

specific membrane proteins has been established through the use of functionalized immunolabels and genetically encoded fluorescent protein constructs. Cholesterol- and sphingolipid-enriched microdomains, which are sometimes called lipid rafts, are also postulated to be present in the plasma membrane and mediate cell signaling, virus budding, and many other disease-related cellular processes. Advanced high-resolution fluorescence microscopy techniques have revealed the presence of biophysically distinct microdomains and cholesterol-dependent nanoclusters within the plasma membranes of living cells. However a chemically specific and spatially well-resolved imaging technique is required to unambiguously establish how specific lipid species are organized within the plasma membrane. Imaging mass spectrometry is a promising approach for visualizing lipid distribution within membranes with chemical specificity and submicron lateral resolution. Previously, the distributions of two isotopically labeled lipids within phase-separated model lipid membranes were successfully imaged with 100-nm-lateral resolution by using high-resolution secondary ion mass spectrometry (SIMS). Here we extend this approach to actual cells and chemically image the distributions of specific lipids within the plasma membrane with better than 100 nm lateral resolution.

### 105-Plat

#### Differential Clustering of Membrane Anchors Observed by FCCS in Live Cells

**Hector H. Huang<sup>1</sup>**, Sara B. Triffo<sup>1</sup>, Adam W. Smith<sup>1</sup>, Jay T. Groves<sup>2</sup>.  
<sup>1</sup>University of Berkeley, Berkeley, CA, USA, <sup>2</sup>Howard Hughes Medical Institute, University of California, Berkeley, CA, USA.

Proper lateral organization of protein signaling elements in the cell membrane is crucial for proper cell behavior. Several important signaling proteins, such as some members of the Src family of kinases, are anchored to the membrane by covalent acyl, alkyl, and glycosylphosphatidylinositol (GPI) moieties. It has been proposed that these membrane anchors help to laterally sort proteins into signaling nanoclusters of unknown sizes. We set out to understand the role of these membrane anchors in the lateral sorting of proteins in the plasma membrane of a live cell. In order to observe this, we genetically encode lipidation motifs from a number of different cell signaling proteins fused to either a monomeric red or green fluorescent protein in living cells. Using Fluorescence Cross-Correlation Spectroscopy (FCCS) we are able to quantify the amount of dynamic colocalization of red and green fluorescent proteins anchored in the cell membrane. By only illuminating an area of the membrane with the cross-section of a focused laser beam, FCCS allows us to observe dynamic colocalization on the nanometer length scale, and unlike Förster Resonance Energy Transfer (FRET), FCCS can detect positive colocalization regardless of orientation and at lengths beyond the Förster radius of FRET pairs. We seek to understand if our fluorescent membrane anchors alone can dynamically colocalize in domains and if these domains can discriminate between different anchors. We have performed pairwise comparison of green-labeled and red-labeled membrane anchors in live cells and have seen different levels of cross-correlation for different anchor pairs.

### 106-Plat

#### Arrays of Nanoapertures for Examining Membrane Organization and Dynamics

**Christopher V. Kelly**, David Holowka, Barbara Baird, Harold G. Craighead.  
 Cornell University, Ithaca, NY, USA.

Nanoscale domains in the plasma membrane may be responsible for a variety of cellular signaling processes via membrane receptor clustering and lipid phase partitioning. However, current experimental approaches are limited in spatial and/or temporal resolution to address many membrane domain hypotheses. We have developed a new approach utilizing an array of nanoapertures to examine membrane organization and dynamics with near-field optical fluorescence microscopy without incorporating a scanning probe or disturbing the membrane. These nanoapertures are glass-filled, cylindrical pores (>50 nm diameter) in a thin aluminum film on a fused silica support, and they provide a planar surface for unperturbed cell adherence and growth. A nanoaperture confines the transmitted excitation light to a sub-diffraction limited spot directly above aperture, providing a 40-fold decrease in the illuminated area versus diffraction-limited illumination of the plasma membrane. Otherwise conventional microscopy excitation sources and fluorescent probes are used to enable fluorescence correlation spectroscopy (FCS) with 50 nanometer and 1 microsecond resolution. Further, these apertures provide two key benefits for FCS in addition to improved resolution: assured alignment of numerous illumination spots for cross-correlations and assured focusing of illumination on the cellular membrane as opposed to focusing within the cytoplasm.

Chromatic aberrations and slight laser misalignment that may complicate far-field, two-color FCS are not of concern here because the illumination profile is determined by the aperture directly. This technique has been applied to both model and living cell membranes to examine the diffusion of lipids and proteins in nanoscale dimensions. In particular, results will be presented demonstrating the effectiveness of this technique to observe diffusion of membrane proteins and lipids in varying phases and cross-correlation that occurs after cross-linking a selected component.

### 107-Plat

#### Modulation of Membrane Domain Sizes and Properties by Hybrid Lipids

**Juyang Huang<sup>1</sup>**, Jian Dai<sup>1</sup>, Mohammad Alwarawrah<sup>1</sup>, Gerald Feigenson<sup>2</sup>.  
<sup>1</sup>Texas Tech University, Lubbock, TX, USA, <sup>2</sup>Cornell University, Ithaca, NY, USA.

At present, there is a fundamental discontinuity between model membranes and real cellular membranes: The macroscopic Lo domains in model membranes and the nanoscopic lipid rafts found in cell membranes not only differ in size, but also in lipid order and protein partition behavior. Recently, new experimental evidence shows that nanometer-size Lo domains can exist in model membranes; however, the lipid mixtures must have at least 4-component and one of the components needs to be a "hybrid lipid", which is a lipid having one saturated acyl chain and one unsaturated chain. In this study, lattice model Monte Carlo simulation was used to simulate 4-component DSPC/DOPC/SOPC/Cholesterol lipid bilayers. The two chains from the same PC are physically linked in the simulation. SOPC (18:0,18:1PC) is a "hybrid lipid" with one chain identical to DOPC (di18:1PC) chains and another identical to DSPC (di18:0PC) chains. We found that: (a) hybrid lipids can significantly shrink the 2-phase region, change the compositions of the coexisting phases, and increase interfacial area; (b) depending on the concentration of hybrid lipids, lipid bilayers can go through a macroscopic to nanoscopic domain size transition; (c) in addition to hybrid lipids, cholesterol also serves as a line tension reducing molecule; (d) hybrid lipids are not very good surfactant molecules: Their preferences to occupy lipid domain interfaces and to orient in the "correct direction" are only modest. Thus, as imperfect surfactants, hybrid lipids can modulate the sizes and biophysical properties of lipid domains in a complex way.

## PLATFORM H: Membrane Receptors & Signal Transduction I

### 108-Plat

#### Role of Water in Transmembrane Domain of G Protein-Coupled Receptors

**Roman Osman<sup>1</sup>**, Arnau Cordero<sup>2</sup>, Themis Lazaridis<sup>3</sup>, Mihaly Mezei<sup>1</sup>, Leonardo Pardo<sup>2</sup>.

<sup>1</sup>Mount Sinai School of Medicine, New York, NY, USA, <sup>2</sup>Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain, <sup>3</sup>City College of New York, New York, NY, USA.

Recent publications of structures of G protein-coupled receptors (GPCR) show conserved waters in the transmembrane domain (TMD), suggesting their structural and functional importance. A structural description of the waters in the TMD was obtained with Grand Canonical Ensemble - Monte Carlo simulations of six GPCRs - bovine and squid rhodopsin,  $\beta_1$ - and  $\beta_2$ -adrenergic receptors, adenosine A2A receptor, and the ligand-free opsin that contains several distinctive features of the presumed active state. A Generic Site analysis identifies all the experimental waters in the TMD as organized in small clusters ranging from a single water to 6 waters within 3.5 Å from the cluster center. The enthalpy of the waters was approximated by the interaction energy with the rest of the system, while the entropy was evaluated by the Inhomogeneous Fluid Solvation Theory. The average free energy is site dependent and represents the transfer energy of discrete waters from bulk to their site(s) in the receptor as defined by the crystallographic coordinates. Notable clusters of waters are found bridging N1.50, D2.50, W6.50 and N7.49. We focus on a single water found in all structures that bridges TM6 with TM7 through H-bonds to the C=O of residues 6.49 and 7.38, which are exposed due to distortions in the helical structures. Comparison of the bridging water in rhodopsin and opsin shows a drastic change in occupancy from 1.0 to 0.44 with concomitant change in their free energy from -3.8 to -1.3 kcal/mol. This change in the energy coincidental with the rearrangement of TM6 highlights the importance of this water in GPCR activation.

Supported by grants from MEC (SAF2010-22198-C02-02) and ISCIII (RD07/0067/0008). AC is supported by ISCIII through the Sara Borrel program.