48-Subg
Ultrafast Recycling of Synaptic Vesicles
Shigeki Watanabe[1], Thorsten Trimbuch[2], Marcial Camacho-Perez[3], Benjamin Rost[3], Christian Rosenmund[3], Erik M. Jorgensen[1].
[1]Department of Biology, University of Utah, Salt Lake City, UT, USA, [2]Neuroscience Research Center, Charité Universitätsmedizin Berlin, Berlin, Germany, [3]Charité Universitätsmedizin Berlin, Berlin, Germany.
Neurons can fire at extremely high rates. To sustain neurotransmission, synaptic vesicles must be recycled locally at synapses. Two models for synaptic vesicle endocytosis have been put forward based on the morphological studies in frog neuromuscular junctions. Heuser and Reese proposed that endocytosis occurs via a slow mechanism using clathrin scaffolds. Ceccarelli and his co-workers proposed a fast mechanism, kiss-and-run. Since then, many studies have sought to identify the mechanism for synaptic vesicle endocytosis. However, instead of resolving the issue, conflicting evidence has accumulated over the years. The molecular studies have suggested clathrin and clathrin-associated proteins are essential. However, the kinetics studies have suggested that both forms co-exist. Our data identify a third pathway that is fast, but requires clathrin to regenerate vesicles.
To investigate how endocytosis takes place, we developed a method, 'flash-and-freeze' fixation that couples optogenetic stimulation with rapid high-pressure freezing and captures endocytosis at millisecond temporal resolution. To our surprise, vesicle membrane is recovered via ultrafast endocytosis within 100 ms following a single stimulus. The large endocytic vesicles then fuse to form an endosome and are resolved by clathrin into synaptic vesicles in 5-6 s. When experiments are performed at 20°C instead of 37°C, ultrafast endocytosis fails, and clathrin regenerates synaptic vesicles directly from plasma membrane. These results suggest that recycling of synaptic vesicles is normally a rapid two-step process: ultrafast endocytosis that removes excess membrane from the surface and then clathrin-mediated biogenesis of synaptic vesicles from endosomes.

49-Subg
Complexin-Mediated Inhibition of Vesicle Fusion: Conserved Functions from Worm to Mouse
Jeremy Dittman[1], Rachel Wragg[1], Daniel Radoff[1], David Snead[1], Yongming Dong[2], Jihong Bai[2], David Eliezer[1].
[1]Biochemistry, Weill Cornell Medical College, New York, NY, USA, [2]Fred Hutchinson Cancer Research Center, Seattle, WA, USA.
Synapses continually replenish their synaptic vesicle (SV) pools while suppressing spontaneous fusion events, thus maintaining a high dynamic range in response to physiological stimuli. The presynaptic protein complexin (CPX) inhibits fusion through interactions between its highly conserved central helix and the SNARE complex. Two poorly conserved domains (the accessory helix and the C-terminal domain) on either side of the central helix (CH) are also required for inhibition of spontaneous fusion. We found that the C-terminal domain (CTD) binds lipids through a novel protein motif, permitting complexin to inhibit spontaneous exocytosis in vivo by targeting complexin to highly curved membranes such as SVs. Membrane curvature enhanced CPX binding and induced conformational changes in a critical amphipathic region of the CTD. The accessory helix (AH) of CPX contributes to the inhibition of exocytosis but the molecular mechanism for this function remains unknown. Several models have been proposed for the role of AH based on the concept that AH competes with VAMP for a binding site on the SNARE complex. Using a series of AH mutations and chimeras with mouse AH together with NMR and CD spectroscopy, electrophysiology, and behavioral assays, we identified key features of the AH and CH required for inhibition of SV fusion by CPX. We propose that the AH stabilizes the CH through nucleation and propagation of helical secondary structure, thereby facilitating binding of the CH to the SNARE complex.

50-Subg
Known Unknowns in Exocytosis
Ronald W. Holz.
Pharmacology, University of Michigan, Ann Arbor, MI, USA.
Despite the immense progress in identifying the essential molecules in the exocytotic pathway and the award of the 2013 Nobel Prize in Physiology and Medicine to colleagues in the field, gaps continue to exist in our understanding of the most fundamental aspects of the process. The intent of the talk is to highlight some of the known unknowns and present recent (surprising) results concerning the regulation of the fusion pore by the very proteins that are being discharged upon fusion.

Subgroup: Permeation & Transport

51-Subg
Voltage-Gated Sodium Channels: Structure and Function of Complexes with Sodium Channel Blockers
B.A. Wallace.
Crystallography, Birkbeck College, London, United Kingdom.
The initiation of the action potential in excitable cells results from the opening of voltage-gated sodium channels. In humans, mutations in sodium channels produce a wide range of neurological and cardiovascular diseases; hence these channels represent key targets for development of pharmaceutical drugs. Sodium channel blockers such as lamotrigine and lidocaine have been shown to have efficacy, respectively, as anti-epileptic and local anaethetic drugs. Sodium channels are also present in some prokaryotes. We have determined the crystal structure of one of these, the open pore form of NavMs, the orthologue from *Magnetococcus marinus*.
In collaboration with the Clapham group, we have recently shown that drugs which are known to block eukaryotic sodium channels also bind to and block the NavMs channel.Crystallographic, computational and electrophysiology methods have been used to determine the functional effects and locations of these and related blocker compounds within the channel cavity. One end is located near the side fenestrations, consistenr with a role for these features in the entry pathway for drugs into the internal cavity of the pore. The other end appears to protrude into the selectivity filter, blocking the passage of sodium ions into the cavity, and ultimately preventing their exit through the intracellular gate. The binding sites identified have been validated by both structure and function studies on designed mutants. We have further shown that the affinities of all the drugs and analogues tested are remarkably similar for the NavMs and human Nav1.1 channels, information which should be valuable for the design of specific and selective drugs.
Bagnéris, DeCaen, Naylor, Pryde, Clapham, Wallace (2014) The prokaryotic NavMs Channel as a structural and functional model for eukaryotic sodium channel antagonism. *Proc. Natl. Acad. Sci. USA* 111:8428-8433.