**86-Plat**

**A Fundamental Force that Regulates Nano-Clustering of Proteins in Biological Membranes**

Kranthi Kiran Mandadapu1, Shachi Katira1, Suryanarayanan Vaikuntanathan1, Berend Smit1, David Chandler1, 2

1Chemistry, University of California, Berkeley, Berkeley, CA, USA, 2Bioengineering, University of California, Berkeley, Berkeley, CA, USA, 3Chemistry, University of Chicago, Berkeley, CA, USA, 4Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA, USA.

Using coarse-grained molecular dynamics simulations, we demonstrate the nature of the membranophobic effect—a proposed phenomenon that governs self-assembly of inclusions within a lipid bilayer, inspired by the statistical mechanics of the hydrophobic effect. We study the nature of this effect on membrane inclusions of various chemistries and sizes. We identify the range of hydrophobic thicknesses over which this phenomenon occurs and characterize the effects of the proposed phenomenon on small inclusions such as cholesterol versus larger, multidomain transmembrane proteins. Our results show that this effect can provide a force for assembly and reorganization in a lipid bilayer based on the in-plane size and hydrophobic thickness of the inclusion, and the melting temperature of the surrounding lipids. We propose that this effect provides a physical framework that can explain lipid raft formation.

**87-Plat**

**Theory of Registered and Antiregistered Phase Separation in Mixed Amphiphilic Bilayers**

John J. Williamson, Peter D. Olmsted.

Physics, Georgetown University, Washington DC, DC, USA.

Wide interest in the phase behaviour of amphiphilic bilayers has arisen since the realisation that it can be exploited by nature, and engineers, to design-in function via membrane domains. A key confounding feature is the presence of two separate, yet coupled, leaflets within the bilayer. The question of inter-leaflet domain alignment (registration) or otherwise is central to proposed cellular roles such as protein localisation and, more fundamentally, creates a zoo of phase behaviour that can only be captured by properly considering the coupled leaflets. Experiment and simulation yield intriguingly disparate observations, but a full theoretical picture is lacking; existing phenomenological theories provide insight but do not link large-scale behaviour to small-scale features. We introduce a theory for phase separation in coupled leaflets by explicitly coarse-graining a lattice model that includes molecule-level structuring and interactions. We show that accounting for hydrophobic mismatch between the mixed species leads to a complex competition of inter-leaflet coupling energies. The free energy obtained helps unify some prima facie contradictory observations by showing that domain antiregistration typically occurs as a metastable state, but can be kinetically preferred during the initial demixing of a bilayer. The role of kinetics in governing registration/antiregistration is explored, and we find that a bilayer in the usual “spindal region” may in fact require a nucleation process to equilibrate. Our results provide a tractable coarse-grained model that explicitly depends on simplified molecular interactions, providing novel insight and encouraging important future work in which the intra- and inter-leaflet behaviour of mixed bilayers is carefully investigated.


**88-Plat**

**How GM1 Affects the Phase State and Mechanical Properties of Phospholipid Membranes**

Nico Fricke, Rumiana Dimova

Theory & Bio-Systems, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.

Even though glycolipids are present at increased concentrations in the outer leaflet of eukaryotic biomembranes, their influence on the mechanical properties of the membrane has not been studied in much detail. In this work, we investigate the effect of GM1, a prominent example among glycolipids, on the physical characteristics such as phase state and bending rigidity of membranes. Both giant vesicles and large unilamellar vesicles made of palmitoyloleyoylphosphatidylcholine (POPC) are explored. We find that for GM1 fractions above ~5 mol%, the membranes are phase separated at room temperature and exhibit GM1-rich microdomains with gel-like nature as observed by fluorescent microscopy. However, cholera toxin B, which is conventionally used as a GM1 marker, is found to be excluded from these domains. So is the fluorescently labeled Bodipy-4-N-(N,N-diethyl)-N-[N-hydroxy-β-D-glucopyranosi]

**89-Plat**

**Examining the Effects of Cholesterol: Laurdan and Patman see it Differently**

Emma R. Moulton, Kelsey J. Hirsche, Monica L. Hobbs, Morgan M. Schwab, John D. Bell.

Phyiology and Developmental Biology, Brigham Young University, Provo, UT, USA.

The liquid-ordered phase induced by cholesterol in phosphatidylcholine bilayers can be detected with Laurdan fluorescence by an increase in the associated Generalized Polarization (GP) value. This increase in GP is usually interpreted as a reduction in the access of water molecules to the bilayer at depths approaching the phospholipid glycerol backbone. Comparisons of Laurdan fluorescence over a broad range of temperatures with various saturated and unsaturated phosphatidylcholines demonstrated that cholesterol has little effect on GP at temperatures below the melting point of the pure lipid (tm). However, above tm, increasing cholesterol concentrations monotonically raised the value of GP. The resulting GP increments generally did not vary with temperature above tm. In contrast, the observations were more complex with Patman, a charged derivative of Laurdan. First, cholesterol raised the value of Patman GP at temperatures below tm and its effect as a function of concentration was bimodal. Second, although cholesterol increased Patman GP above tm (similar to Laurdan), the effect size was smaller and was bimodal as a function of cholesterol concentration. Finally, the GP values at high versus low cholesterol concentration converged as temperature was raised well beyond tm. In some cases, such as with unsaturated lipids ≥ 54°C above tm, this convergence of Patman GP values reached a cross-over point; Patman GP was reduced by the presence of cholesterol beyond this point. These results suggest that the charge associated with Patman, or possibly the slightly deeper location of the probe in the bilayer, provides a means for identifying additional effects of cholesterol on the membrane that are not visible with Laurdan.

**90-Plat**

**Orientational Texture of Membrane Domains: Effect of Lipid Composition and Binding of a Bacterial Toxin**

Adam C. Simonsen1, Jes Dreier2, Vita Solovyeva1, Jonas C. Jeppesen1, Jonathan Brewer3

1MEMPHYS, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense M, Denmark, 2MEMPHYS, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark.

The principles governing the in-plane organization of biomembranes remains enigmatic more than 20 years after the proposition of the raft hypothesis. The recent discovery of orientational texture of membrane gel domains represents a previously hidden level of membrane complexity[1]. Using polarized two-photon fluorescence imaging we have shown that gel domains in phospholipid membranes may contain long-ordered orientational texture patterns originating from the projection of the tilted acyl chains on the bilayer plane. Fourier analysis of the signal variations with respect to polarization angle enables the lipid orientation to be resolved spatially. We find that the texture of gel domains can exhibit topological defects including a vortex, pairs of half-integer vortices, and line defects[2]. Membrane texture resembles texture found in liquid crystals and Langmuir monolayers and have also been associated with hexatic positional order of the lipids. The texture pattern in membranes is closely linked to the lipid composition as demonstrated by the occurrence of uniformly aligned domains for some compositions. Specifically, a close correlation has been found between the hydrophobic thickness mismatch at the border of domains and the texture pattern[3]. Recently we have explored the possibility that the Shiga toxin protein from the bacteria Shigella dysenteriae may remodel texture patterns in membranes. A
significant effect of the toxin is found, possibly related to decreased lipid mobility [4].

Here we will provide an overview of membrane texture in a range of systems and describe our efforts to understand and systematize the observations.


91-Plat
Plasma Membrane Vesicle Critical Temperature Scales with Growth Temperature in a Zebrafish Cell Line
Margaret Burns, Jing Wu, Kathleen Wisner, Sarah Veatch.
Biophysics, University of Michigan, Ann Arbor, MI, USA.

Giant plasma membrane vesicles (GPMVs) isolated from mammalian cell lines contain coexisting liquid phases at low temperature, a single liquid phase at elevated temperatures, and undergo robust micron-sized critical fluctuations near the miscibility transition which is typically close to room temperature. In past work, we have measured the temperature dependence of critical composition fluctuations and speculated that mammalian cells tune their membrane composition to maintain a room temperature critical point in order to experience <100nm super-critical composition fluctuations under growth conditions of 37°C [1]. Here, we present evidence that cells actively tune membrane critical temperatures (Tc) to a specific temperature difference below growth temperatures by preparing GPMVs from ZF4 cells, a zebrafish cell line that is capable of growing at temperatures ranging between 20°C and 32°C. As was the case in mammalian cell lines, ZF4 cells produce GPMVs with coexisting liquid phases at low temperature and micron-sized critical fluctuations near the miscibility transition. ZF4 derived GPMVs transition temperatures shift to lower values when cells are grown at lower temperature, such that Tc was 16.8 ± 1.2°C below growth temperatures for ZF4 cells grown between 20 and 32°C. We also examined the time-course of Tc adjustment by preparing GPMVs from cells adapted for growth at 28°C then subsequently grown at 20°C. Transition temperatures adjust with a time-constant of 1.5 days, in good correspondence with the doubling time under these growth conditions.


Platform: Ion Channels, Pharmacology, and Disease

92-Plat
Molecular Dynamics of Amantadine Block in M2 of Influenza A: WT VS S31N
Mitchell L. Gleed1, Harris Ioannidis2, Antonios Kolocouris1, David D. Busath1.
1Physiology and Dev. Biol., Brigham Young University, Provo, UT, USA, 2Dept. of Pharmaceutical Chemistry, National and Kapodistrian University of Athens, Athens, Greece.

The ability of amantadine to block wild-type influenza A M2 is profoundly influenced by its position and orientation in the channel, which in turn affect the drug’s hydration state, according to molecular dynamics simulations. Umbrella sampling results reveal that amantadine has strong binding free energy in wild-type M2, accompanied by low lateral water density, when it is initially oriented with its amine toward the C-terminus of the protein and its adamantane cage is positioned between Ser31 alpha carbons and in contact with the Val27 side chains. In critical regions of the reaction coordination, simulations show that the drug frequently rotates to this orientation even when started in the opposite orientation. In contrast, the prevailing influenza A M2 mutant (S31N) shows no significant difference in the likelihood of amantadine orientation-reversal between simulations of opposite initial orientation. Furthermore, in free simulations of the S31N, the drug shows a high propensity to drop deeper into the channel, where lateral water density becomes high. High lateral water density should facilitate proton transport past the drug: the drug binds but does not block. The differences in amantadine behavior between the Ser31 and Asn31 variants suggest that residue 31 plays a key role in orientation- position-specific block of M2 by amantadine and that an Asn31 mutation increases mobility and rotational freedom of amantadine in the mutant M2 (S31N).

Consequently, the success of future anti-influenza drugs may depend on their ability to adopt specific orientations as they plug the Val27-lined opening of the channel.

93-Plat
Potentiation of CFTR Gating by an Energetically Additive Mechanism
Han-Y I Yeh1,2, Junn-Ting Yeh1,2, Tzyh-Chang Hwang2,3.
1National Yang-Ming University, Taipei, Taiwan, 2Dalton Cardiovascular Research Center, Columbia, MO, USA.
CFTR is the only member of the ATP-binding cassette (ABC) protein superfamily that functions as an ion channel. Once its regulatory (R) domain is phosphorylated, CFTR will harness the free energy from ATP binding/hydration in the nucleotide-binding domains (NBDs) to power the conformational changes of the gate located in the transmembrane domains (TMDs). We inadvertently found that some permeant anions such as nitrate or bromide, when applied to the cytoplasmic side of the channel, can increase the Po of CFTR by raising slightly the opening rate but decreasing considerably the closing rate. Since nitrate also decelerates non-hydrolytic closing, we conclude that the effect of nitrate is independent of ATP hydrolysis. Surprisingly, despite their markedly different physical properties, the gating effects of nitrate are remarkably similar to those of VX-770, a well-characterized CFTR potentiatior now used in clinics. Nonetheless, while VX-770 is equally effective when applied from either side of the membrane, nitrate potentiates gating mainly from the cytoplasmic side, implicating a common mechanism for gating modulation but via two distinct sites of action. Since nitrate increases the Po of ∆R, as well as ∆NBD2-CFTR, we exclude the R domain and NBD dimer as potential targets for nitrate. Furthermore, for both G551D and ∆NBD2-CFTR, two mutants with extremely low Po, we show that the fold-increase of the Po by nitrate plus VX-770 is virtually the same as the product of fold increases by individual reagents. Collectively, our data suggest that nitrate and VX-770 independently affect CFTR gating by shifting the equilibrium of gating conformational changes of the TMDs towards the open channel configuration. These results may not only shed light on the future development of novel therapeutic reagents, but also provide insights into the coupling mechanism of gate opening/closing and NBD dimerization/dissociation.

94-Plat
Affinity Calculations for Lipophilic Modulators Binding to Isolated Sites on GABA(A) Receptors
Sruthi Murlidaran, Reza Salari, Grace Brannigan.
Computation and Integrative Biology, Rutgers University, Camden, NJ, USA.
The γ-amino butyric acid type A (GABA(A)) receptor is an ionotropic receptor critical for inhibitory signaling in the central nervous system. GABA(A) receptor sites are positively modulated by numerous general anesthetics and likely contribute to the effects of general anesthesia. Several site-directed mutagenesis and photoaffinity labeling experiments have suggested numerous potential binding sites for general anesthetics, as well as other modulators of GABA(A) receptors including neurosteroids and thyroid hormone. The absolute affinities of the modulator for each site are unknown, however, making it challenging to determine which of the many sites is occupied at clinical concentrations. Measurement of modulator affinity for individual sites is challenging experimentally, while isolation of individual sites in computational methods is relatively straightforward. Here we use the Alchemical Free Energy Perturbation (AFEP) method combined with molecular dynamics simulations to calculate absolute binding affinities of propofol and sevoflurane for pore and intersubunit sites on a homology model of an α1β2γ2 GABA(A) receptor, after initial coordinates were determined with automated docking. We calculate significant differences in general anesthetic affinity for pore and intersubunit sites at various interfaces, consistent with a complex dose-response curve in which low affinity sites are associated with inhibition and high affinity sites are associated with potentiation.

95-Plat
SLC6A14 Modifies Fluid Secretory Capacity of Cystic Fibrosis Affected Epithelium by Enhancing CFTR Channel Function
Saumel Ahmadi1,2,3, Catherine Luk1, Sunny Xia1,2, Michelle Di Paola1,3, Timothy Chung1, Johanna Rommens1,2, Christine Bear1,2,4.
1Molecular Structure and Function, The Hospital for Sick Children, Toronto, ON, Canada, 2Physiology, University of Toronto, Toronto, ON, Canada, 3Biochemistry, University of Toronto, Toronto, ON, Canada, 4University of Toronto, Toronto, ON, Canada.

Cystic fibrosis (CF) is usually caused by F508del mutation in the CFTR gene. Normally, CFTR protein aids in salt transport across the apical surface of epithelia, keeping their surface hydrated. F508del results in decreased surface expression of CFTR, leading to dehydration of the surface. This makes the lung...