7-Subg
Different Acylation Motifs Direct Multiply Orthogonal Co-Localization of Lipid Anchored Proteins in Live Cell Membranes
Jay T. Groves.
Univ California Berkeley, Berkeley, CA, USA.
No Abstract.

8-Subg
Plasma Membrane Heterogeneity and Receptor Mediated Signaling
Barbara Baird.
Cornell Univ, Ithaca, NY, USA.
The complex role of plasma membrane structure in orchestrating receptor-mediated signal transduction is addressed in collaborative studies investigating how antigen crosslinking of IgE-receptors on mast cells initiates signaling pathways leading to multiple cellular responses. Segregation of liquid ordered regions from disordered regions of the plasma membrane provides protection from transmembrane phosphatases and thereby a mechanism for crosslinking-dependent phosphorylation of IgE-receptors by active Lyn kinase in the first signaling event. Defined clustering of IgE-receptors with patterned lipid bilayers enables fluorescence visualization of co-redistributing signaling components with spatial resolution on the micron scale. Nanoscale resolution of clustering components is visualized with scanning electron microscopy and super-resolution fluorescence microscopy. Single molecule dynamics can be characterized in nanofabricated devices. These integrated approaches for examining membrane structural heterogeneity and functional consequences will be discussed.

Subgroup: Exocytosis & Endocytosis

9-Subg
Motion and Capture of Granules in Synaptic Boutons
Edwin S. Levitan.
Univ Pittsburgh, Pittsburgh, PA, USA.
We have been using GFP imaging to study neuropeptide granule and signaling dynamics in the intact *Drosophila* neuromuscular junction. Expression of a GFP-tagged neuropeptide first revealed that a brief tetanus increases resident granule mobility for many minutes. Mobilization and the accompanying post-tetanic potentiation of release are induced by Ca^{2+}-induced Ca^{2+} release from presynaptic endoplasmic reticulum, which then activates Calmodulin-dependent protein kinase II (CamKII). Monitoring CamKII with a FRET-based indicator shows that the enzyme is activated in the cytoplasm and then translocates to active zones, where small synaptic vesicles undergo exocytosis. CamKII also induces replacement of granules in the nerve terminal by tapping into the flow of granules transiting through *en passant* synaptic boutons. Surprisingly, this activity-dependent capture occurs while granules are moving in the retrograde direction. To determine the route taken by granules to generate this retrograde flux, single granules were tracked for minutes. At rest, granules explore the nerve terminal in a circuitous journey to and from the most distal bouton with rare capture events along the way. Thus, granule distribution and replacement are not limited by synthesis or axonal transport. Rather, granule mobility and capture are locally controlled in nerve terminal boutons to support release.

10-Subg
Priming Snares For Ca2+-Triggered Vesicle Exocytosis
Thomas F.J. Martin.
University of Wisconsin-Madison, Madison, WI, USA.
No Abstract.

11-Subg
Lumenal Vesicle Formation in the Endocytic Pathway
Phyllis Hanson.
Washington University, St. Louis, MO, USA.
The ESCRT (endosomal sorting complex required for transport) machinery comprises a set of protein complexes that are responsible for sorting and trafficking into multivesicular bodies within the endocytic pathway as well as the topologically related processes of viral budding and cytokinesis. Recent studies suggest that ESCRT-III and the AAA+ ATPase VPS4 play a central role in driving lumenal vesicle formation and release. This talk will discuss recent studies that explore the role and regulation of these components in creating lumenal vesicles, and will discuss the significance and implications of this pathway for neuronal cell biology.

12-Subg
Membrane Curvature and Fission By Dynamin: Mechanics, Dynamics and Partners
Vadim A. Frolov[1], Pavel V. Bashkirov[1,2], Sergei A. Akimov[1,2], Joshua Zimmerberg[1].
[1]National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, USA, [2]A.N. Frumkin Institute of Physical Chemistry and Electrochemistry of RAS, Moscow, Russian Federation.
Membrane fission requires creation of high membrane curvature even before the actual rearrangement of lipid bilayer occurs. We analyze here how this curvature stress enforces rearrangements in the inner monolayer of tightly squeezed membrane necks leading to ''self-fusion'' of the inner monolayer and formation of canonical stalk-type membrane intermediate. We further resolve experimentally how a single fission machinery assembled by dynamin, the prototype protein orchestrating membrane fission in different intracellular processes, imposes curvature stresses leading to fission of nanotubes pulled from a lipid bilayer. We show that dynamin is capable of producing critical curvature stress, leading to non-leaky membrane fission, and that the geometrical arrangement of dynamin on the tube membrane corresponds to the theoretically predicted configuration optimal for the triggering of membrane fission. Using dynamin mutant with altered membrane insertion profile, we confirm another prediction of the model on the critical role of the hydrophobic insertion in lowering the main energy barrier for the fission. Finally, we analyze the dynamic coupling between the GTPase cycle of dynamin and the membrane curvature and outline the pathways of mechano-chemical energy transduction in the membrane fission mediated by dynamin.

13-Subg
Measuring Exocytosis At Single Cells and in Intact Tissue
Mark Wightman.
University of North Carolina, Chapel Hill, NC, USA.
Exocytosis is a fundamental mechanism of intercellular communication. It involves the release of small packets of molecules that are packaged within the cell in vesicles that are released upon receipt of an appropriate stimulus. Because individual exocytotic events at most cells consist of release of a few attomoles or less, these events have been difficult to directly measure. Almost two decades ago we circumvented this problem by placing a carbon-fiber microelectrode adjacent to a single chromaffin cell in primary culture. With an appropriate potential applied to the electrode, catecholamines were oxidized by the electrode following exocytosis. The current arising from these events was shown to be due to the release of the catecholamines contained within a single vesicle. The detailed view of exocytosis provided by this approach provided new insights into this fundamental process. Subsequent research showed that this approach could be extended to exocytosis at neurons whose vesicles contain only zeptomole amounts of catecholamines. Research today is exploring exocytotic release events in more intact tissue to understand the competing events of release, uptake and diffusion.