Subgroup: Membrane Structure & Assembly

1-Subg
New Tools For Studies Of Membrane Protein Dimerization In Mammalian Membranes
Kalina Hristova.
Johns Hopkins University, Baltimore, MD, USA.
Receptor Tyrosine Kinases (RTKs) play key roles in cell growth, differentiation, metabolism, and migration. These single-pass receptors conduct biochemical signals by dimerizing in the plasma membrane. The process of lateral dimerization, which controls the distribution between inactive monomers and active dimers, serves as a key regulator of the biochemical processes that determine cell fate. Enhanced dimerization leads to persistent autocrine activation and tumorigenesis, or impaired growth. An understanding of the dimerization process as a function of interaction energies, protein concentration and ligand concentration, is lacking. Our laboratory is developing methodologies that yield quantitative information about RTK dimerization and activation in cellular membranes. These methods will enable biomedical researchers to study the quantitative aspects of signal transduction in the context of the biological membrane.

2-Subg
Small, Dynamic Domains in Lipid Membranes near a Miscibility Critical Point
Aurelia R. Honerkamp-Smith[1], Sarah L. Veatch[2], **Sarah L. Keller[1]**.
[1]Dept. of Chemistry, Univ. of Washington, Seattle, WA, USA, [2]Dept. of Chemistry and Chem. Biology, Cornell Univ., Ithaca, NY, USA.
We study giant lipid vesicles as a model of plasma membranes in cells. We find that liquid domains appear on the surface of vesicles containing at minimum a high melting temperature lipid, a low melting temperature lipid, and cholesterol. These three components separate into two phases. Near a miscibility critical point, the edges of the domains fluctuate, which indicates a low line tension at the boundary between the domain and the surrounding membrane. At higher temperatures, above the critical point, domains are replaced by submicron fluctuations. We find that the size of the largest fluctuations (the correlation length) and their compositions (the order parameter) scale in a way that is consistent with the universality class of the two-dimensional Ising model. This knowledge has a direct application: we can predict at what temperature our membranes should contain domains of any particular size, even at length scales below our optical resolution.
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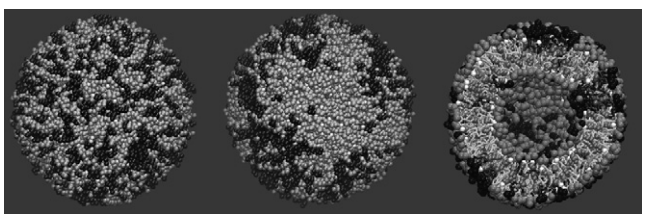
3-Subg
Fascinating Vesicles
Siewert J. Marrink.
Univ Groningen, Groningen, Netherlands.
Lipid vesicles, or liposomes, are widely used in biophysical studies as mimics of either complete cells or cell parts, and have a potential wide range of biotechnological applications. Here I will present the latest results of our group on simulations of vesicles, based on the coarse grained MARTINI forcefield[1]. I will discuss equilibration issues of vesicles[2], and show the formation of raft-like domains in ternary systems composed of saturated and unsaturated lipids together with cholesterol[3]. The effect of osmotic pressure on the structure and stability of lipid vesicles and the response of membrane-embedded mechano-sensitive protein channels will also be discussed[4].



Domain formation in a three component vesicle. Starting from a randomized mixture (left), a saturated-PC/cholesterol enriched liquid-ordered domain is formed on a microsecond time scale (middle and rightmost image). Light/dark grey denotes saturated/poly-unsaturated lipids. Cholesterol is depicted with a white hydroxyl group. Water not shown.

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4-Subg
Thermodynamics of Membrane Partitioning and Self-association of Transmembrane Helices: Impact of Lipid Composition
Katsumi Matsuzaki.
Kyoto Univ., Kyoto, Japan.
Membrane partitioning and self-association of transmembrane helices (TMHs) have been extensively investigated using model TMHs to elucidate the driving forces of membrane protein folding. We designed the inert hydrophobic model TMH $(AALALAA)_3$ to obtain thermodynamic parameters related to the steps. The formation of the antiparallel dimer was detected by fluorescence resonance energy transfer between fluorescent labeled peptides [1]. Stronger dimerization was observed in thicker membranes and at lower temperatures ($\Delta G = -9 - -26$ kJ mol^{-1}), driven by large negative ΔH values ($-18 - -80$ kJ mol^{-1}). The enthalpy changes for helix-helix interaction can be well explained by electrostatic interaction between helix macrodipoles in different dielectric environments. The incorporation of cholesterol and PE also facilitated the dimerization by large negative ΔH values.
The partitioning process was also investigated based on the transfer of the helix between vesicles [2]. Under hydrophobic mismatch conditions up to ~7 Å, the helix partitioning became unfavorable up to $+7$ kJ mol^{-1}, hampered by an increase in entropic (up to $+20$ kJ mol^{-1}) and enthalpic (up to $+66$ kJ mol^{-1}) terms in thinner and thicker membranes, respectively. The obtained thermodynamic parameters were reasonably explained assuming that hydrophobic mismatch induces aqueous exposure or membrane burial of the helix termini, respectively. I also present a design of a water-soluble TMH for the direct measurement of the partitioning process. Furthermore, I will introduce a novel method for quick fluorescent labeling of membrane proteins to detect TMH interactions in living cell membranes [3].
[1] Y. Yano and K. Matsuzaki, *Biochemistry* 45, 3370 (2006).
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5-Subg
Structure And Bending Rigidity Of Fully Hydrated Lipid Bilayers With Added Peptides And Cholesterol Using Diffuse X-ray Scattering
Stephanie Tristram-Nagle.
Carnegie Mellon University, Pittsburgh, PA, USA.
Our lab uses x-ray synchrotron radiation to study fully hydrated stacks of ~2000 lipid bilayers. Like cell membranes, these oriented bilayers fluctuate when they have their full complement of water. These fluctuations produce diffuse x-ray scattering which allows us to determine both structure and the rigidity (bending modulus K_C). We have more recently been adding two classes of peptides, channel-forming and fusion, to these lipid bialyers. By comparing our experimental form factor data to form factors obtained from MD simulations by Peter Tieleman we confirm that the channel-former, alamethicin, inserts into DOPC membranes in a transmembrane fashion. We also find that alamethicin thins DOPC by 3 Å and diC22:1PC membranes by 4 Å at 1:10 P/L mole ratio. Two peptides from the gp41 protein on the ectodomain of the HIV-1 virus, fusion peptide FP23 at the N-terminus, and the cholesterol-sequestering CRAC motif peptide near the transmembrane region, were also added to lipids of varying thickness and chain unsaturation (BJ (2007) 93:2048, BBA (2008) 1778:1120). The CRAC-motif LWYIK peptide thinned SOPC membranes by 3 Å at 1:9 P/L ratio. All peptides caused a softening of the membranes, but the decrease in the bending modulus, K_C, was greatest for the FP23 peptide, which we have suggested is related to its special ability to promote highly curved fusion intermediates at the HIV/T-cell fusion site. We have also added cholesterol to bilayers and have obtained the remarkable result that cholesterol does not stiffen unsaturated DOPC bilayers, in striking contrast to the well known result that cholesterol greatly stiffens saturated DMPC bilayers (Phys. Rev. Letts. (2008) 100:198103).

6-Subg
Control of Hydrophobic Helix Topography in Membranes by Lipid Composition
Khurshida Shahidullah, **Erwin London**.
Stony Brook University, Stony Brook, NY, USA.
The sequence of hydrophobic helices can modulate topography in terms of both the stability of the transmembrane (TM) configuration (relative to a *membrane-bound* non-TM state) and the transverse position of the helix in

a membrane. Both of these parameters can affect membrane protein function. Studies in our lab have shown that single hydrophilic residues within a hydrophobic sequence can have a profound effect upon both stability of the TM configuration and TM helix transverse position, such that a non-TM topography, or a shifted state in which the hydrophilic residues moves to the boundary of the membrane, respectively, forms. We have also found that the composition of membrane lipids can control topography. Most strikingly, physiologic levels of anionic lipids stabilize the TM configuration of hydrophobic helices that are flanked by cationic juxtamembrane residues, but not when the helices are flanked by uncharged residues. Interestingly, the effects of the lipids PS and PG are not identical, suggesting that factors in addition to Coulombic electrostatic interactions are involved. Our most recent studies indicate that anionic lipids can also effect the transverse position of hydrophobic helices. When a helix is flanked by cationic residues, the presence of anionic lipids suppresses the transverse TM helix shifts induced by hydrophilic residues within a hydrophobic helix. These studies show that anionic lipids can have significant and headgroup structure-specific effects upon membrane protein topography.

7-Subg
Control of Membrane Remodeling at the Golgi Through Sensors of Membrane Curvature: The ALPS Motif
Bruno Antonny[1], Joelle Bigay[1], Jean-François Casella[1], Guillaume Drin[1], Bruno Mesmin[1], Vincent Morello[1], Pierre Gounon[2], Romain Gautier[1].
[1]Institut de Pharmacologie Moleculaire et Cellulaire CNRS, VALBONNE, France, [2]Université de Nice Sophia Antipolis, Nice, France.
During the formation of coated vesicles the curvature of the lipid membrane strongly increases. This change can be sensed by specific motifs named ALPS that are present in some regulators of COP vesicles. ALPS motifs are intrinsically unstructured but fold as amphipathic helices at the surface of curved lipid membranes (radius < 50 nm). The sharp response to membrane curvature relies on the atypical polar face of the helix. Made mostly by Ser, Thr and Gly residues, this face cannot contribute to membrane binding. Thus membrane adsorption relies mostly on the insertion of the hydrophobic residues, which is favored by the lipid packing defects induced by membrane curvature. Two examples will be described where ALPS motifs permit to organize in time and in space reactions at the surface of membranes. The first example is the disassembly of the COPI coat. The lifetime of a coat must be finely tuned such as to be compatible with the capture of cargo and with membrane remodeling (budding, fission). We have identified two ALPS motifs in ArfGAP1, which controls COPI disassembly by catalyzing GTP hydrolysis in Arf1, one component of this coat. The remarkable sensitivity of ArfGAP1 to membrane curvature suggests that Arf1-GTP molecules are gradually eliminated from the center of the coat but not at the periphery during membrane budding. The second example is the tethering of transport vesicles by the long coiled-coil protein GMAP-210. We demonstrate that GMAP-210 can bridge small vesicles through its N-terminal ALPS motif to membranes primed with Arf1-GTP through its C-terminal GRAB domain. Interestingly, ArfGAP1 can disrupt this interaction when membrane curvature increases. This suggests that GMAP-210 acts as a molecular vector connecting in an asymmetric and reversible manner flat and curved membranes.

8-Subg
From Hydrophobic Matching to Interfacial Tuning: New Ideas for the Mutual Adaptation Between Membranes and Peptides
Jesús Salgado.
University of Valencia, Paterna (Valencia), Spain.
It is widely accepted that membrane proteins and lipid bilayers are complementary in terms of the distribution in space of their hydrophobic and polar regions. Similarly, it is also accepted that the hydrophobic parts of the protein and the membrane must adapt to each other. Classically these ideas are rationalized under the concept of hydrophobic matching, which predicts a number of possible mechanisms by which proteins can vary their effective hydrophobic length, or membranes can change their hydrophobic thickness. Such effects have been studied in detail for simplified systems, like transmembrane peptides or protein fragments, which generally show that optimizing peptide orientation is the principal adaptation response.
Based on simple computational methods, we show that the relative positioning, including orientation, of a peptide in a membrane can be easily and accurately predicted if the bilayer interfaces are taken into account. This allows studying in detail the adaptations of peptides to membranes, showing that, together with the classical coarse adjustment achieved by changes of the peptide tilt, there can be fine tuned adjustments through the azimuthal rotation. The latter tuning effect occurs mainly by optimizing positions of residues near the interface, and because it involves small changes of free energy, it provides a mechanism for high peptide dynamics. Additionally it strengthens the importance of the bilayer interface for the mutual adaptation of membranes and proteins and gives a new framework for the definition of so called flanking (or anchoring) residues.

Subgroup: Permeation & Transport

9-Subg
Coupling and uncoupling of Cl− and H+ movements through CLC transporters and channels
Christopher Miller.
Brandeis Univ, HHMI, Waltham, MA, USA.

10-Subg
Mechanism of Ion Recognition by Over-coordination: "The Caress of the Surroundings"[1]
Sameer Varma, **Susan Rempe**.
Sandia National Laboratories, Albuquerque, NM, USA.
Transferring Na^+ and K^+ ions from their preferred coordination states in water to states having different coordination numbers incurs a free energy cost. In several examples in nature, however, these ions *readily* partition from aqueous-phase coordinations into spatial regions having much higher coordination numbers. In particular, crystallographic data for the celebrated potassium channels show that their binding sites coordinate K^+ using eight carbonyl ligands, all within 3.0 Å from K^+. This makes for twice as many ligands as seen preferentially around K^+ in aqueous phase,[2,3] which would seemingly suggest an enormous uphill transition on the free energy surface.
We combine quantum, classical, and structural informatics studies to interrogate ion partitioning from low coordinations in water to over-coordinated[4,5] binding sites in proteins. Our results define the important role of the ligand surroundings in driving transitions in ion coordination structure, which underlies ion recognition in some proteins like potassium channels.[6]
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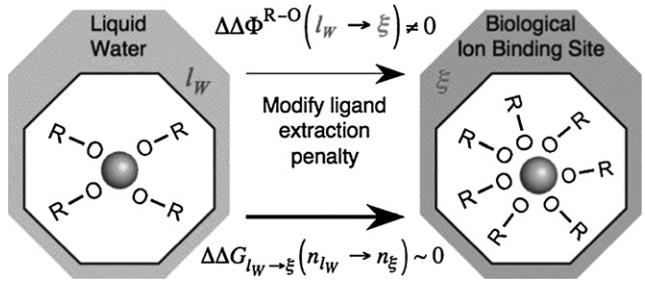

11-Subg
CTR Structure and Mechanism
Christopher J. De Feo[1], Stephen G. Aller[2], Yariv Barkan[3], Maya Shcushan[3], Gnana Siluvai[4], Nir Ben-Tal[3], Ninian J. Blackburn[4], **Vinzenz M. Unger**[1].
[1]Yale University, New Haven, CT, USA, [2]The Scripps Research Institute, La Jolla, CA, USA, [3]Tel-Aviv University, Tel-Aviv, Israel, [4]Oregon Health and Sciences University, Beaverton, OR, USA.
Copper uptake proteins (CTRs), mediate cellular acquisition of the essential metal copper in all eukaryotes. Using electron cryomicroscopy, we determined a 3D-structure of hCTR1 at ~7A resolution. The structure suggests that CTR1 proteins transport copper through a movement of Cu(I)-ions between defined binding sites and that intracellular copper chaperones are capable of directly obtaining copper from CTR1. To test these ideas, we used EXAFS to determine copper binding sites in hCTR1. We find that trimeric hCTR1 can stably bind 2 Cu(I)-ions through 3-coordinate Cu-S bonds. Moreover, EXAFS data obtained using Se-Cys-labeled CCS is consistent with the idea that the chaperone can obtain Cu(I) directly from copper loaded hCTR1. Modeling of a hypothetical hCTR1-CCS complex furthermore suggests that CCS may be able to associate with the membrane. Such a partitioning would greatly increase the efficiency of copper transfer to the chaperone because it would allow CCS to find hCTR1 through a 2D-diffusional search rather than a 3D-random walk. In support of this idea, we find that hCCS can bind to bilayers in vitro. Lastly, we generated a C-alpha model of the membrane embedded region of hCTR1 to aid future mechanistic studies. The model is consistent with the results of an extensive Trp-scan analysis of the membrane domain of hCTR1 and yCTR3 in that the overwhelming majority of residues found to be structurally and/or functionally important participate in helix packing interactions or face the copper permeation pathway along the 3-fold axis the CTR trimer.