We further quantified the Dyn1-eGFP interaction with lipid membrane nanotubes at low protein/lipid ratios. We found that under such circumstances Dyn1-eGFP oligomers stably bound to membrane templates were generally smaller than a single helical rung. Yet they responded to GTP addition and produced stochastic membrane scission. We discuss possible mechanistic interpretations of these findings.
Ethanol intoxication in humans is thought to act primarily through interactions with membrane protein receptors, however we recently showed that short-chain alcohols dramatically alter liposome fusion rates to a planar membrane (Paxman et al. 2017. Biophys J. 112:121-132) reopening a previously long-studied possible target for ethanol. Objective: To determine the mechanisms whereby alcohols alter the fusion of liposomes to a planar membrane. Methods: We used the nystatin/ergosterol fusion assay, differential scanning calorimetry (DSC), and molecular dynamics (MD) to measure membrane-membrane and alcohol-membrane interactions. Results: Previously we showed that ethanol excites fusion when applied on the \(cis\) (liposome) side, and inhibits on the \(trans\) (extracellular) side. Presumably, excitation is due to lowering the activation energy of fusion by alcohol acting on the vesicular membrane and inhibition is due to a slight rise in activation energy due to alcohol in the planar membrane. We expected symmetric addition to excite fusion similar to \(cis\) addition, since in both cases alcohol has access to both membranes. However, symmetric addition generally decreases fusion rates, more similar to \(trans\) addition. Since fusion in our protein-free model system is driven by an osmotic gradient (high \(cis\)), we now hypothesize that alcohol’s action in planar membranes is modified by water movement through the membrane. When alcohol is added \(trans\), water movement aids its entry into the planar membrane where it strongly inhibits fusion. But with \(cis\) addition, water movement tends to flush alcohol out of the membrane decreasing the inhibition and allowing excitation, due to action on the vesicle membrane, to dominate. Measurements of fusion rates with decreased osmotic gradients are consistent with this hypothesis. Conclusions: Alcohols, and especially ethanol, alter membrane-membrane interactions in a way that significantly changes fusion rates at physiologically relevant doses.
61-POS Board 61

DIFFERENTIAL CO-RELEASE OF TWO NEUROTRANSMITTERS FROM A VESICLE FUSION PORE IN MAMMALIAN ADRENAL CHROMAFFIN CELLS

Zhuan Zhou¹; Qihui Wu¹; Bin Liu¹; Quanfeng Zhang¹;
¹Institute of Molecular Medicine, Peking University, Beijing, China

Co-release of multiple neurotransmitters from secretory vesicles is common in neurons and neuroendocrine cells. However, whether and how the transmitters co-released from a single vesicle are differentially regulated remain unknown. In matrix-containing dense-core vesicles (DCVs) in chromaffin cells, there are two modes of catecholamine (CA) release from a single DCV: quantal and sub-quantal. By combining two microelectrodes to simultaneously record co-release of the native CA and ATP from a DCV, we report that (1) CA and ATP were co-released during a DCV fusion; (2) during kiss-and-run (KAR) fusion, the co-released CA was sub-quantal, while the co-released ATP was quantal; (3) knockdown and knockout of the DCV matrix led to quantal co-release of both CA and ATP, even in the KAR mode. These findings strongly imply that, in contrast to the sub-quantal CA release in chromaffin cells, fast synaptic transmission without transmitter-matrix binding is mediated exclusively via quantal release in neurons.
**Wednesday, May 8, 2019**  
**Poster Session II**  
**14:50 – 16:20**  
**University Botanical Gardens of Padova**

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Wednesday. Presenting authors with odd-numbered poster boards should present from 14:50 – 15:35 and those with even-numbered poster boards should present from 15:35 – 16:20. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

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Posters should be set up the evening of May 6 and removed by noon on May 10.
THE PROTOTYPICAL G PROTEIN-COUPLED M2 MUSCARINIC RECEPTOR ACTS AS A NOVEL REGULATORY ELEMENT OF THE MITOCHONDRIAL FUNCTION THROUGH ITS CARBOXYL TERMINAL DOMAIN

Paolo Annibale; Roberto Maggio2; Martin J Lohse1;  
1Max Delbrück Center Berlin, Signaling Processes of Receptors, Berlin, Germany  
2Universita` degli Studi dell'Aquila, Department of Life, Health and Environmental Sciences, L'Aquila, Italy

Muscarinic acetylcholine receptors are prototypical G protein-coupled receptors activated by the endogenous neurotransmitter acetylcholine. We show that the carboxyl terminal fragment of the muscarinic M2 receptor, containing the transmembrane regions VI and VII (M2tail), is expressed by virtue of an internal ribosome entry site. The M2tail fragment does not follow the normal route to the plasma membrane, as does the parental wild type M2 receptor, but is sorted to the mitochondria where it appears to controls oxygen consumption and the formation of oxygen radical species. To the best of our knowledge, the expression of a carboxyl-terminal M2 receptor that proves capable of regulating mitochondrial function, constitutes a novel mechanism that cells may use for controlling their metabolism under variable environmental conditions.
COMPARATIVE ANALYSIS OF MEMBRANE CONSTRICTION BY DYNAMIN ISOFORMS

Rebeca Bocanegra 1; Ariana Velasco2,3; Sara de Lorenzo1; Ormaetxea Julene2,3; José L Carrascosa1;5; Anna Shnyrova2,3; Borja Ibarra1; Vadim Frolov2,3,4; 1IMDEA Nanoscience, Madrid, Spain 2Biofisika Institute (CSIC, UPV/EHU), Leioa, Vizcaya, Spain 3University of the Basque Country, Biochemistry and Molecular Biology, Leioa, Vizcaya, Spain 4Basque Foundation for Science, Bilbao, Vizcaya, Spain 5National Center for Biotechnology (CSIC), Madrid, Spain

The proteins of dynamin superfamily are large GTPases widely implicated in fission and fusion of endomembranes. Their activity is necessary for internalizing essential nutrients, organelle transformations and maintenance, dynamins are intimately involved in signaling and membrane trafficking networks in the cell, in life and pathology. The superfamily founding members, dynamins 1 and 2 (Dyn1 and 2), remain the most characterized dynamins primarily involved in orchestrating membrane fission in the clathrin dependent endocytosis. As an endocytic vesicle buds, dynamin molecules are recruited to its neck, where it self-assembles a helical coat generating a dynamin-lipid tube. The helix constriction driven by GTP hydrolysis promotes fission of the neck and release of the vesicle. While the above patterns of dynamin activities common for Dyn1 and 2 are well understood, in vitro analyses revealed important functional differences between neuron-specific Dyn1 and ubiquitous Dyn2 isoforms. Here we performed systematic mechanistic comparison of membrane remodeling activities of Dyn1 and 2 reconstituted using lipid membrane nanotubes. By combining fluorescence microscopy and optical tweezers approaches, we quantified the nanotube constriction by dynamin isoform in apo state and in the presence of different nucleotide. Our analyses revealed significant differences between membrane constriction and curvature stabilization activities of Dyn1 and 2. We discuss possible relevance of these differences to physiological functions of the proteins.
EXTRASYNAPTIC EXOCYTOSIS FROM THE AXON OF IDENTIFIED SEROTONERGIC NEURONS

Montserrat G Cercós¹; Citlali Trueta¹;  
¹Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Neurophysiology, Mexico City, Distrito Federal, Mexico

Serotonin exocytosis is activated by single impulses from presynaptic terminals and by high-frequency trains of impulses from extrasynaptic sites at the neuronal soma. Vesicle-containing sites at the axons of serotonergic neurons suggest extrasynaptic secretion, but axonal extrasynaptic exocytosis has not been functionally demonstrated and its regulation mechanisms are unknown. Here we show axonal extrasynaptic exocytosis and analyze its firing-frequency and calcium-source dependence. Exocytosis and intracellular calcium were analyzed from the fluorescence of FM styryl dyes and Fluo-4, respectively, in identified leech serotonergic neurons. Ten impulses at 1 or 20 Hz produced one or two axonal exocytosis phases, respectively, suggesting different vesicle pools arriving from increasing distances to the membrane, which were corroborated by electron microscopy. Two calcium transients underlie exocytosis upon stimulation at both frequencies: a fast transient synchronized with electrical activity, which activates exocytosis from the closest vesicle pool and determines the mobilization of distant pools, and a slower and longer submembrane transient, produced by a positive feedback mechanism activated by released serotonin, which allows exocytosis of all mobilized vesicles. The fast calcium transient in response to 20-Hz stimulation was twice larger than that upon 1-Hz stimulation, and in contrast to the later, propagated through the axoplasm. Depletion of intracellular calcium stores revealed that this propagation requires calcium-induced calcium release (CICR), and is necessary for mobilizing distant vesicles towards the membrane. Blockade of L-type calcium channels, generally associated with extrasynaptic exocytosis, did not impair axonal exocytosis or the propagation of the fast calcium transient upon 20-Hz stimulation, suggesting that calcium entry through non-L synaptic channels activates CICR. Axonal extrasynaptic exocytosis has intermediate mechanisms between synaptic and somatic exocytosis and expands the possibilities of single neurons to produce different effects in neural circuits.
CELL ADHESION REMODELLING DURING OSMOTIC PERTURBATIONS

Celine Dinet¹; Margarita Staykova¹;
¹Durham University, Physics Department, Durham, United Kingdom

Throughout our body, tissue cells cope with sudden changes of environmental osmotic stresses by regulating their volume and surface area. Previous studies on neurones and other cell types have shown that in response to hyperosmotic shocks membrane invaginations called vacuole like dilations (VLD’s) are formed at discrete low adhesion sites to compensate for excess plasma membrane [1]. More recent work claimed that this phenomenon is passive and involves the disruption of membrane adhesion [2]. In this work, we aim to learn about the impact of such phenomenon on the adhesion contacts remodelling and reversibility.

To address this, we are using a biomimetic model system of cell to cell adhesion comprised of giant unilamellar vesicles (GUV’s) adhering to a supported lipid bilayer or to another GUV via specific biotin-neutravidin or cadherins bonds. The osmolarity of the outer medium is changed and the remodelling of the membrane as well as that of the adhesion contacts are followed using optical microscopes. We observe that the membrane adhesion contacts are pushed away from the sites of VLD formation than regenerated, and the VLD formation, size and dynamics depends on the magnitude of the stress and the adhesion density. [1] C.E.Morris et al., ‘The Invagination of Excess Surface Area by Shrinking Neurons’, Biophys J. 85(1), 223-225, (2003). [2] AJ. Kosmalaska et al., ‘Physical principle of membrane remodelling during cell mechanoadaptation’, Nat Commun. 6: 7292, (2015).
A RANDOM-WALK MODEL FOR HIGHLY DYNAMIC SIMULATIONS OF THE VESICULAR RELEASE PROCESS

Johan Dunevall¹; Anna Larsson²; Soodabeh Majdi²; Sebastian Barg¹; Andrew G Ewing²;  
¹Uppsala University, Medical Cell Biology, Uppsala, Uppsala län, Sweden  
²University of Gothenburg, Chemistry and Molecular Biology, Göteborg, Västra Götalands län, Sweden

One technique that has proven extremely useful for the study of exocytosis during the last 25 years is single cell amperometry (SCA). In SCA benefits from the many neurotransmitters and/or hormones (signaling molecules), such as, catecholamines that are electroactive and can be oxidized or reduced at the surface of a polarized electrode. By recording the current passed through the electrode surface as a function of time a current vs. trace is obtained, which allows for the number of released molecules to be quantified. Also, due to the excellent temporal resolution (< ms) of SCA, rapid fluctuation in current (flux of molecules) can be monitored progressively during a release event. However, in the data obtained by this method, information about the regulatory mechanism of exocytosis is hidden and to be able to fully understand the release process highly dynamic models must be used. We present here a random-walk-based model where all geometric features, such as vesicle and pore radius as well as intra- and extravesicular diffusion coefficients can be altered as a function of time. This modelling tool can be used to increase the understanding of what factors are important for the regulation of exocytosis. In addition, the model can be used to estimate the fluctuation of the fusion pore radius as a function of time from experimentally obtained data. This can be extremely useful to understand how exocytosis is regulated during partial release, where only a fraction of the vesicular content is released during a fusion event.
Considering its importance, it is surprising how little is known about the molecular-level mechanism of membrane fusion and its intermediate state called hemi-fusion model. In general, trapping of a hemi-fused model in time is challenging to realize without the assistance of external factors. To mitigate nature’s complexity, a common approach to investigate membrane fusion is using bottom up supramolecular model systems that are self-assembled from artificial molecules. Here we propose a highly promising artificial model system to systematically study hemi-fused intermediate: Frenkel excitonic nanotubes self-assembled from amphiphilic cyanine dye 3,30-bis(2-sulphopropyl)-5,50,6,60-tetrachloro-1,10-dioctylbenzimidacarbocyanine (abbreviated as C8S3). The system’s unique collective optical properties (Frenkel excitons) arises from the ensemble of the aggregated molecules, which are highly sensitive to the details of the molecular packing. Specifically, the dye monomers self-assemble into two distinct, well-defined morphologies; double-walled nanotubes, which self-assemble further into hemi-fused hierarchical structures, which are bundles of single-walled nanotubes. Most importantly, those two morphologies show two distinct spectroscopic signatures. This allows the use of steady-state spectroscopy as an elegant tool for in-situ investigations of the system’s structural properties during the self-assembling process. In this study, counterions with varying properties (e.g. polarizability) were employed to control the self-assembling process. We observed quantitative correlation between concentration of cation and formation of hemi-fused structure. Furthermore, for a given concentration of salt series, we found that solvated ion pairs drive the self-assembly process faster towards thermodynamic product than electrostatically bound ion pair. On the other hand, the size of the directly participating ion species (cation) had no effect on the self-assembly. Our results emphasize the high potential of the reported model system and environmental parameters for engineering a cell mimic for crucial intermediate of membrane fusion: hemi-fusion model.
CHARACTERIZATION OF SPONTANEOUS CALCIUM EVENTS AND CORRELATION TO QUANTAL RELEASE IN PRIMARY NEURONS

Roberta Mancini¹; Yong Qian²; Quentin Bourgeois-Jaarsma¹; Rizky Lasabuda¹; Robert E Campbell²; Alexander J. Groffen¹,³;
¹Center for Neurogenomics and Cognitive Research, Functional Genomics, Amsterdam, Noord-Holland, The Netherlands
²University of Alberta, Chemistry, Alberta, Canada
³VU Medical Center, Clinical Genetics, Amsterdam, Noord-Holland, The Netherlands

Ca²⁺ is an important signalling molecule which is critical for evoked neurotransmission and regulates spontaneous release. Resting neurons exhibit spontaneous local Ca²⁺ fluctuations, but their relation to quantal release events is uncertain. Here we measured miniature excitatory postsynaptic currents (mEPSCs) and spontaneous Ca²⁺ events (SCEs) simultaneously in the same neuron using the Ca²⁺ indicator syGCaMP6f. This approach did not show a strict correlation. Second, we developed a new fluorescent probe sypHyJReX which can detect Ca²⁺ signals and secretion events for a single synaptic vesicle. The latter approach showed spontaneous Ca²⁺ events that were immediately followed by vesicle fusion events in the same synapse, suggesting that spontaneous Ca²⁺ events can trigger vesicle release events. However, the lack of a strict correlation with mEPSCs suggests that these events may contribute to only a small fraction of the spontaneous release events, or alternatively, involve non-glutamatergic vesicles.
BIOCHEMICAL AND STRUCTURAL ANALYSIS OF A HUMAN DYNAMIN 2 MUTANT, R719W, ASSOCIATED WITH HEREDITARY SPASTIC PARAPLEGIA (HSP)

John Jimah¹; Abigail Stanton¹; Jenny Hinshaw¹; Lieza Chan¹;
¹National Institutes of Health, Structural Cell Biology Section, Laboratory of Cell and Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, USA

Classical dynamin GTPases (dynamin 1, 2 and 3) mediate membrane fission during endocytosis. Currently, all known disease-associated mutations in dynamins occur in dynamin 2. One such mutation, R719W, is linked to HSP, a disease that causes muscle weakness and stiffness resulting in the inability to walk. The **objective of this study** is to uncover the molecular mechanism of dynamin 2 in normal and disease states. Structural modeling of dynamin 2 situates residue R719 in the bundle signaling element (BSE), a three-helix bundle that links dynamin’s GTPase domain to the stalk domain. Upon GTP hydrolysis by wt dynamin, the BSE swings downward by 68° relative to the GTPase domain (powerstroke) resulting in rearrangements in the polymer that leads to constriction of dynamin-lipid tubules from an inner luminal diameter of 7 nm to 4 nm. Cells expressing the R719W mutant are deficient in endocytosis, which may be due to reduced GTP hydrolysis-dependent powerstroke. Our **experimental methods** involve obtaining structures of dynamin 2 wt and R719W mutant assembled on lipid membranes to elucidate the mechanism of assembly and membrane constriction. **Preliminary results** from cryo-EM studies reveal that the extent of membrane constriction may differ between dynamin 2 wt and R719W. This work will increase our understanding of the structural features of dynamins necessary for membrane fission and the molecular basis of dynamin-associated diseases.
BIOPHYSICAL FEATURES UNDERLYING THE EXTREME CALCIUM SENSITIVITY OF SYNAPTOTAGMIN-7

Jefferson Knight$^1$; Hai Tran$^1$; Lauren Anderson$^1$; 
$^1$University of Colorado Denver, Dept. of Chemistry, Denver, Colorado, USA

Synaptotagmin (Syt) family proteins are crucial regulators of membrane fusion during exocytosis. Eight of the seventeen Syt isoforms bind membranes in a Ca$^{2+}$-dependent manner via their tandem C2 domains, C2A and C2B. Among these, Syt-7 is the most Ca$^{2+}$ sensitive, binding anionic lipids and triggering SNARE-mediated membrane fusion even at sub-micromolar Ca$^{2+}$ concentrations. Our goal was to understand how the C2A and C2B domains of Syt-7 work together to achieve this extreme Ca$^{2+}$ sensitivity. We compared membrane binding properties of individual and tandem C2 domains between Syt-7 and Syt-1 using fluorescence-based liposome binding assays, stopped flow dissociation kinetics, insertion depth measurements, and aggregation assays. Results indicate that the greatest difference between the two proteins is the Ca$^{2+}$-dependent membrane binding strength of the C2A domains: Syt-7 C2A bound physiological liposomes with ~25-fold greater Ca$^{2+}$ sensitivity and dissociated ~100-fold more slowly than Syt-1 C2A. The C2B domain of Syt-7 also bound membranes somewhat more strongly than Syt-1 C2B, both in the presence and absence of the polyanionic lipid phosphatidylinositol-(4,5)-bisphosphate (PIP$_2$), although differences between the C2B domains were not as stark as between the C2A domains. Perhaps surprisingly, Syt-7 exhibited less interdomain cooperativity than Syt-1, as assessed via direct comparisons of chelator-induced dissociation kinetics from model liposomes lacking PIP$_2$. We suggest this is due to the relative energetic dominance of the C2A domain in Syt-7 but the C2B domain in Syt-1. Inclusion of 1% PIP$_2$ in the lipid composition potentiated the ability of the Syt-7 C2AB tandem to bind and aggregate liposomes even in the absence of Ca$^{2+}$, an effect which is presumably prevented in vivo by other competing interactions. Overall, our results suggest conserved structural features that support divergent membrane binding energetics and Ca$^{2+}$ sensitivities between these two important synaptotagmins.
SINGLE MOLECULE MASS PHOTOMETRY OF DYNAMIN AND MEMBRANE INTERACTION REVEALS ITS POLYDISPERSITY AND OLIGOMER-SPECIFIC INTERACTIONS

Manish S Kushwah1; Philipp Kukura1;
1University of Oxford, Department of Chemistry, Oxford, Oxfordshire, United Kingdom

Dynamin, the prototypical member of dynamin superfamily, is a large multi-domain GTPase, and is essential for membrane fission during clathrin-mediated endocytosis. Electron and fluorescence microscopy studies along with conductance measurements have provided detailed preliminary insights into dynamin polymerization in both solution and on membranes, as well as effects of nucleotide binding, membrane constriction and membrane fission upon GTP hydrolysis. Furthermore, extensive x-ray crystallography and cryo-electron microscopy of dynamin in various nucleotide-bound states have provided a detailed picture of GTP-hydrolysis catalyzed membrane fission. A quantitative understanding of dynamin polymerization, the critical initial step in translating from a solubilised to a functional form, however, remains elusive and requires an approach enabling dynamic observation of the dynamin polymerization and its interactions at the molecular level. Here, we use mass photometry, a label-free, imaging-based method to accurately determine the molecular mass of single molecules (Young and Hundt et al., 2018), to measure oligomeric distributions of dynamin-1 over a range of concentrations and conditions. We find an equilibrium between monomer, dimer and tetramer at lower concentrations (<100nM), moving towards 2-8mers at higher concentrations (<500nM). Interestingly, dimers and tetramers dominate (>50nM) making them the most stable species and GTP binding shifts the equilibrium towards tetrameric species (200-500nM). The abundances of these species as a function of concentration reveals the energetics of oligomerization and effect of GTP binding on associated equilibria. We next compared binding of oligomers to lipid vesicles (~20nm diameter) and supported bilayers and found that the dimer shows preference to bilayers over vesicles, with the trend is reversed for the tetramer, indicating a curvature-dependent binding affinity of the oligomers. These results represent a first step towards a quantitative view of dynamin polymerization.
THE LIFE OF A BETA CELL WITHOUT DYNAMIN

Fan Fan\textsuperscript{1}; Yumei Wu\textsuperscript{2}; Natalia Tamarina\textsuperscript{3}; Shawn M Ferguson\textsuperscript{2}; Louis Philipson\textsuperscript{3}; Pietro De Camilli \textsuperscript{2}; Xuelin Lou\textsuperscript{1};
\textsuperscript{1}Medical College of Wisconsin, Department of Cell Biology and Neurobiology, Milwaukee, Wisconsin, USA
\textsuperscript{2}Yale University, School of Medicine, Department of Cell Biology, CNNR program, New Haven, Connecticut, USA
\textsuperscript{3}University of Chicago, Department of Medicine, Kovler Diabetes Center, Chicago, Illinois, USA

We study the molecular basis of vesicle trafficking in neurons and endocrine cells. GWAS studies predict that membrane trafficking defect in pancreatic $\beta$ cells lies at the central of diabetes. Here we evaluate the effect of imbalanced membrane trafficking on insulin secretion by fully shutting down dynamin-mediated trafficking. We generated a mouse model (cTKO) that lacks all three mammalian dynamin genes ($DNM$ 1, 2 and 3) selectively in adult $\beta$ cells and examined insulin granule trafficking using multiple sensitive approaches both in vitro and in vivo. Deletion of 3 dynamin genes led to impaired (but not abolished) endocytosis and aberrant clathrin-coated endocytic intermediates. Interestingly, individual insulin granules showed higher mobility and slower fusion kinetics, indicating impaired exocytosis and fusion pore dynamics. Moreover, cTKO mice developed hyperglycemia and glucose intolerance. This work illustrates that dynamin functions in both membrane fission and fusion of dense core vesicles, highlighting a fundamental role of dynamin-mediated trafficking balance in preserving insulin secretion and glucose homeostasis.
VISUALIZATION OF ENDOPLASMIC RETICULUM MEMBRANE TOPOLOGY BASED ON SUPER-RESOLUTION MICROSCOPY DATA

Zach Marin\textsuperscript{1,2}; Michael Graff\textsuperscript{1}; Joerg Bewersdorf\textsuperscript{1,2}; David Baddeley\textsuperscript{1,3};
\textsuperscript{1}Yale University, Cell Biology, New Haven, Connecticut, USA
\textsuperscript{2}Yale University, Biomedical Engineering, New Haven, Connecticut, USA
\textsuperscript{3}University of Auckland, Auckland Bioengineering Institute, Auckland, New Zealand

Membrane fusion and fission processes within the endoplasmic reticulum (ER) are essential for maintaining the complex structure and function of this important organelle, but difficult to study due to the ER’s convoluted topology. The ER exhibits a broad continuum of morphologies ranging from flat, sheet-like domains to highly curved parking-garage structures, tubules, and nanoscale holes. The ER is also highly dynamic, and can transition between morphologies on a timescale of seconds to minutes. While many proteins that are key players in ER organization have been identified, the mechanisms of ER membrane fusion and fission are still poorly understood--it even seems likely that different fission and fusion mechanisms are active in different parts of the ER.

Due to the structural heterogeneity in the ER, we believe that fusion and fission processes must be studied in the context of local membrane morphology. To enable this, we have developed super-resolution microscopy techniques that provide a detailed view of ER morphology and dynamics. Single-molecule switching nanoscopy (SMSN), in particular, provides 3D data at 20-nm resolution. Rather than directly imaging membrane surfaces, however, SMSN gives us the positions of proteins that localize to the ER membrane. Here, we present our work toward creating and visualizing biophysically-constrained representations of a membrane passing through an SMSN point cloud. These algorithms are implemented in the PYthon Microscopy Environment (PYME), an open-source nanoscopy imaging and analysis suite. This tool enables us to quantify the curvature of the ER membrane, which we can use to understand possible mechanisms of ER fusion events, and other membranous organelles.
UNDERSTANDING DYNAMIN CONSTRICION VIA PROCESSIVE STEPPING

Jeffrey Noel¹; Oleg Ganichkin¹; Alexander Mikhailov²; Oliver Daumke¹,³;
¹Max Delbrueck Center, Berlin, Germany
²Kanazawa University, Nano Life Science Institute, Kanazawa, Ishikawa, Japan
³Freie Universitat, Berlin, Germany

The final scission step in clathrin-mediated endocytosis is catalyzed by the molecular machine dynamin. While there have been many suggestions for how the final topological change of the membrane tube is effected, all proposals assume some degree of constriction by dynamin. We quantify the elasticity of the dynamin polymer and conclude that passive elastic deformation of the tube is likely insufficient to reach sufficiently small radii for scission. Thus, as discussed over the past decade, dynamin must use GTP to actively deform the membrane. We show through molecular modeling that the conformational change between the G-domain and BSE induced by GTP hydrolysis is sufficiently large to allow for a processive stepping motion between helical turns. We further characterize the G-domain/BSE motion with single-molecule FRET and stopped-flow kinetic measurements. These experimental inputs and detailed molecular simulations are combined into a polymer simulation that can explore the interaction of many G-domains simultaneously. We show that under certain conditions, the dynamin model can perform processive stepping leading to constriction of its membrane tube template.
SPATIAL SURVIVAL ANALYSIS OF SECRETORY GRANULE BEHAVIOR
RECORDED BY TIRF IMAGING IN HUMAN BETA-CELLS

Thi Huong Phan¹; Giuliana Cortese²;
¹University of Padova, Department of Information Engineering, Padova, Italy
²University of Padova, Department of Statistical Sciences, Padova, Italy

Exocytosis on beta-cells is one of the fundamental cellular processes that releases insulin from secretory granules. Studying survival of granules on the plasma membrane and their spatial correlation within cells during the exocytosis is of great interest. We analyze TIRF images recorded from 8 human beta-cells, containing granules and syntaxin information. One of the main objectives of this project is to investigate the relationship between the survival rates of granules and syntaxin levels, while adjusting for spatial correlation among granules within cells. To address the biological problem, an imaging procedure is performed to obtain survival datasets in which granules are followed from the first moment they arrive at the plasma membrane until they are no longer visualized in TIRF-images. Briefly, granule centroids are firstly detected in each single video image, then their trajectories are recorded by linking the same granules in subsequent frames. To study the concentration of syntaxin at granules in the plasma membrane, on-granule syntaxin levels are quantified for each single granule.

In the survival analysis perspective, we propose a semiparametric proportional hazard model with individual frailties, where the clustering structure, as well as the spatial correlation between granules are modeled via the variance-covariance matrix of frailties. Finally, we apply the standard Cox model and our method to analyze the granule data. We discover that there exist two distinct groups of granules identified by their presence/absence at beginning of the experiment. For granules that are present at the beginning of the experiment, a higher level of syntaxin contributes to obtain a higher survival rate; moreover, disappearing of these granules from the plasma membrane are very strong spatially correlated within cells. In contrast, syntaxin level seems to have no effect on survival rates of granules that appear during the experiment and also no spatial correlation is found.
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INTRINSIC CURVATURE DIFFERENCE DRIVEN DEMIXING OF MEMBRANE LIPID COMPONENTS

Radha Ranganathan¹; Miroslav Peric¹;
¹California State University Northridge, Physics, Northridge, California, USA

Stress due to intrinsic curvature difference between lipids in binary lipid membranes is a possible mechanism for membrane fission. With this as a founding hypothesis, we examine the effect of incorporating the inverse-cone shaped oxidized phospholipid, 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine (PGPC) in each of DPPC, POPC, and DOPC bilayers. The intrinsic curvatures of these bilayer lipids differ from each other due to their state of saturation. Laurdan fluorescence spectra were evaluated by a relatively new approach of fitting the spectra to lognormal functions. In the pure PC bilayers, Laurdan emits from the unrelaxed state at 432 nm and from the charge transfer state at 445 nm in the DPPC gel phase and also in DOPC and POPC at T < 20°C. At higher temperatures in the liquid phase the emissions are at 490 and 445 nm at about equal levels. More significantly PGPC causes the 432 nm peak to reappear in the liquid phase. The areas of the emission peaks were evaluated. The 432 nm peak from the unrelaxed state indicates the presence of tighter packed lower polarity regions that can result from interlipid hydrogen bonding. A possible model is that PGPC and bilayer PC do not mix uniformly because of shape differences. Inter PGPC hydrogen bonding via its polar terminal and ester groups promotes component segregation and stabilizes the unrelaxed state of Laurdan (432 nm emission). With further increase in PGPC concentration, curvature mismatch between domains increases stress and PGPC separates as micelles (confirmed by DLS) and the Laurdan spectrum appears similar to that in the pure bilayer PC, indicative that Laurdan mostly remains in the pure PC bilayer. Differences in the spectral emission characteristics between the mixing behaviors of PGPC with the different bilayer PCs support the model of intrinsic curvature stress driven separation of lipid components.
AUTOMATED IDENTIFICATION OF SECRETORY GRANULES IN TIRF IMAGES OF PANCREATIC BETA CELLS

Fabio Scarpa¹; Elisa Candeo¹; Morten Gram Pedersen¹;
¹University of Padova, Department of Information Engineering, Padova, Italy

Introduction: The loss of first-phase insulin secretion is an early sign of developing type 2 diabetes (T2D). Using a high-resolution TIRF microscopy, we can visualize a subset of docket insulin granules in human beta cells for which localized Ca2+ influx triggers exocytosis with high probability and minimal latency. This pool of granules is absent in beta cells from human T2D donors, and also their insulin release mechanism is slow and not synchronized. To evaluate granule dynamics underlying secretory activity in beta cells, Syntaxin and the insulin granules were marked with fluorescent proteins EGFP and NPY-Cherry. This activity was recorded in a sequence of images. Along the sequence, one can observe a luminous pulse followed by the disappearance of light, which likely represents an exocytosis event, through which the insulin is secreted. Until now, the location of the granules and their disappearance were identified manually. Methods: We propose an algorithm able to detect automatically the peaks of fluorescence and to recognize light disappearance, corresponding to granule location and hormone secretion respectively. The proposed algorithm is mainly based on a Laplacian of Gaussian filter and local thresholding. The former increases sharpness and emphasizes small circular objects, while the latter recognizes the objects with maximum brightness, i.e. granules. This analysis on single image is followed by an analysis on the stack of images, able to identify where and when some granules eventually disappear. Results and conclusions: The algorithm was evaluated on a data-set composed by 9 sequences of images, provided by the University of Uppsala, Sweden. Compared to manual analysis, the proposed algorithm identified granules with 90% accuracy, 79% sensibility and 100% specificity. The automated process is very fast (few seconds per image), objective and reproducible.
KETAMINE EVOKES VESICLE FUSION PORE NARROWING AND FLICKERING IN ASTROCYTES

Matjaz Stenovec$^{1,2}$; Eva Lasie$^1$; Boštjan Rituper$^1$; Jernej Jorgacevski$^{1,2}$; Marko Kreft$^{1,2,3}$; Robert Zorec$^{1,2}$;
$^1$University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Ljubljana, Slovenia
$^2$Celica Biomedical, Cell Engineering Laboratory, Ljubljana, Slovenia
$^3$University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia

Ketamine, a dissociative anesthetic, elicits analgesic, psychotomimetic and rapid antidepressant effects that are of particular neuropharmacological interest in psychiatry. Recent studies in astrocytes revealed that prolonged ketamine application reduces Ca$^{2+}$ signaling and exocytotic release of gliotransmitters. Here high-resolution cell-attached membrane capacitance measurements were used to examine at single vesicle level how ketamine affects the interaction between the vesicle and the plasmalemma in cultured rat astrocytes. Ketamine evoked long-lasting epochs of repetitive opening and closing of the vesicle fusion pore, termed bursts that were both time- and concentration-dependent. These were recorded following an acute application of anesthetic dose (25 µM) and a 30 min application of (sub)anesthetic doses (0.025-25 µM) of ketamine. In these bursts, unitary fusion pore openings were characterized by a decreased fusion pore conductance, indicating that the fusion pore was stabilized in a narrow configuration, too narrow to allow the passage of relatively large gliotransmitters, such as BDNF reported previously (Stenovec et al. 2015). Ketamine-evoked increase in burst occurrence correlated well with a decreased occurrence of full vesicle fissions, indicating that narrow fusion pore predominantly hindered the retrieval of vesicles into endocytotic pathway. While the nature of this, by now unrecognized, mechanism of ketamine treatment in astroglia is unknown, it may play a role in vesicular release and (re)uptake of molecules, and possibly contributes to the powerful ketamine antidepressant action in patients suffering from major depressive disorder.
ABNORMALLY INCREASED AMOUNT AND VELOCITY OF EXTRASYNAPTIC SECRETION IN OLFAC TORY NEURONAL PRECURSORS FROM PATIENTS WITH SCHIZOPHRENIA OR BIPOLAR DISEASE

Montserrat G Cercós¹; Citlali Trueta¹; Gloria Benítez-King²;
¹Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Neurophysiology, Mexico City, Distrito Federal, Mexico
²Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Neuropharmacology, Mexico City, Distrito Federal, Mexico

The alterations that underlie the pathophysiology of neuropsychiatric disorders include the dysregulation of structural and functional properties of neurons. Among these, the secretion of neurotransmitters and hormones, which plays a key role for neuronal communication and development, is altered. Neuronal precursors from the human olfactory epithelium have been recently characterized as a reliable model for studying the etiopathogenesis of neuropsychiatric disorders. Our previous work has shown that melatonin enhances the development of morphological and functional features of cloned olfactory neuronal precursors (ONPs) from a healthy subject. Here we studied extrasynaptic secretion in primary cultures of ONPs obtained from schizophrenia or bipolar disorder. Secretion was evaluated from the cumulative changes of FM1-43 fluorescence in response to depolarization. Potassium-evoked secretion from the soma and axons of ONPs obtained from schizophrenia or bipolar patients had an increased amplitude and velocity, compared with ONPs from age- and gender-matched healthy control subjects (HCS). This suggests the fusion of larger amounts of vesicles, which rested closer or were mobilized faster to the plasma membrane. As a first approach to study the alterations underlying this increase in secretion, we measured the thickness of microfilaments stained with rhodamine-phalloidin. The actin microfilament bundles of ONPs from patients were significantly thicker than those of HCS. Interestingly, treatment for 12 h with melatonin modulated the abnormally increased secretion in ONPs from schizophrenia and bipolar patients and brought it to levels similar to those found in HCS ONPs. Melatonin also induced the thickening of actin microfilament bundles. Our results suggest that cytoskeletal modifications change vesicle mobilization to the membrane, thus producing alterations in neurotransmitter systems observed in neuropsychiatric disorders.
ROLE OF MEMBRANE MEDIATED FORCES IN RECEPTOR-MEDIATED ENDOCYTOSIS

Jef Wagner\textsuperscript{1};
\textsuperscript{1}Union College, Physics and Astronomy, Schenectady, New York, USA

Many virus particles can gain entry to the cell through passive receptor mediated endocytosis, where the wrapping the virus particle is driven through the binding of receptors embedded in the cell membrane with ligands on surface of the virus. The time it takes for the membrane to fully wrap a virus depends strongly on the diffusion rate of the receptors in the membrane, since new receptors must migrate to the wrapping site. This study uses a monte-carlo simulation to explore at how interactions between the receptors effect the time it takes for a virus to be fully wrapped.
Endocytosis and Degradation of Kv1.5 Channels Induced by Activation of Protein Kinase C

Shetuan Zhang¹; Tingzhong Wang¹;
¹Queen’s University, Biomedical and Molecular Sciences, Kingston, Ontario, Canada

The voltage-gated potassium channel Kv1.5 plays important roles in the rhythmic beating of the atria, vascular tone of the pulmonary artery, and insulin secretion of pancreas. The activity of ion channels in a cell is determined by the function of single channels and the total number of channels in the plasma membrane. While the function and modulation of ion channels have been extensively studied, much less is known about how the number of ion channels in the plasma membrane is regulated. Using the patch clamp recording technique and biochemical methods in human embryonic kidney cells stably expressing Kv1.5 channels, we discovered that activation of protein kinase C (PKC) with PMA (phorbol 12-myristate 13-acetate, 10 nM) abolished Kv1.5 channel expression and current within 3 hours. Biochemical experiments revealed that PKC activation enhanced ubiquitination of Kv1.5 proteins. Vps24 (also known as charged multivesicular body protein 3, CHMP3) is a protein that sorts transmembrane proteins into lysosomes via the multivesicular body (MVB) pathway. It contributes to the endosomal sorting complexes required for transport (ESCRT)-III protein complex for protein sorting into the MVB. STAMBP (STAM-binding protein, also known as AMSH, associated molecule with the SH3 domain of STAM) is a deubiquitination isopeptidase that participates in the endosomal sorting of several cell-surface molecules. Our results showed that overexpression of Vps24 or STAMBP accelerated, whereas knockdown of Vps24 or STAMBP by siRNA transfection impeded PKC-mediated Kv1.5 degradation. Lysosome inhibitor bafilomycin A1 (1 µM) prevented PKC-mediated Kv1.5 degradation. Truncation of the N-terminus of Kv1.5 up to residue 209 (deltaN209) abolished PKC-mediated reduction in Kv1.5 current and expression. We conclude that PKC activation targets N-terminus of Kv1.5, leading to channel ubiquitination which triggers endocytosis and degradation of Kv1.5 channels through ESCRT machinery (Supported by the Canadian Institutes of Health Research Grant MOP-72911).
DYNAMIN 1 PUTS ALL VESICULAR RELEASE VIA SUBQUANTAL MODE IN ADRENAL CHROMAFFIN CELLS

Zhuan Zhou¹; Quanfeng Zhang¹; Qihui Wu¹;
¹Institute of Molecular Medicine, Peking University, Beijing, China

Dynamin 1 (dyn1) is required for clathrin-mediated endocytosis in most secretory (neuronal and neuroendocrine) cells. There are two modes of Ca²⁺-dependent catecholamine release from single dense-core vesicles: full-quantal (quantal) and sub-quantal in adrenal chromaffin cells (ACCs), but their relative occurrences and impacts on total secretion remain unclear. To address this fundamental question in neurotransmission area using both sexes of animals, here we report 1) dyn1-KO increased quantal size (QS, but not vesicle size/content) by >250% in dyn1-KO mice; 2) the KO-increase QS was rescued by dyn1 (but not its deficient mutant or dyn2); 3) the ratio of quantal versus subquantal events was increased by KO; 4) following a release event, more protein contents were retained in WT vs KO vesicles; and 5) the fusion pore size d₀ was increased from 9 nm to =9 nm by KO. Thus, Ca²⁺-induced exocytosis is a sub-quantal release in sympathetic adrenal chromaffin cells, implying that neurotransmitter release is generally regulated by dynamin in neuronal cells.
Thursday, May 9, 2019  
Poster Session III  
14:30 – 16:00  
University Botanical Gardens of Padova

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Thursday. Presenting authors with odd-numbered poster boards should present from 14:30 – 15:15 and those with even-numbered poster boards should present from 15:15 – 16:00. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

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Posters should be set up the evening of May 6 and removed by noon on May 10.
SNARE PRIMING INHIBITION VIA PHOSPHATIDIC ACID INDUCED SEC18 CONFORMATIONAL CHANGE

Andres S. Arango1,2; Robert P Sparks3; Matthew L Starr3; Zhiyu Zhao1,2; Muyun Lihan1,2; Rutilio Fratti1,3; Emad Tajkhorshid2,4;
1University of Illinois at Urbana-Champaign, Center for Biophysics and Quantitative Biology, Champaign, Illinois, USA
2University of Illinois at Urbana-Champaign, Beckman Institute, NIH Center for Macromolecular Modeling and Bioinformatics, Champaign, Illinois, USA
3University of Illinois at Urbana-Champaign, Department of Biochemistry, Champaign, Illinois, USA
4University of Illinois at Urbana-Champaign, Department of Biochemistry, Center for Biophysics and Quantitative Biology, Champaign, Illinois, USA

Membrane fusion is mediated by SNARE proteins, and is vital for a multitude of cellular transport phenomena. Post fusion, cis-SNAREs are recycled for continued fusion, in an ATP dependent process called priming. The unique job of priming all cellular SNAREs is the responsibility of AAA+ ATPase NSF or Sec18. In a joint computational and experimental effort, we show that Phosphatidic Acid (PA) inhibits the priming of cis-SNARE complexes by inducing conformational changes in Sec18 protomer. We further studied priming inhibition via a new small molecule inhibitor of Sec18 called IPA, which inhibits fusion, priming, and competitively inhibits Sec18 binding to PA. We identify potential PA binding sites to Sec18 using computational flooding of short tailed PA, as well as using an HMMM PA rich membrane to capture unbiased spontaneous membrane insertion of Sec18 monomers. In addition, to further sample potential binding sites of both PA as well as IPA on Sec18 we employed ensemble molecular docking using AutoDock Vina, with consecutive molecular dynamics simulations performed for the top poses using NAMD for characterization of ligand stability. Finally, we performed Random Accelerated Molecular dynamics simulations on the most stable resultant poses to estimate dissociation constants, which were compared to results from liposome binding experiments providing insight into the mechanism of priming regulation via Sec18. Our experimental and computational results show that PA induces conformational change of Sec18, inhibiting priming.
ASTROGLIAL ACTIVATION BY INTERFERON GAMMA AUGMENTS CELL SURFACE EXPRESSION OF ANTIGEN PRESENTING MHCII MOLECULES VIA INCREASING EXOCYTOTIC BUT DECREASING ENDOCYTOTIC VESICLE INTERACTIONS WITH THE PLASMALEMMAMico Božic2; Robert Zorec1,2; Matjaž Stenovec1,2;

1Celica BIOMEDICAL, Ljubljana, Ljubljana, Slovenia
2University of Ljubljana, Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, Ljubljana, Slovenia

Astrocytes are homeostatic cells in the central nervous system that partake in neuroinflammation upon activation; a process also termed reactive astrogliosis. It was shown previously that interferon gamma (IFNgamma), an inflammatory cytokine, induces expression of major histocompatibility type II molecules (MHCII) engaged in antigen presentation at the cell surface. To elucidate the vesicular pathway involved in MHCII delivery and segregation at the plasmalemma of cultured rat astrocytes, we investigated the subcellular localization of MHCII by confocal and structured illumination (SIM) microscopies and examined the dynamics of single vesicle interactions with the plasmalemma by high-resolution cell-attached membrane capacitance measurements. Astrocyte activation with IFNgamma increased the expression of MHCII, which upon immunolabelling co-localized highly with LAMP1-EGFP, a lysosomal marker, and minutely with immunolabelled Rab4A, EEA1 and TPC1, markers of various endosomes. As revealed by SIM measurements, numerous MHCII-positive vesicles in IFNgamma-activated astrocytes had larger diameter, consistent with their lysosomal identity. Contrastively, these were scarce in controls. MHCII localized to the cell surface only in IFNgamma-activated astrocytes. Membrane capacitance measurements revealed distinct types of reversible (kiss-and-run) and irreversible (full) exo-/endocytotic events in both groups. Following IFNgamma treatment reversible fusion of vesicles with larger diameter that exhibited a prolonged fusion pore dwell time and an increased fusion pore conductance was observed. Furthermore, IFNgamma activation reduced the frequency of full endocytotic events. ATP stimulation, which increases intracellular calcium activity, increased the frequency of exocytotic and decreased the frequency of full endocytotic events in both groups. Therefore, in IFNgamma-activated astrocytes, the altered balance between exo- and endocytosis is favoring exocytosis, predominantly of larger lysosomes, which contributes to prolonged residence of MHCII molecules at the plasmalemma, promoting antigen presentation in reactive astrocytes.
CRYO-EM AND KINETIC ANALYSIS OF OPA1-MEDIATED MITOCHONDRIAL INNER MEMBRANE FUSION

Luke Chao;
1Massachusetts General Hospital, Molecular Biology, Boston, Massachusetts, USA
2Harvard Medical School, Genetics, Boston, Massachusetts, USA

Mitochondrial membrane dynamics and ultrastructure are intimately tied to the organelle functional state. OPA1 is the mitochondrial inner membrane fusogen and plays roles in cristae morphology. We present preliminary single-particle Cryo-EM reconstructions of OPA1 indicating multiple nucleotide-dependent conformational states. With an in vitro reconstitution system, we distinguish nucleotide and lipid-dependent membrane tethering by OPA1, and discuss work to relate protein conformational state with the hemifusion and pore-opening steps in membrane fusion.
LIPID NANOTUBES FROM FREESTANDING LIPID MEMBRANES: OPTICAL TWEEZERS MANIPULATION AND QUANTITATIVE DETERMINATION OF MEMBRANE TENSION

Aurora Dols-Perez; Guillermo J Amador; Victor Marin; Roland Kieffer; Daniel Tam; Marie-Eve Aubin-Tam
1Delft University of Technology, Department of Bionanoscience, Kavli Institute of Nanoscience, Delft, Zuid-Holland, The Netherlands
2Delft University of Technology, Laboratory for Aero and Hydrodynamics, Delft, Zuid-Holland, The Netherlands

Lipid tubes, tubules or nanotubes are highly curved lamellar structures in the nanometer-micrometer scale with great importance in many biological processes. They play a vital structural role in different cellular organelles such as the endoplasmic reticulum, mitochondria and Golgi apparatus, but also in communication processes such as inter and intracellular exchanges and cellular migration. Their biophysical study is often carried using vesicles, supported lipid bilayers or living cells. In these systems, it is challenging to achieve asymmetric lipid distribution, dynamic buffer control and zero curvature. Using a freestanding lipid bilayer in a microfluidic device, these challenges can be solved and present additional advantages such as easy access to both sides of the membrane, possibility to create several membranes in a same device, possibility to circulate different solutions, and full compatibility with optical techniques. In this work, we show the combination of these novel freestanding lipid membranes formed inside a microfluidic chip with optical tweezers for the study of lipid nanotubes. Nanotubes were formed by pushing and pulling beads through or from the membranes, reaching lengths above half a millimeter. The quantification of the forces implied in the process, the tension of the membrane and the bending rigidity was possible without the need of additional sensors apart from the optical tweezers. Our method provides a robust platform, not only for nanotubes studies, but also for further study of protein-membrane interactions under controlled conditions on each side of the membrane, and modulated membrane complexity.
STABILITY OF LD CONTACT SITE

Laura J Endter¹; Herre Jelger Risselada¹;
¹Georg-August University, Theoretical Physics, Goettingen, Niedersachsen, Germany

Lipid Droplets (LDs), although long time unterated in its role in eukaryotic cells, have now emerged to be extensively studied and still pose intriguing questions to the researchers engaged in unraveling their secrets. Being closely linked to regulatory processes regarding the lipid metabolism and homeostasis they are not only fascinating objects in a biophysical point of view but are also a possible target for medical applications for pathological conditions such as obesity and diabetes [1, 2]. Once formed, the LD phospholipid monolayer appears to stay in close contact with the lipid bilayer of the endoplasmic reticulum. Aiming to determine the stability of the LD connection to the endoplasmic reticulum (ER) and the influence of seipin on that contact side we deploy coarse grained simulations using the Martini model. With calculations of free energy differences [3, 4] and barriers we may be able to shed some light on the question of the nature of the structure and the mechanistic pathway of formation and maintenance of the LD-ER contact site.[1] M. Schuldiner and M. Bohnert, Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids, 1862, no. 10, 1188–1196 (2017).[2] A. R. Thiam and L. Fore^t, Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids, 1861, no. 8, 715–722 (2016).[3] Y. G. Smirnova, M. Fuhrmans, I. A. Barragan-Vidal, and M. Mu¨ller, J. Phys.D. Appl. Phys., 48, no. 34, 343001 (2015).[4] H. J. Risselada, Biophys. J., 112, no. 12, 2475–2478 (2017).
THE INTERACTIONS BETWEEN THE NUCLEAR PORE COMPLEX AND THE PORE MEMBRANE (POM) REGION WITHIN THE NUCLEAR ENVELOPE

Joseph S Glavy¹;
¹University of Texas at Tyler, Pharm. Sci, Tyler, Texas, USA

The nuclear envelope separates into three domains: the outer nuclear membrane (ONM), the inner nuclear membrane (INM) and the pore membrane (POM). The POM is the region of the nuclear envelope where INM and ONM merge occurs. The POM curvatures are coated with select membrane proteins believed to anchor Nuclear Pore Complexes (NPCs) like the prongs of a diamond ring. POM proteins are involved in the initiation of pore complex construction, stabilization, emancipation and eventual reformation of the NPC. The three-dimensional structure of purified complexes was determined by single particle electron microscopy. To uncover their dynamic interacting layers of the NPC including areas within POM curvatures, we crosslinked the engaged complexes within the human NPC (hNPC) and the subsequent identification of crosslinked peptides through crosslinking mass spectrometry (XL-MS). These data, along with a high resolution structure of the hNPC were integrated to determine how the layers of the hNPC come together forming encrusted rings and their connectivity to the POM curvatures.
FUSION PORE REGULATION BY CAMP AND EPAC2 IN PANCREATIC BETA CELLS

Alenka Gucek; Nikhil R Gandasi; Muhmmad O Hmeadi; Marit Bakke; Stein O Doskeland; Anders Tengholm; Sebastian Barg;
1Uppsala University, Medical Cell Biology, Uppsala, Uppsala län, Sweden
2University of Bergen, Biomedicine, Bergen, Hordaland, Norway

The central feature of regulated exocytosis is formation of a fusion pore, an aqueous channel connecting vesicular lumen with the extracellular space. After exocytosis has been triggered, this complex proteo-lipidic structure acts as a molecular sieve that regulates cargo release based on size. There is evidence that dysregulated fusion pore expansion is involved in common diseases, including type-2 diabetes and Parkinson’s disease. Here we have used high-resolution TIRF imaging in pancreatic beta-cells to study molecular mechanisms that regulate fusion pore behavior. A pH-sensitive granule marker was used to measure fusion pore behavior in real time. The time course of GFP-labeled proteins at the release site was quantified in parallel, and the data correlated with pore behavior. We report that elevated cytosolic cAMP favours stable fusion pores that allow rapid release of nucleotides, but restrict exit of peptides. The effect is mediated by the cAMP-dependent Rap-GEF Epac2, because cAMP-dependent fusion pore regulation is absent when Epac2 is inactivated pharmacologically or in Epac2-knockout mice. We show further that overexpression of Epac2 and several proteins involved in endocytosis favor cAMP-dependent pore restriction, and are recruited specifically to restricted fusion pores during exocytosis. We conclude that Epac2 controls cAMP-dependent fusion pore restriction and thereby affects differentially the release of hormones and transmitter molecules during exocytosis.
DYNAMICS OF THE READILY RETRIEVABLE POOL OF SURFACE SYNAPTIC VESICLE PROTEINS ANALYSED BY FRAP

Martin Kahms1; Debora C Moschner1; Juergen Klingauf3;
1University of Muenster, Institute of Medical Physics and Biophysics, Muenster, Nordrhein-Westfalen, Germany

During synaptic transmission, synaptic vesicles (SVs) fuse with the plasma membrane at the active zone and are subsequently retrieved at the peri-active zone by compensatory endocytosis with high fidelity. However, the mechanisms of SV protein re-sorting and organization into patches of readily retrievable pool (RReTP) of SVs in the peri-active zone prior to endocytosis remain poorly understood. Here, we analyzed the dynamic organization of SV proteins of the RReTP and investigated their sorting determinants. We deleted and swapped domains of various SV proteins and demonstrated trans-membrane domains of these proteins to be dispensable for correct sorting into budding SVs. This suggests that retrieval efficacy is mainly determined by the cytosolic domains of SV proteins. Furthermore, we performed fluorescence recovery after photobleaching (FRAP) experiments at presynaptic boutons expressing pHluorin-tagged synaptic proteins. This enables the selective bleaching of the surface fraction of SV proteins and analysis of their mobility as well as their affinity towards the RReTP. We found that most SV proteins can readily diffuse within axonal and presynaptic membranes, but are enriched at the RReTP, albeit with rather low affinity. In contrast, we observed that two major large SV proteins, the vacuolar ATPase (v-ATPase) and the vesicular glutamate transporter (v-Glut), display limited mobility with their diffusion restricted to the pre-synaptic compartment, and are mostly part of the RReTP. Thus, we hypothesize that these molecules serve as central hubs, which recruit other endocytic synaptic cargo molecules at the peri-active zone and orchestrate the molecular composition of the readily retrievable pool.
REGULATION OF EXOCYTOSIS BY AMISYN, A PI(4,5)P2 AND SYNTAXIN-BINDING PROTEIN

Ilona Kondratiuk1,2; Shrutee Jakhanwal3; Reinhard Jahn3; Ira Milosevic1,2;
1European Neuroscience Institute (ENI), Gottingen, Niedersachsen, Germany
2University Medical Center Gottingen (UMG), Gottingen, Niedersachsen, Germany
3Max Planck Institute for Biophysical Chemistry, Gottingen, Niedersachsen, Germany

Higher functions of the brain, for example learning and memory, are mediated by fast and precisely coordinated neurotransmitter release through the process of regulated exocytosis. Intense research in the past three decades has identified numerous proteins involved in exocytosis, including the Sec1/Munc18 (SM) protein family (Munc18, Munc13), synaptotagmins that sense calcium, and the SNARE complex proteins: synaptobrevin-2/VAMP-2, syntaxin-1 and SNAP-25 that mediate membrane fusion. While the key exocytic proteins are highly conserved through evolution, the regulation of exocytosis has advanced and requires more proteins in the higher organisms, such as vertebrates. In addition to the core set of exocytic machinery, exocytosis is regulated by complexin, tomosyn and amisyn (STXBP6), cytosolic proteins that bind the SNARE complex. Amisyn is reported to be an important negative regulator of exocytosis, yet little is known about this brain-enriched protein. We found that, in addition to the C-terminal SNARE motif that interacts with syntaxin-1 and forms ‘fusion-inactive’ SNARE complex, amisyn contains an N-terminal pleckstrin homology (PH) domain. The PH domain of amisyn is phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) specific, and it mediates its interaction with the plasma membrane. Given that amisyn is a conserved protein present only in vertebrates, it makes it necessary to characterize it better. We have generated amisyn knock-out mice to study amisyn-dependent processes at the vertebrate neurons and neurosecretory cells. We found that amisyn is important for the priming of secretory vesicles and the size of vesicle pools, but not fusion kinetics. Curiously, the inhibition is not due to amisyn’s SNARE motif binding to syntaxin-1, but the full-length protein is needed for the proper control of exocytosis. We are currently further investigating the mechanisms of amisyn-dependent inhibition and its implication to neurotransmission.
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ASSESSMENT OF THE FUNCTIONALITY AND STABILITY OF SOLUBILIZED NICOTINIC ACETYLCHOLINE RECEPTOR DETERGENT COMPLEXES IN LIPIDIC CUBIC PHASE (LCP) AS A GUIDE FOR CRYSTAL FORMATION

Jose A. Lasalde-Dominicci1,2,4; Orestes Quesada-Gonzalez3,4;
1University of Puerto Rico, Department of Biology, San Juan, Puerto Rico
2University of Puerto Rico, Chemistry, San Juan, Puerto Rico
3University of Puerto Rico, Physical Sciences, San Juan, Puerto Rico
4Molecular Sciences Research Center- UPR, San Juan, Puerto Rico

LCP is a crystallization platform for membrane proteins that has increased exponentially the number of crystal structures in the past decade. However, little is known about the mechanism by which membrane protein detergent complexes diffuse in the LCP to form crystals. Also, the relation between detergent solubilization and nAChR stability is still poorly understood. We used three families of lipid-like detergents with different chain length to isolate nAChR from Torpedo californica's electroplax tissue (TcET). The functionality of affinity-purified nicotinic acetylcholine receptors detergent complexes (nAChR-DCs) was assessed prior to LCP preparation using the two electrode voltage clamp (TEVC). The stability and Fractional Mobility (FM) of the nAChR-DCs in LCP was assayed for 30 days using fluorescence recovery after photobleaching (FRAP). Our results show that phospholipid-analog detergents with 16 carbon acyl chains sustain nAChR-DC mobility unchanged for the 30 day period, while in most of cholesterol-analog detergents, and CF analogs the FM decays on the first 15 to 20 days of the experiment. We found an interesting correlation between the elongation of the acyl chain on phospholipid-analog detergents and an increment on the FM, particularly observed on the LFC detergent family. The most abundant fatty acids in the TcET phospholipid species PC, PE, PG, PI, and PS were 16:0 and 22:6. These results are consistent with the fact the nAChR-LFC16 complex is the most stable complex among all the detergents that we have evaluated. We are currently producing nAChR-LFC16 crystals for structural studies using LCP, vapor diffusion techniques and the RMP@LMx technique (U.S. patent 10,155,221) developed in our laboratory (www.nachrs.org). This research was supported by the NIH NIGMS grants 1R01GM098343, 5R25GM061151 and COBRE P20GM103642
INVESTIGATION OF FORCES DURING MEMBRANE FUSION BY USING LIPID VESICLES AS AFM PROBES

Ines Lüchtefeld1; Tomaso Zambelli1; Janos Vörös1;
1ETH Zurich, Institute for Biomedical Engineering, Zurich, Switzerland

The interaction and fusion of phospholipid membranes plays an important role in many essential physiological processes such as neurotransmitter release at the synapse or drug delivery to cells with cationic liposomes. In spite of the physiological importance, the process of establishing a fusion pore between two membranes is still not fully understood. Especially vague is the understanding about the role of the contact time and contact force of two membranes for the establishment of hemi- and full fusion states. Therefore, we propose a new experimental system to investigate the influence of contact forces during membrane fusion. The system consists of an atomic force microscopy (AFM) cantilever with an integrated microfluidic channel, namely the FluidFM. It is used to firstly attach a giant unilamellar vesicle (GUV) to the tip of the cantilever by applying negative pressure inside of the channel, and to subsequently bring this GUV into force-controlled contact with a supported lipid bilayer, a surface immobilized GUV, or a cell. Using fluorescence increase and recovery measurements we are able to observe fusion states dependent on the contact force, contact time, and other parameters like membrane tension and ion concentration. By bringing two lipid membranes into close and force-controlled proximity for defined time periods, the presented experimental system is not only able to model processes like cationic liposome assisted drug delivery, but can also mimic the zippering function of the cytosolic domains of the SNARE complex. Consequently, the presented experimental system is able to facilitate new quantitative insights into various membrane fusion processes, like the contact time and force necessary for protein free fusion, as in liposome assisted drug delivery, or the role of the transmembrane domains of the SNARE complex components.
Fission of biological membranes is an essential process that maintains their necessary shapes, sizes, compositions, and connectivity. Additionally, pathogens must hijack membrane fission pathways to infect and escape from cells. Despite its importance, many aspects of fission remain poorly understood, including how fission proteins assemble on membranes and convert chemical energy to the mechanical force needed to sever lipid bilayers. I have utilized a combination of chemical synthesis, biophysics, cryo-electron microscopy (cryo-EM), and molecular dynamics simulations to investigate how one class of fission proteins (ESCRT-III) remodels membranes and alters the physical properties of lipid bilayers to effect fission. Cryo-EM reconstructions of ESCRT-III-bound membranes with brominated analogs of lipids, which strongly scatter electrons, reveal lipid leaflet asymmetry induced by high curvature and molecular details of lipid packing. The structures of ESCRT-III proteins assembled on membranes containing brominated lipids provide insight into how the proteins are able to alter the structure membranes to overcome the energetic barrier to fission. Finally, we compare experimental results to calculations using continuum models in order to shed light on how these structures lead to fission. An improved understanding of the mechanism of membrane fission could provide new therapeutic targets for diseases and infections involving malfunctioning membrane-remodeling proteins.
DIRECT OBSERVATION OF DYNAMIN-INDUCED MEMBRANE FISSION BY HIGH-SPEED ATOMIC FORCE MICROSCOPY

Yuliang Zhang¹; Javier Vera Lillo³; Vadim Frolov³,⁴; Aleksandr Noy¹,²;
¹Lawrence Livermore National Laboratory, Biology and Biotechnology Division, Livermore, California, USA
²University of California Merced, School of Natural Sciences, Merced, California, USA
³University of the Basque Country, Biofisika Institute (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, Leioa, Vizcaya, Spain
⁴Basque Foundation for Science, Ikerbasque, Bilbao, Vizcaya, Spain

Dynamin1 is a large GTPase mediating membrane fission step during endocytosis. The ability of Dynamin1 to self-assemble into a helix engulfing the neck of the endocytic vesicle is critical for its function. Even though the structure of dynamin1 in the assembled state on the membrane has been reported recently, how the cooperative GTP hydrolysis by Dynamin1 polymer leads to fission remains a subject of considerable controversy. We have used high-speed atomic force microscopy to investigate real-time dynamics of GTP-dependent rearrangements of dynamin1 self-assembled on lipid nanotubes. The movies show GTP-induced local conformational rearrangements of the polymer leading to rapid membrane fission. Our observations are consistent with the hypothesis that fission is stochastically coupled to GTP-driven dynamics of short dynamin scaffolds that induce acute and transient curvature stress.
TARGETING FUSION TO SPECIFIC MEMBRANE DOMAINS

Rafaela R. M. Cavalcanti$^{1,2}$; Rafael B. Lira$^2$; Karin A. Riske$^1$; Rumiana Dimova$^2$;
$^1$Universidade Federal de São Paulo, Biophysics, São Paulo, Brazil
$^2$Max Planck Institute of Kolloids and Interfaces, Theory & Bio-Systems, Golm-Potsdam,
Brandenburg, Germany

Membrane fusion is a fundamental process in cell life but certain molecular details are still unknown. In addition, because cell membranes are commonly organized in domains, understanding the fusion process in non-homogenous systems can be especially challenging. The use of giant unilamellar vesicles (GUVs) as a biomimetic system can assist the better understanding of the modifications occurring at the membrane fusion to specific regions and monitored changes in membrane composition using cationic fusogenic liposomes (LUVs, ~100 nm) and GUVs exhibiting coexisting fluid and gel phases. Fusion efficiency was assessed using microscopy-based fluorescence resonance energy transfer (FRET). We observed high FRET signal at the negatively charged fluid domains. Fusion was associated with content mixing (full fusion) and GUV budding, indicating increase in membrane area. The high fusion efficiency lead to dissolution of the (initially neutral) gel domains. In GUVs with neutral fluid domains and negatively charged gel domains, we observed low FRET, low but detectable content mixing and mainly LUV docking on the gel domains. In these vesicles, the phase separated domains persisted due to the low fusion efficiency. We also explored fusion to homogeneous fluid charged GUVs with increasing cholesterol fraction: no significant change in fusion efficiency was observed. In conclusion, we demonstrate targeting of fusion to specific membrane domains and modulation of fusion efficiency by controlling membrane charge and phase state. This can play an important role in regulating the interaction between cells and liposomes used in drug delivery systems.
ESCRT-MEDIATED REMODELLING AND SEVERING OF PULLED MEMBRANE NANOTUBES

Mark Remec Pavlin\textsuperscript{2}; Jamie Shiah \textsuperscript{1}; James Hurley\textsuperscript{1,2,3};
\textsuperscript{1}UC Berkeley, Molecular and Cell Biology, Berkeley, California, USA
\textsuperscript{2}UC Berkeley, Graduate Group in Biophysics, Berkeley, California, USA
\textsuperscript{3}Lawrence Berkeley National Laboratory, Molecular Biophysics and Integrated Bioimaging, Berkeley, California, USA

The endosomal sorting complex required for transport (ESCRTs) constitute an evolutionarily conserved set of proteins that have been identified as crucial for many membrane remodeling and membrane scission events in eukaryotes. Notably, structural work has characterized a variety of polymers formed by these proteins, including spirals, rings, cones and tubes that can deform membranes in vivo and in vitro. Here, we utilize a home-built optical trap and confocal fluorescence microscopy setup to functionally characterize the behavior of ESCRT proteins on membrane nanotubes. We show that ESCRTs are capable of nucleating onto, applying force onto, and eventually severing membrane nanotubes. Fluorescence imaging and force measurement of this process provide deeper mechanistic insight into the molecular function of ESCRT proteins as they remodel and sever membranes.
MULTIPLE ISOFORMS OF SYNAPTOTAGMIN REGULATE PEPTIDE SECRETION FROM NOCICEPTIVE NEURONS

Judit Meszaros¹; Elizabeth P Seward¹;
¹University of Sheffield, Biomedical Science, Sheffield, United Kingdom

In response to inflammation and nerve injury, pain signaling is transduced from the periphery to the CNS through nociceptors. While much is known about the changes in ion channel function which underlie pain signaling, less is known about what changes occur at the level of the exocytotic machinery to support enhanced signaling, hyperalgesia and peptide secretion. Here we present research aimed at characterizing molecules regulating peptide secretion from nociceptors, focusing on the well-known vesicular regulator of exocytosis, synaptotagmin (SYT). We found that SYT4, -11, -2 and -7 are expressed in nociceptors isolated from adult dorsal root ganglia (DRG). Co-localization studies with CGRP indicated that multiple pools of peptidergic vesicles exist within nociceptors, characterized through their differential expression of distinct Syt isoforms. CGRP secretion, as measured by ELISA from DRGs prepared from heterozygous or litter-mate knockout (KO) SYT4 or Syt7 mice showed no significant difference, implying either that these two isoforms of SYT play a modulatory rather than essential role in controlling peptide secretion from nociceptors or that compensatory changes have taken place in the gene knockout animals. To investigate the involvement of SYT4 and SYT7 in peptide release in greater detail, we switched to expressing SYT4-pHluorin or SYT7-pHluorin in combination with NPY-mCherry in adult nociceptors and imaging fusion using TIRFM. We compared results obtained with the SYT-pHluorins with synapto-pHluorin (VAMP2-pHluorin). Analysis of single events showed that fusion of vesicles expressing SYT4 or SYT7 are relatively slow and long-lived and have the characteristics associated with ‘kiss-and-run’ exocytosis. Expressing SYT7-pHluorin in SYT4 KO nociceptors significantly altered the timing and kinetics of single fusion events. In the absence of SYT4, exocytosis of SYT7-bearing vesicles is slowed and reduced, consistent with the view that SYT4 is increasing the pool of releasable peptidergic vesicles in nociceptors.
It has been shown experimentally that amyloid fibrils, which are naturally present in seminal fluid, may increase the infectious viral titer of HIV-1 by four to five orders of magnitude [1]. These fibrils termed SEVI (semen-derived enhancer of viral infection) interact with HIV-1 virions and promote their attachment to target cells, resulting in accelerated fusion. While the inhibition of this process would be of interest in the context of HIV, fusion enhancement is desirable for targeted retroviral gene transfer and drug delivery.

The infection enhancement mechanism is not yet fully understood. While the positive surface charge of the amyloid fibrils evidently plays a role in bringing the negatively charged viruses and cells in contact, the increased infectivity also appears to depend on the intrinsic properties of the fibril structures [2], such as fibril stiffness and fibril length. To reconstruct the interaction landscape that amyloid fibril covered viruses face when undergoing fusion with a host cell, we employ elastic continuum models in conjunction with coarse grained molecular dynamics simulations.

THE ROLE OF SPECIFIC LYSOSOMAL LIPID - BIS(MONOACLYGLYCERO)PHOSPHATE (BMP) - ON MEMBRANE FUSION AND ITS ASSOCIATION WITH ACID ENVIRONMENT

Tayana M. Tsubone¹; Ana Paula Ramos²; Rosangela Itri¹;
¹University of Sao Paulo, Institute of Physics, São Paulo, Brazil
²University of Sao Paulo, Department of Chemistry, Ribeirão Preto, São Paulo, Brazil

The autophagic/lysosomal system includes a variety of vesicular compartments that undergo dynamic fusion events. It because during autophagy, cytoplasmic materials are sequestered by the autophagosome and transported to the lysosome for digestion. The specific stages of autophagy are induction, formation of the isolation membrane (phagophore), formation and maturation of the autophagosome and, finally, fusion with a late endosome or lysosome. However, the characteristics and factors modulating these autophagosome–lysosome fusion remain, for the most part, unknown. Koga and co-workers suggested that changes in the lipid composition may inhibit autophagic vesicular fusion. However, the complexity of vesicular fusion events that take place in autophagy, makes it difficult to study in intact cells. To gain insights on the properties that govern membrane lysosomal fusion events, we have investigated the biophysical properties of mimetic membranes that contain bis(monoacylglycerol)phosphate (BMP) - a hallmark phospholipid of lysosomal membranes - and their consequences on membrane fusion. Considering that BMP has a unique structural characteristic, here we have used DOPG (that is a structural isomer of BMP) for comparison purpose. Interestingly, our results have shown that membrane fusion is significantly influenced by pH, unlike the data from DOPG vesicles. The explanation for this phenomena can be related to properties of BMP on packing membranes differently at low pH. Surface pressure vs surface area isotherms obtained from Langmuir monolayers of BMP in pH 4.5 indicates that the surface pressure rises more slowly than in BMP pH 7.4 isotherm suggesting that the presence of protons on the head group disturbs lipid film packing and reduce the surface area available for each molecule due their higher compaction. In fact, at pH 4, BMP exhibits lower surface charge density than DOPG, indicating that the decreased electrostatic repulsion on the charged head groups of BMP.

Acknowledgements. Thanks FAPESP for financial support with scholarship grant (2016/23071-9) and R.I. acknowledges CNPq research fellowship.
MITOCHONDRIA SHAPE CHANGES AND THE INNER MEMBRANE-LOCATED MECHANOSENSOR, MITO(BK$_{Ca}$) - A HYPOTHESIS

Agnieszka Walewska$^1$; Piotr Koprowski$^1$; Adam Szewczyk$^1$;  
$^1$Nencki Institute of Experimental Biology, Laboratory of Intracellular Ion Channels, Warsaw, Mazowieckie, Poland

In our studies, we recorded by patch-clamp technique the single-channel activity of mitochondrial large-conductance calcium-activated potassium channels (mitoBK$_{Ca}$) residing in the inner membrane of mitochondria of the human glioma U-87 MG cells. We have found that subpopulation of mitoBK$_{Ca}$ channels was mechanosensitive and application of hydrostatic pressure to the recording pipette resulted in increased open probability due to longer open times. However, not all mitoBK$_{Ca}$ channels were mechanosensitive possibly due to the expression of different splice variants of BK$_{Ca}$. Our finding indicated to possible engagement of the mitoBK$_{Ca}$ channel in mitochondrial dynamics in which changes in membrane tension and shape might play a role. Here, we hypothesize that mechanical forces that might arise during remodeling and volume changes of cristae, mitochondria fusion and fission could impact local tension within the inner mitochondrial membrane. This, in turn, can modulate the activity of mitoBK$_{Ca}$ and provide regulation of ion flow to modulate mitochondrial membrane potential or/and volume. This work was supported by the Polish National Science Center, grant no. 2017/26/M/NZ3/00727.
MOLECULAR MECHANISM OF NEUROTRANSMITTER RELEASE: LOCKED UNTIL THE LAST MILLISECOND

Qiangjun Zhou\textsuperscript{1}; Thomas C Südhof\textsuperscript{1,2}; Axel T Brunger\textsuperscript{1,2};
\textsuperscript{1}Stanford University, Molecular and Cellular Physiology, Stanford, California, USA
\textsuperscript{2}Howard Hughes Medical Institute, Stanford, California, USA

Synaptic transmission is essential for the process of communication between two neurons. It occurs upon fusion of synaptic vesicles with the plasma membrane, a highly regulated and ultrafast process occurring in less than a millisecond. Synaptotagmin, complexin, and neuronal SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) proteins mediate evoked synchronous neurotransmitter release, but the molecular mechanisms mediating the cooperation between these molecules remain unclear. Through a combination of high-resolution structural, biochemical and electrophysiological studies, we showed how these proteins form the primed pre-fusion SNARE–complexin–synaptotagmin-1 complex. Furthermore, combined with functional studies on the dominant-negative effects of certain synaptotagmin-1 mutants, we proposed an unlocking mechanism that is triggered by Ca\textsuperscript{2+} binding to the synaptotagmin-1 molecules, leading to SNARE complex zippering and membrane fusion. Finally, these studies also explained the high speed of Ca\textsuperscript{2+}-triggered fusion – everything is in the right place until the synaptotagmin-1 “brake” at the tripartite interface is released. However, the molecular organization of the primed pre-fusion complexes, and the association of these complexes with the synaptic and plasma membranes remains unclear. Over the past two years, I have continued my research investigating the synaptic vesicle fusion machinery \textit{ex vivo} and \textit{in situ}. My recent work shows more details of the primed state of synaptic vesicles on the plasma membrane in cultured neurons using cellular cryo-electron.