# Symposium 11: Cargo Transport by Single Molecular Motors

**1857-Symp**
**Single Molecule Imaging In Live Cells Reveals Selection Of Microtubule Tracks By Kinesin Motors**
**Kristen Verhey**.
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Long-distance transport of vesicular and protein cargoes in cells requires microtubules and their associated molecular motors. The basic mechanistic principles of tubulin polymerization and motor motility have been discovered from in vitro studies. The challenge is to translate the mechanics of these individual parts into the workings of ensembles of molecules inside cells. By imaging single kinesin motors in live cells, we reveal new properties about the interactions of motors and microtubules. We show that single Kinesin-1 motors move preferentially on a subset of microtubules available in COS cells. Preferential motility does not occur on dynamic microtubules marked by end binding protein 3 (EB3), which decorates the plus tips of growing microtubules. Rather, retrospective immunofluorescence staining demonstrates that single Kinesin-1 motors utilize stable microtubules marked by specific post-translational modifications. Preferential motility on stable microtubules is not a general property of kinesin motors as neither the kinesin-2 family member KIF17 nor the kinesin-3 family member KIF1A moved on a subset of microtubules. Selective transport enables Kinesin-1 to carry vesicles containing the marker protein vesicular stomatitis virus (VSV)-G along stable microtubules in COS cells whereas KIF17 transport of vesicles containing the voltage-activated potassium channel Kv1.5 occurs along both stable and dynamic microtubules in HL-1 atrial myocytes. These results support the hypothesis that a tubulin code of post-translational modifications can direct kinesin transport events in cells.

**1858-Symp**
**Steps, Force And Motile Mechanism Of Cytoplasmic Dynein**
**Hideo Higuchi**.
University of Tokyo, Tokyo, Japan.
Native cytoplasimic dynein moves with 8-nm steps by overlapping hand-over-hand mechanism (Toba et al. PNAS 2006). The 8-nm steps were confirmed in vesicle transport using FIONA method of quantum labeled vesicles. The contribution of individual dynein head to each step of the dynein is unclear because the step size is always masked by 8-nm of microtubule pitch. To avoid the masking, a recombinant single-headed dynein was prepared and bound separately onto a bead. The movement of dynein heads on the bead was characterized by laser trap and measurement with nanometer accuracy. The two molecules of single-headed dynein move forward and backward with various step sizes. As the dynein heads on a bead can bind on the microtubule, the histograms of step sizes from -30 to +30 nm were well fitted to a single Gaussian curve. The mean step size decreased from ~5 nm to 0 nm if the stalling force is increased from 0 to 2 pN meanwhile the half width, ~11 nm, of the step size distribution was however not changed. Based on this finding, it is suggested that the directional movement is generated by a 5-nm working distance of a dynein head while the other dynein head freely diffused 11 nm. The suggestion could explain recent controversial data about step size of different preparations of dynein.

**1859-Symp**
**Class V Myosins in Budding Yeast: Theme and Variations**
**Kathleen M. Trybus**.
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A prominent feature of most class V myosins is their ability to take multiple steps on actin without dissociating, known as processivity. Recent evidence showed that there are also non-processive class V myosins, both in humans and lower organisms. These motors need to work in ensembles to ensure continuous, unidirectional movement. The budding yeast *Saccharomyces cerevisiae* has two class V myosins, both of which are non-processive, but for different reasons. Myo2p is a dimeric motor with a low duty cycle, meaning that it spends a small portion of its cycle time strongly attached to actin. Myo4p has a high duty cycle motor, but is single-headed and thus cannot move processively as a single molecule. We propose that the association of Myo4p with its adapter protein She3p accounts for why it is single-headed. She3p is required for transport of all cargo of Myo4p (mRNA and cortical ER), and thus it has become a subunit of the motor. Myo2p, in contrast, moves many different cargoes (e.g. organelles and secretory vesicles), each with a unique adapter protein. Using a combination of *in vitro* and *in vivo* techniques, we probe how the features of each motor are uniquely suited for its particular cellular role. The involvement of other proteins that act as ''processivity factors'' will also be discussed.

**1860-Symp**
**Regulation Of Switching Of Membrane Organelles Between Cytoskeletal Transport Systems In Melanophores**
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**Vladimir Rodionov[1]**.
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Intracellular transport is driven by organelle-bound molecular motors that move cargo organelles along microtubules (MTs; motors of kinesin and dynein families) or actin filaments (AFs; myosin family motors). While transport along each cytoskeletal track type is well characterized, switching between the two types of transport is poorly understood. Here we used a combination of particle tracking and computational modeling approaches to measure parameters that determine how fast membrane organelles switch back and forth between MTs and AFs, and compare these parameters in different signaling states. As a model system we used melanophores, which aggregate thousands of membrane-bounded melanosomes in the cell center or redisperse them throughout the cytoplasm. Dispersion involves successive transport of melanosomes along the radial MTs and randomly arranged AFs. For aggregation, melanosomes that are transported along AFs transfer back onto MTs for movement to the MT minus ends clustered in the cell center. We performed tracking of individual pigment granules moving along MTs or AFs, determined major movement parameters (velocities and durations of runs to the plus or minus ends of MTs, and along AFs) using the Multiscale Trend Analysis, and incorporated them into a computational model for pigment transport. Comparison of the results of computational simulations of pigment distribution along the cell radius with experimentally obtained changes of pigment levels showed that regulation involves a single parameter: the transferring rate from AFs to MTs. This result suggests that MT transport is the defining factor whose regulation determines the choice of the cytoskeletal tracks during the transport of membrane organelles.

# Symposium 12: Regulated Intramembrane Proteolysis (RIP)

**1861-Symp**
**Intramembrane Proteolysis by the Rhomboid Serine Protease GlpG**
**Eitan Bibi**.
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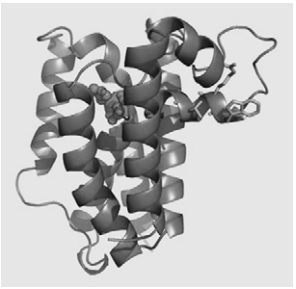
Intramembrane proteases catalyze peptide bond cleavage of integral membrane protein substrates. This activity is crucial for many biological and pathological processes. Rhomboids are evolutionarily widespread intramembrane serine proteases that cleave type I or II membrane protein substrates. In addition to high-resolution structural studies of the E. coli representative GlpG, this presentation will discuss the ability of rhomboids to cleave unfolded multi-spanning membrane proteins.



**1862-Symp**
**Structure and mechanism of Site-2 Protease**
**Yigong Shi**.
Tsinghua University School of Medicine, Beijing, China.

**1863-Symp**
**Biochemical And Structural Characterization Of Intramembrane Proteases**
**Iban Ubarretxena**.
Mount Sinai School of Medicine, New York, NY, USA.
In regulated intramembrane proteolysis membrane proteins are cleaved within their transmembrane region, resulting in soluble fragments that regulate cell physiology. The intramembrane proteases responsible for cleavage are widespread with important roles in human biology and disease. Rhomboids catalyze the activation of epidermic growth factor receptor ligands in D. melanogaster. How rhomboids recognize their substrates and select which peptide bond to cleave is not understood. We have studied the substrate specificity and peptide bond selectivity of purified rhomboids from several organisms using chimeric

transmembrane substrates. Analysis of the proteolytic products by mass spectrometry reveals that cleavage occurs in the membrane/water interface at sites shared by both eukaryotic and prokaryotic rhomboids. Mutagenesis of the substrates reveals a helical amino acid motif that is crucial for substrate recognition and peptide bond selection. With insight from computational data a model for the substrate-enzyme complex will be presented.
Gamma-Secretase catalyzes the production of amyloid beta-peptides involved in Alzheimer's disease. Using negative-stain single-particle electron microscopy we have determined the structure of a native-like 500kDa gamma-secretase complex comprising presenilin, nicastrin, APH-1, and PEN-2 that is fully catalytically active. Antibody labeling of the extracellular domain of nicastrin was employed to ascertain the topology of the reconstruction. Active site labeling with a gold-coupled transition state analog inhibitor demonstrates that gamma-secretase contains a single active site facing a large conical internal cavity. This cavity, surrounded by a ~35Å thick transmembrane protein wall, extends from the extracellular side of the membrane to past the membrane centre, where it narrows to finally close at the cytoplasmic side. Based on our structure we suggest a model for gamma-secretase function, in which a hydrophobic transmembrane helix substrate is hydrolyzed by catalytic aspartyl moieties at the interface of a water-accessible internal cavity away from the surrounding lipid environment.

**1864-Symp**
**Intramembrane Aspartyl Proteases: Structure, Mechanism and Inhibition**
**Michael S. Wolfe**.
Brigham & Women's Hospital, Boston, MA, USA.
Gamma-secretase catalyzes proteolysis within the boundaries of the lipid bilayer as the last step in the formation of amyloid beta-protein, the major protein component of the cerebral plaques of Alzheimer's disease. This enzyme is comprised of four different membrane proteins, with presenilin as the catalytic component of an unusual intramembranous aspartyl protease. The discovery of presenilin homologues that do not require other protein cofactors for proteolytic activity has cemented the idea of presenilin as the gamma-secretase subunit responsible for hydrolysis within the membrane. Small molecule probes, biochemical analysis, molecular biology and biophysical approaches have been combined to advance our understanding of the structure, function, and mechanism of these biologically important and medically relevant membrane-embedded enzymes. (This work was supported by grants AG17574, NS41355 and AG15379 from the NIH and IIRG 4047 from the Alzheimer's Association.)

# Platform AA: Membrane Structure

**1865-Plat**
**Direct Observation Of Plasma Membrane Rafts Via Live Cell Single Molecule Microscopy**
**Mario Brameshuber**[1], Julian Weghuber[1], Hannes Stockinger[2], Gerhard Schuetz[1].
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The organization of the cellular plasma membrane at a nanoscopic length scale is believed to affect the association of distinct sets of membrane proteins for the regulation of multiple signaling pathways. Based on in vitro results, conflicting models have been proposed which postulate the existence of stable or highly dynamic platforms of membrane lipids and proteins; commonly, these structures are termed membrane rafts. The lack of experimental evidence confirming the existence of putative rafts in living cells has yielded increasing skepticism, casting doubt on a major portion of the recent literature. Here we directly imaged and further characterized lipid rafts in the plasma membrane of living CHO cells by single molecule TIRF microscopy. Using a novel recording scheme for ''Thinning Out Clusters while Conserving Stoichiometry of Labeling'' (1), molecular homo-association of GPI-anchored mGFP was detected at 37°C and ascribed to specific enrichment in lipid platforms. The mobile mGFP-GPI homo-associates were found to be stable on a seconds timescale and dissolved after cholesterol depletion using methyl-beta-cyclodextrin or cholesterol-oxidase.
Having confirmed the association of mGFP-GPI to stable membrane rafts, we attempted to use an externally applied marker to test this hypothesis. We used Bodipy-GM1, a probe that was recently reported to be enriched in the liquid-ordered phase of plasma membrane vesicles. When applied to CHO cells at different surface staining, we found that also Bodipy-GM1 homo-associated in a cholesterol-dependent manner, thus providing further evidence for the existence of membrane rafts.
(1). Moertelmaier, M., Brameshuber, M., Linimeier, M., Schütz, G.J. & Stockinger, H. Thinning out clusters while conserving stoichiometry of labeling. Appl Phys Lett 87, 263903 (2005).

**1866-Plat**
**Using Cell Surface Protein Distributions To Investigate The Physical Basis Of Plasma Membrane Lateral Heterogeneity**
**Sarah Veatch**, Ethan Chiang, David Holowka, Barbara Baird.
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It is widely recognized that proteins and lipids are heterogeneously distributed on the cell surface, yet little is known regarding the underlying physical principles that give rise to this membrane organization. Recently, we characterized robust and dynamic critical fluctuations in isolated plasma membrane vesicles and proposed that critical fluctuations provide a plausible physical basis for <50 nanometer-sized lateral heterogeneity in the plasma membranes of intact cells (1). In order to test this hypothesis, we have visualized the submicron lateral distributions of gold particle-labeled proteins and lipids on the surface of intact, chemically fixed, RBL-2H3 mast cells using scanning electron microscopy, and we have quantified their distributions using a correlation function analysis. Correlation functions are routinely used to decipher interactions in complex systems, and we use them here to validate different possible physical bases of plasma membrane lateral heterogeneity. We find that proteins and lipids in resting cells are significantly correlated at short distances (<30nm) and uncorrelated at long distances (>50nm), consistent with previous studies. In addition, the detailed shapes of experimentally derived correlation functions provide additional information into the organizing principles that give rise to membrane heterogeneity. We evaluate the validity of different proposed mechanisms of membrane organization by fitting our experimental findings to predictions of various models. Models investigated include critical fluctuations, micro-emulsions, and membrane coupling to cytoskeletal components. Implications for cellular processes such as signaling will be discussed.
1. Veatch, S. L., P. Cicuta, P. Sengupta, A. Honerkamp-Smith, D. Holowka, and B. Baird. 2008. Critical fluctuations in plasma membrane vesicles. ACS Chemical Biology 3:287-293.

**1867-Plat**
**Unsaturated Phosphatidylcholine Acyl Chain Structure Affects the Size of Ordered Nanodomains (Lipid Rafts) Formed by Sphingomyelin and Cholesterol**
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How lipid composition affects the size of ordered domains (lipid rafts) in ternary lipid mixtures with cholesterol is unclear. We used FRET, DPH anisotropy, and quenching by the nitroxide-bearing molecule tempo to study raft size and raft thermal stability. Because FRET pairs with a relatively long distance range were used, FRET was not be able to detect very small nanodomains. In contrast, such domains could be detected by nitroxide quenching, which is of very short range, and anisotropy, which measures order of the lipids in immediate contact with the fluorescent probe. Ordered domain formation in lipid vesicles containing 1:1:1 sphingomyelin/DOPC/cholesterol or sphingomyelin/POPC/cholesterol were compared. These mixtures form co-existing ordered and disordered domains at lower temperatures and homogeneous disordered fluid domains at high temperature. The melting temperature, above which ordered domains disappear, was similar for these mixtures as measured by anisotropy and tempo quenching, but much higher for sphingomyelin/DOPC/cholesterol than for sphingomyelin/POPC/cholesterol when measured by FRET. This was true for more than one donor/acceptor FRET pair. We conclude that these mixtures form ordered domains with a similar stability. However, while domains large enough to detect by FRET form in sphingomyelin/DOPC/cholesterol under a wide variety of conditions, sphingomyelin/POPC/cholesterol has a tendency to form very small nanodomains. Because POPC is an abundant lipid in mammalian cells, this may be one reason that cellular ordered domains/rafts are very small.

**1868-Plat**
**Effect of Substrate Properties on the Topology, Lateral Diffusion and Phase Behavior of Supported Lipid Bilayers**
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Lipid bilayers supported by planar solid substrates have been extensively used as model systems for cell membrane. Recently, use of corrugated surfaces as substrates has received attention not only to overcome the basic problems related with the planar supported lipid bilayers such as the inaccessibility of