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
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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
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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
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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