1973-Plat
Imaging of Lipid Blayer Mixtures and Actual Cell Membrane Fragments by Nanosims
Monica M. Lozano, Steven G. Boxer.
Stanford University, Stanford, CA, USA.
Lipids, proteins, and cholesterol are the major components of biological membranes; however, little is known about their lateral organization and how it changes during cell-cell interactions, signaling, development and division on the nanometer length scale that is relevant for function. These studies develop a new method for determining the spatial organization and organization of model membranes using secondary ion mass spectrometry (SIMS) imaging in an effort to explain the rich phase behavior of complex biological membranes. Furthermore, these experiments set the stage for measuring the native distribution of cholesterol and proteins within membrane fragments taken from cells. Advanced fluorescence and atomic force microscopies (FM and AFM, respectively) used thus far to interrogate model cell membranes have limited molecular specificity (can only detect fluorescently labeled molecules in FM), spatial resolution (governed by optics in FM or tips in AFM) and both lack compositional information. SIMS imaging, in particular using the NanoSIMS by Cameca, offers imaging capability with high sensitivity (sub-ppm), specificity (based on isotopic labeling), and spatial resolution (~50nm). Using this unique technique, the composition and organization of cholesterol, lipids and proteins within the plane of the lipid bilayer of model and actual cell membranes with nanometer resolution is investigated. Cholesterol in plasma membranes is responsible for modulating acyl chain order, membrane elasticity and lateral organization and its level is tightly regulated. In order to investigate the spatial distribution of cholesterol in actual cell membranes, supported cell membranes fragments are imaging using the NanoSIMS. These measurements are the first example of the direct analysis of the organization of a cell membrane at the nanometer length scale that is relevant for function setting the stage for quantitative analysis of systems of increasing complexity and direct biological relevance.

1974-Plat
Near-Critical Fluctuations and Cytoskeleton-Assisted Phase Separation Lead to Subdiffusion in Cell Membranes
Eugene P. Petrov, Jens Ehrg, Petra Schwille.
Technische Universitaet Dresden, Dresden, Germany.
We address the relationship between membrane microheterogeneity and anomalous subdiffusion in cell membranes by carrying out large-scale lattice-based Monte Carlo simulations of two-component lipid membranes. We study the diffusion of lipid molecules in free membranes and find that near-critical fluctuations in the membrane lead to transient subdiffusion of lipid molecules spanning several orders of magnitude in time [1, 2]. We observe that the membrane-cytoskeleton interaction strongly affects phase separation, enhances subdiffusion, and eventually leads to hop diffusion of lipids [2]. Thus, we present a minimum realistic model for membrane rafts showing the features of both microscopic phase separation and subdiffusion.

1976-Plat
Inactivation and Voltage-Dependent Rectification Mechanisms in the KcsA Potassium Channel
Celine Boiteux, Simon Bernard.
Biozentrum, University of Basel, Basel, Switzerland.
Potassium channels regulate ion permeation by varying their conductance notably through a mechanism known as C-type inactivation, which implies that shortly after activation, their selectivity filter stops conducting ions at rates that depend on various stimuli. This inactivation process plays a critical role in controlling the length and frequency of cardiac action potentials, as well as the firing patterns in neurons.

It’s been shown that the prokaryotic KcsA channel undergoes C-type inactivation like its eukaryotic counterparts (Gao et al., PNAS 102:17630 (2005)), suggesting KcsA as a prototypic model for structural studies of inactivation gating. The detailed microscopic process underlying C-type inactivation remains unexplained despite the accumulation of experimental evidences showing the key role played by the selectivity filter and some neighboring residues. In particular, the interactions between Asp80, Glu71 and Trp67, as well as the water molecules trapped in the P-loop of the channel, seem to be strongly linked to the stability of the filter and its ability to adopt a stable inactivated state (Cordero-Morales et al., Nat. Struct. & Mol. Biol. 14:1062 (2007)). Models of inactivation gating that were proposed on the basis of x-ray crystallography studies involve transitions between conducting states, containing two ions, and non conducting ones, containing a single ion (Zhou et al., Nature 414:23 (2001); Cuello et al., Nature 466:272 (2010)).

Using molecular dynamic simulations and free energy calculations, we investigated the possible transitions between different ion occupancy states involving the conducting and putatively inactivated conformations of KcsA. A comprehensive study of key mutations showing different inactivation phenotypes allows us to propose a structural model describing the inactivation mechanism of KcsA, as well as the voltage dependent rectification observed in WT and some mutants.

1977-Plat
The Second Highly-Conserved Threonine Residue of the Potassium Channel Signature Sequence Mediates Activation-Coupled to C-Type Inactivation
Luis G. Cuello, Dominique G. Gagnon, D. Marien Cortes.
Texas Tech University Health Science Center, Lubbock, TX, USA.
In the amino-acid sequence of a K+ channel pore domain are encoded most of the structural elements necessary to 1) catalyze the permeation of K+ ions and 2) execute activation and C-type inactivation gating. In KcsA, a C-type inactivation process similar to that found in eukaryotic channels has been identified [1] and crystallographic evidences indicate that the collapse of the channel selectivity filter (SF) is responsible for it [2]. It was also established that an allosteric communication between the channel activation gate and the SF underlies activation coupled to C-type inactivation in KcsA and other K+ channels [3]. In KcsA, F103 seems to mediate this allosteric communication by interacting with T75 of the same subunit, the second highly conserved Threonine in the SF of K+ channels (TTXIVGD). In order to gain functional and structural insights regarding this allosteric communication, we have mutated KcsA-T75 to a Glycine or an Alanine and...