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].
[1]University of Linz, Institute for Biophysics, Linz, Austria, [2]Medical University of Vienna, Department of Molecular Immunology, Vienna, Austria.
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.
Cornell University, Ithaca, NY, USA.
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
Priyadarshini Pathak, Erwin London.
Stony Brook University, Stony Brook, NY, USA.
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
Emel I. Goksu[1], Barbara A. Nellis[1], Wan-Chen Lin[2], Jay T. Groves[2], Subhash H. Risbud[1], Marjorie L. Longo[1].
[1]UC Davis, Davis, CA, USA, [2]UC Berkeley, Berkeley, CA, USA.
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

the proximal leaflet and lack of appropriate protein insertions but also to understand the bilayer-nanoparticle interactions. Here, we demonstrate the formation of multiphase lipid bilayers on nanoporous silica xerogels and compare it with mica supported bilayers. It was observed that the lipid bilayer follows the surface contours by AFM (Atomic Force Microscopy). This was also confirmed by the quantitative fluorescence analysis. The lateral diffusion coefficient of the lipids on silica xerogel was found to be lower than on mica by both FRAP (Fluorescence Recovery After Photobleaching) and FCS (Fluorescence Correlation Spectroscopy) experiments. The basic reason for this reduction was the bilayer following the surface contours. The domains on silica xerogel were observed to be symmetric and larger than the domains on mica. This reflects the possible effect of the support on the phase behavior of the lipid mixture. Ternary mixtures containing cholesterol were also prepared and the substrate effect on phase behavior was investigated.

1869-Plat

Understanding The Nucleation and Growth of Lipid-Lipid Phase Separation at Nanometer-Length-Scale: A Small Angle Neutron Scattering Study

Sumit Garg[1], Lionel Porcar[2], Paul Butler[3], Ursula A. Perez-Salas[1].
[1]Argonne National Laboratory, Argonne, IL, USA, [2]Institut Laue-Langevin, Grenoble, France, [3]National Institute of Standards and Technology, Gaithersburg, MD, USA.

Although lipid-lipid phase separation has been studied extensively in model lipid membranes, most of the available information is limited to micron-size domains. Lack of information on the nanometer-length-scale is a big hurdle in understanding the fundamental basis for lipid domain nucleation, in the first place, and growth. How do small unstable domains grow? What is the effect of temperature and membrane curvature? To answer these questions, this work presents Small Angle Neutron Scattering (SANS) studies on small Unilemellar Vesicles (ULVs) (diameter varying from 30nm to 400nm) made of 1:1 and 3:7 ratios of deuterated DPPC (dDPPC) and hydrogenated DLPC respectively. Small vesicles with varying sizes not only provide a means to control the curvature, but also limit the amount of available lipids for domain growth. Experiments were performed in contrast matched conditions, such that the scattering length density of the solvent and that of the homogeneous lipid vesicle (above the melting transition temperature, Tm, of the mixture) were exactly equal. As the sample temperature was lowered below Tm, the lipids started to phase separate and an excess scattering characteristic of lipid segregation into nano-size domains was observed. Notable trends were observed in the scattering curves as the temperature was lowered below the Tm of the mixtures. Interestingly, these results show that the phase separation behavior varies significantly between the small and large size vesicles. Insight in to the basic mechanism for the formation of lipid-lipid phase separation as a function of temperature and curvature will be discussed based on the analysis of the scattering intensities and Differential Scanning Calorimetry experiments.

1870-Plat

Cholesterol Displacement By Ceramide In Sphingomyelin-containing Liquid-ordered Domains, And Generation Of Gel Regions In Giant Lipidic Vesicles

Jesús Sot[1], Maitane Ibarguren[1,2], Jon V. Busto[1,3], Ruth L. Montes[1], **Felix M Goni**[1,3], Alicia Alonso[1,3].
[1]Unidad de Biofisica (CSIC-UPV/EHU), Leioa, Spain, [2]Departamento de Bioquímica y Biología molecular, Leioa, Spain, [3]Departamento de Bioquímica y Biología Molecular. Universidad del País Vasco, Leioa, Spain.

Fluorescence confocal microscopy and differential scanning calorimetry are used in combination to study the phase behaviour of bilayers composed of PC:PE:SM:Chol equimolecular mixtures, in the presence or absence of 10 mol% egg ceramide. In the absence of ceramide, separate liquid-ordered and liquid-disordered domains are observed in giant unilamellar vesicles. In the presence of ceramide, gel-like domains appear within the liquid-ordered regions. The melting properties of these gel-like domains resemble those of SM:ceramide binary mixtures, suggesting Chol displacement by ceramide from SM:Chol-rich liquid-ordered regions. Thus three kinds of domains coexist within a single vesicle in the presence of ceramide: gel, liquid-ordered, and liquid-disordered. In contrast, when 10 mol% egg diacylglycerol is added instead of ceramide, homogeneous vesicles, consisting only of liquid-disordered bilayers, are observed.

1871-Plat

How Membrane Curvature Can Sort Proteins

Stefan Semrau, Timon Idema, Cornelis Storm, Thomas Schmidt.
Leiden University, Leiden, Netherlands.

While it is a well known fact that membrane proteins interact via electrostatic, hydrophobic and van der Waals forces, only recently attention was drawn to a new kind of fundamental interaction that is mediated by the membrane itself. Proteins that locally impose a membrane curvature create a deformation that is probed by other, similar proteins. Since it is very difficult to quantitatively measure and discriminate the different types of interactions in the case of proteins, we study a model system which allows the exclusive observation of membrane mediated interactions alone. Building on our earlier experiments on fully phase separated vesicles (Semrau et al., PRL, 2008) we study biomimetic vesicles with bulging liquid ordered domains. These domains deform the surrounding membrane and create a repulsive force between them. This leads to a wealth of measurable effects: we observe hindered domain fusion, a preferred domain size and, most importantly, an intriguing sorting mechanism. Domains of different sizes spontaneously demix and form regions of equally sized domains. Quantitative measurements of the repulsive force allow us to build a model for the sorting that is independently confirmed in Monte Carlo simulations. The observed sorting provides a new mechanism for protein sorting in the endoplasmic reticulum and for membrane mediated protein aggregation.

1872-Plat

Lateral Stress and Spontaneous Curvature in Mixed Membranes

Horia I. Petrache[1], Michael F. Brown[2].
[1]Indiana University Purdue University Indianapolis, Indianapolis, IN, USA, [2]University of Arizona, Tucson, AZ, USA.

A key aspect of membrane mechanics is the balance of attractive and repulsive forces involving lipid head groups and acyl chains [1]. In particular, demethylation of PC lipids and addition of polyunsaturated chains [2] have been shown to alter substantially the activity of membrane receptors [3, 4] and of ion channels. How to best describe membrane forces? An appealing theoretical description is the lateral stress profile, which is easily calculated from computer simulations. However the lateral stress profile is not yet accessible experimentally. Experimental observables (which can be related to the lateral pressure profile if desired) are (i) the *spontaneous curvature* values as obtained from X-ray scattering, and (ii) the lateral *mean-torque profile* obtained from solid-state ^{2}H NMR [1]. We report and analyze experimental data on PE/PC and saturated-polyunsaturated lipid mixtures to show how measured shifts in the chain mean-torque profiles correspond to shifts in the balance of forces between headgroups and acyl chains. We first determine changes in the mean-torque profile caused by variations in lipid composition. We then determine a geometric parameter called the projected segmental area, directly related to the spontaneous curvature of individual monolayers. Last, we show that lateral compression energies are sufficiently large to provide a thermodynamic driving force for protein conformational changes. Our approach reveals that a universal chain packing profile exists for saturated acyl chains, and that the measurable shift in the force balance gives rise to the observable membrane mechanics. This approach provides a new framework for relating lipid composition to membrane function.

[1] H.I. Petrache *et al.* (2000) *Biophys. J.* **79**, 3172.
[2] H.I. Petrache *et al.* (2001) *J. Am. Chem. Soc.* **123**, 12611.
[3] A.V. Botelho *et al.* (2002) *Biochemistry* **41**, 6354.
[4] M.F. Brown (1994) *Chem. Phys. Lipids* **73**, 159.

Platform AB: Molecular Dynamics

1873-Plat

Automated Event Detection and Activity Monitoring in Long Time-Scale Molecular Dynamics

Willy Wriggers[1], Kate A. Stafford[1], Yibing Shan[1], Stefano Piana-Agostinetti[1], Paul Maragakis[1], Kresten Lindorff-Larsen[1], Patrick J. Miller[1], Michael P. Eastwood[1], Ron O. Dror[1], David E. Shaw[1,2].
[1]D. E. Shaw Research, New York, NY, USA, [2]Center for Computational Biology and Bioinformatics, Columbia University, New York, NY, USA.

Molecular dynamics trajectories of biological systems contain many events of great scientific interest, including conformational transitions, folding processes, and translocations of ligands and reaction products. In proteins these events often correspond to high-level tertiary or quaternary structure rearrangements, which alter the contacts between amino acid residues. Due to advances in computer architecture and software, molecular dynamics trajectories representing such structure-changing events have become easy to generate, but their length complicates scientific interpretation. The goal of this work is to simplify an important part of the analysis workflow (and to complement traditional visual inspection) by automating the mining of long trajectories. We present