separation of helices associated with hydration of the crevice promotes inactivation.

Platform AM: Exocytosis & Endocytosis

1793-Plat Vesicle Diffusion Close to Supported Lipid Bilayers: A Model For Endo- and Exocytosis

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Understanding underlying mechanisms of vesicle diffusion near membranes can provide a comprehensive interpretation of endocytosis, exocytosis and synaptic fusion. We experimentally and theoretically characterize the dynamics of freely diffusing small vesicles near supported planar bilayers using total internal reflection-fluorescence correlation spectroscopy (TIR-FCS). The population distributions of vesicles diffusing near planar bilayers are affected by changing ionic strength, buffer pH, and the composition of the planar bilayers. As a result, hydrodynamic interactions of vesicles with the planar bilayers are changed, altering vesicle movements near the bilayers. These experimentally determined dynamics of vesicles at physiological conditions agree with theoretical expectations. Effective surface charge on neutral bilayers are also analyzed by comparing experimental and theoretical data, and we demonstrate the possibility that vesicle dynamics can be modified by surface charge redistribution of the planar bilayer. Based on these results, we hypothesize that small vesicles, when they are not in contact with cellular membrane, do not randomly diffuse around membranes, but may diffuse in a controlled manner depending upon the local and dynamic biological conditions. These studies allow us to further investigate the molecular dynamics involved in membrane fusion and fission.

1794-Plat Nanometer-scale Rearrangements Of Fission Pore During Normal And Aborted Endocytosis

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We report measurements, with a time resolution of milliseconds, of fission intermediates during formation and pinching-off of single endocytic vesicles. Fission pore conductance changes are detected by patch-clamp admittance measurements and associated with nanometer-scale rearrangements of the surrounding membrane structure (membrane neck). Principle modes of membrane neck deformation/transformation are considered and compared to resolved fission pore dynamics and electron microscopy of endocytic intermediates. Two kinetically distinct stages of the conductance time-course were designated to correspond to:

1. the uniform elongation of a highly constricted membrane neck (calculated fission pore diameter < 3 nm), followed by
2. the abrupt decrease of pore diameter finalizing the pinching-off event.

Disruption of the endocytic machinery by the dynamin-interacting drug dynasore precluded elongation of membrane neck and resulted in oscillations of fission pore diameter insufficient to complete fission. Energetic aspects of membrane neck elongation and constriction will be discussed.

1795-Plat Membrane Lipid Segregation in Endocytosis

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We explore the equilibrium mechanics of a binary lipid membrane that wraps a spherical or a cylindrical particle. One of the lipid membrane components induces a positive intrinsic spontaneous curvature, while the other induces a negative local curvature. Using a Hamiltonian approach, we derive the equations governing the membrane surface shape and lipid concentrations near the wrapped object.

Asymptotic expressions and numerical solutions for membrane shapes are presented. We determine the regimes of bending rigidity, surface tension, intrinsic lipid curvature, and effective receptor binding energies that lead to efficient wrapping and endocytosis. Our model is applicable to the invagination of Clathrin coated pits and to receptor-induced wrapping of colloids such as spherical virus particles.

1796-Plat The Relationship Of The Membrane Of Docked Synaptic Vesicles To The Presynaptic Membrane At Neuromuscular Junctions At Nanometer Spatial Resolution

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We examined by electron tomography the relationship of the membrane of docked vesicles to the presynaptic membrane in tissue sections from resting frog and mouse neuromuscular junctions fixed in aldehyde and stained with heavy metals with the aim of determining its constancy. We first measured the distance from the external surface of the presynaptic membrane to the luminal surface

of the vesicle membrane for 42 docked frog vesicles and 12 docked mouse vesicles throughout their entire region of closest apposition with the presynaptic membrane. In each case the distance was similar to the sum of the distances across the presynaptic membrane and the vesicle membrane away from the region of apposition. Thus, for most, if not all, of the docked vesicles in frog and mouse neuromuscular junctions the external surface of the vesicle membrane is in contact with the cytoplasmic surface of the presynaptic membrane with little or no intermingling between them. Next, we measured the area of closest apposition of the docked vesicles to the presynaptic membrane. For frog there was a 6-fold difference in area from vesicle to vesicle; in mouse there was a 3-fold difference. Thus, while the structural interaction between docked vesicles and presynaptic membrane is constant, the area of such interaction is highly variable. Finally we measured the distance between the presynaptic membrane and the "ribs" in active zone material, which are connected to the docked vesicles, for the docked vesicles in our frog and mouse samples. The greater the distance, the smaller the area of closest apposition between the vesicle and presynaptic membrane, which might be expected if the ribs regulate vesicle docking as previously proposed (Harlow et al., Nature *409*: 479–484, 2001).

1797-Plat Increased Motion and Travel, Rather than Stable Docking, Characterize the Last Moments Before Secretory Granule Fusion

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The state of secretory granules immediately before fusion with the plasma membrane is unknown, although the granules are generally assumed to be stably bound (docked). We had previously developed methods using TIRFM and image analysis to determine the position of chromaffin granules immediately adjacent to the plasma membrane with high precision, often to within ~10 nm, or less than 5% of the granule diameter (300 nm). These distances are of the dimensions of large proteins and are comparable to the unitary step sizes of molecular motors. Here we demonstrate with quantitative measures of granule travel in the plane parallel to the plasma membrane that secretory granules change position within several hundred milliseconds of nicotinic-agonist-induced fusion. Furthermore, just before fusion, granules frequently move to areas that they have rarely visited. The movement of granules to new areas is most evident for granules that fuse later during the stimulus. The movement may increase the probability of productive interactions of the granule with the plasma membrane or may reflect the pull of molecular interactions between the granule and the plasma membrane that are part of the fusion process. Thus, instead of being stably 'docked' before exocytosis, granules undergo molecular scale motions and travel immediately preceding the fusion event.

1798-Plat The Structures of COPII Coats and Cages

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COPII vesicles are responsible for packaging and transporting over 10,000 different cargo molecules (about one-third of the eukaryotic genome) of widely varying sizes and shapes from the endoplasmic reticulum (ER) to downstream compartments of the secretory pathway. Proteins involved in generating COPII vesicles include the GTPase Sar1, the cargo adaptor Sec23/24, and the cage scaffold Sec13/31. We previously showed that Sec13/31 can self-assemble into a novel cuboctahedron cage, leading us to suggest that Sec13/31 may form cages of increasing size based on the simple rule that the geometry of the cage is dictated by four Sec13/31 heterotetramers combining to form a vertex. We have now solved the structure of an icosidodecahedral COPII coat assembled from both Sec13/31 and Sec23/24 by cryo-electron microscopy (cryoEM) and single particle reconstruction. This new structure reveals for the first time the structure of the adaptor layer and reveals possible mechanisms for the coordination of adaptor interaction with cage formation and for the collection of cargo of varying size. We fit a new crystal structure of the Sec13/31 heterotetramer into the cuboctahedron and icosidodecahedron structures, and this lead us to propose a molecular mechanism by which the COPII system can accommodate cargo of diverse sizes and shapes including ion channels, insulin receptors, chylomicron particles and procollagen.

1799-Plat High Resolution 3D Single Molecule Imaging With Multifocal Plane Microscopy Reveals Complex Endocytic And Exocytic Pathways In Live Cells

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The study of intracellular events on the endocytic/exocytic pathway is of fundamental importance to understanding cellular function. Past research efforts have mainly focused on studying these events either on the plasma membrane or at the cell interior. However, the 3D trafficking itineraries that span from the plasma membrane to the intracellular compartments have not been well studied. The main reason for this is that current microscopes are not well adapted for imaging rapidly moving intracellular objects in 3D. Recently we developed a microscopy modality, multifocal plane microscopy (MUM), in which several planes can be simultaneously imaged within the cell in conjunction with visualization of the plasma membrane plane by total internal reflection fluorescence illumination [1]. This has allowed us to quantitatively track and characterize 3D intracellular events that are otherwise difficult to capture with conventional imaging techniques.

Our data shows evidence for a direct delivery pathway where a tubule extends from a sorting endosome to an exocytic site on the

plasma membrane. In some exocytic events, we observed a 'triggering' mechanism where a collision of a small vesicle preceded the fusion event. Using quantum dot labeled IgG molecules (QD-IgG) we have imaged the entire 3D trafficking itinerary on the endocytic pathway at the single molecule level. We observed that QD-IgG molecules exhibit complex itineraries before and after endocytosis. Quantitative analysis of the data reveals that QDs can be tracked with high accuracy, which is difficult to achieve with other conventional imaging techniques. The present technique provides an invaluable tool to study the 3D trafficking pathways in cells and provides insight into the intracellular events that precede (follow) exocytosis (endocytosis).

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1800-Plat Identification Of Early Endocytic Structures After Stimulation Of Pancreatic Acinar Cells

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Our recent work challenges the classical model of granule fusion and collapse during secretion in epithelial cells (Palade 1975). We show that granules don't collapse but persist at the plasma membrane for many minutes (Thorn *et al.* 2004) with exocytosis being terminated by closure of the fusion pore (Larina *et al.* 2007). However, the next step, that of endocytic recovery of the granule membrane, still remains unclear. Here we used the HRP-DAB method to follow early steps of endocytosis using transmission electron microscopy. In pancreatic tissue fragments stimulated for 1 minute with Acetylcholine and left to recover for 10 minutes we observed unstained granules fused to the plasma membrane with open fusion pores; consistent with the protracted fusion events seen in live cells (Thorn *et al.* 2004). Electron-dense DAB reaction product was observed in large vesicles that were predominantly located towards the apical end of the cells. The measured diameter of these electron-dense vesicles was 450 +/− 17 nm (mean diameter +/− SEM, n=44, from 3 independent preparations). These labeled vesicles are statistically smaller in size compared to the total population of zymogen granules (748 +/− 11 nm, n=230, mean diameter +/−SEM). We conclude that the labeled vesicles are surprisingly large and, while this might suggest that whole granules are endocytically recaptured, it is still unclear why they are smaller than the total population of zymogen granules.

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Platform AN: Protein Folding & Stability

1801-Plat A Coarse Grained Model Of Protein Collapse With Explicit Solvent

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We study the collapse transition of a lattice based protein model including an explicit coarse-grained model of a solvent. This model accounts for explicit hydrophobic interactions, and it is studied by Monte Carlo simulation. The protein is modeled as self-avoiding random walk with nearest neighbor interactions on a two dimensional lattice. Without the solvent, universal quantities of the chain around the collapse transition temperature are well known. Hydrophobicity is then modeled through a lattice of solvent molecules in which each molecule can have Q states depending of an orientation variable. Only one state is energetically favored, when two neighboring solvent molecules are both in the same state of orientation. The monomers are placed in interstitial position of the solvent lattice, and are only allowed to occupy sites surrounded by solvent cells of the same orientation. The potential of mean force between two interstitial solute molecules is calculated, showing a solvent mediated attraction typical of hydrophobic interactions. We then show that this potential increases with the energy of hydrogen bond formation as it appears in the model, while its characteristic range decreases. More importantly, we show that the chain embedded in the solvent undergoes a collapse transition, with the temperature of the transition being shifted relative to that of the chain in isolation. We calculate several critical exponents near the collapse transition, and we observe that their values are not conserved in presence of the explicit solvent.

1802-Plat How Do Chemical Denaturants Affect the Mechanical Folding and Unfolding of Proteins?

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Single molecule atomic force microscopy (AFM) has evolved into a powerful tool to investigate the folding/unfolding dynamics of proteins. In single molecule AFM experiments, proteins are forced to undergo unfolding/folding reactions along a pre-defined reaction coordinate determined by the stretching force acting on the protein of interest. Here we combined single molecule AFM with chemical denaturation to investigate the effect of chemical denaturants guanidinium chloride (GdmCl) on the mechanical folding and unfolding kinetics of a small protein GB1. Upon increasing the concentration of GdmCl, we observed a systematic decreasing in the mechanical stability of GB1, indicating the softening effect of the chemical denaturants on the mechanical stability of proteins. This mechanical softening effect originates from the reduced free energy barrier between the folded and unfolding transition state, which decreases linearly as a function of the denaturant concentration. Chemical denaturants, however, do not shift the mechanical unfolding pathway or alter the distance between the folded and