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408-Pos Board B194
Ultraviolet Resonance Raman Study of Lipid Mediated Peptide Folding
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The interactions between proteins and biological membranes play an important role in many aspects of biochemistry. Thus, the ability to monitor the structural dynamics of membrane proteins is of great interest. In general, hydrophobic peptides are disordered and tend to aggregate in aqueous environments. For example, the amyloid-β (Aβ) peptide, a major component of the insoluble plaques associated with Alzheimer's disease, is intrinsically disordered under physiological conditions. However, Aβ adopts α-helical structure in membrane mimicking environments. This is not surprising as the hydrophobic region derives from the transmembrane (TM) region of the amyloid precursor protein. More interesting is the fact that low concentrations of organic solvents or surfactants promote aggregation and formation of β-sheet structure. The ability to simultaneously monitor lipid association and study its effect on the secondary structure of amyloidogenic proteins would be of great interest. Recent studies have shown a significantly enhanced amide I mode in the deep-UV resonance Raman (dUVRR) spectra of transmembrane proteins is a marker for lipid association. Positively charged hydrophobic peptides, including the hydrophobic Aβ(25-40) fragment of Aβ, spontaneously insert into anionic lipid bilayers. The application of dUVRR spectroscopy to monitor lipid-association, insertion and folding of these peptides will be presented.

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Analysis of the Area Per Lipid in Protein-Membrane Systems
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Molecular dynamics (MD) simulation is a powerful research tool to investigate structural and dynamical properties of biological membranes and membrane proteins. The lipid structures of simple membrane systems in recent MD simulations are in good agreement with those obtained by experiments. However, for protein-membrane systems the complexity of protein-lipid interactions makes investigation of lipid structure more difficult. Although the area per lipid is one of the important structural properties of membranes, the area in protein-membrane systems cannot be calculated easily by conventional approaches like Voronoi tessellation method. Here, we developed a new method, based on a combination of the two-dimensional Voronoi tessellation and Monte Carlo integration methods. Monte Carlo integration enables us to estimate the cross-sectional area of arbitrary-shaped target molecules. We applied the method to all-atom MD trajectories of the sarcoplasmic reticulum Ca2+-pump and the SecY protein-conducting channel. The calculated lipid surface area was in agreement with experimental values and consistent with other structural parameters of lipid bilayers. We also observed a response of lipid bilayers to the conformational transition of SecY. We believe that our method is useful to analyze time courses of protein-lipid interactions in MD simulations of membrane proteins.

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Docking of PKC(Alpha)-C1 and PKC(Beta)-C2 Domains to POPC/POPS/POG Lipid Membranes
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Protein kinase C (PKC) isoenzymes are a large family of serine/threonine kinases that play important roles in cellular signaling. However, the detail process of their docking at cell membranes is still not well understood. In this study, eight independent atomistic molecular dynamics (MD) simulations were performed to systematically investigate the docking of PKC(alpha)-C1 and PKC(beta)-C2 domains to lipid bilayers composed of POPC, POPS, and POG (1-palmitoyl-2-oleoyl-sn-glycerol). POG concentration varied from 0 to 25% (i.e. 0%, 6.25%, 12.5%, and 25%), and the ratio of POPC/POPS was kept at 3/1. Our result shows that PKC(beta)-C2 domain adapts a parallel configuration when it docks to the lipid bilayer in the absence of POG; however, at the presence of POG, it adapts a perpendicular configuration. This difference in docking configurations is due to the increase of spacing between lipids headgroups at the presence of POG, which allows the perpendicular docking of PKC(beta)-C2 domain. Furthermore, PKC(beta)-C2 domain shows no significant conformational change during the docking in both cases. On the other hand, PKC(alpha)-C1 domain only docks to a lipid bilayer that contains POG. In the absence of POG, the domain stays in a parallel configuration on the surface of the bilayer. The docking of PKC(alpha)-C1 domain to lipid bilayers containing POG shows a high specificity of PKC(alpha)-C1 domain toward diacylglycerol.

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Organization and Lipid Interaction of the Model Amphipathic α-Helix Bundle Protein apoLp-III
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Amphipathic α-helix bundle domains are found in many proteins responsible for neutral lipid transport and storage. Important examples are found in apoE, the perilipins, and the representative exchangeable apolipoprotein apoLp-III. Here we characterized the organization and lipid interaction of apoLp-III in Langmuir monolayers modeling the phospholipid monolayer surrounding the neutral lipid particle, i.e. lipoprotein. Surface-sensitive X-ray techniques showed that apoLp-III is partially unfolded at the interface as the unfolded protein was best represented by two distinct regions. This surprising result does not result from the high degree of glycosylation of apoLp-III as the recombinant protein behaved in a similar fashion. This suggests that either apoLp-III is partially unfolded or that unfolded protein is associated with the protein monolayer. Injection of apoLp-III underneath a previously formed (phospho)-lipid monolayer results in a rapid increase of the surface pressure. We characterized this increase in pressure as a function of effective lipid molecular shape and lipid packing density. These results should shed important light on the interaction of amphipathic α-helix bundle domains with phospholipid monolayers. This work will be extended in the future to include additional apolipo-and lipid droplet proteins containing amphipathic α-helix bundle domains.

Membrane Dynamics & Bilayer Probes I

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Membrane Fluidity Profiles as Deduced by Saturation-Recovery EPR Measurements of Spin-Lattice Relaxation Times of Spin Labels: Multifrequency Approach
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New capabilities using saturation-recovery (SR) EPR at X-band (9.4 GHz) and W-band (94 GHz) to obtain profiles of the membrane fluidity have been demonstrated for dimyristoylphosphatidylcholine (DMPC) membranes with and without 50 mol% cholesterol. Phosphatidylcholine (n-PC) spin labels were used. Results were compared with profiles of the rotational diffusion coefficient, R_{perp}, obtained from simulation of EPR spectra using Freed's model. The spin-lattice relaxation rate (T_1^{-1}) obtained from SR EPR measurements of phospholipid spin labels in deoxygenated samples depends primarily on the rotational correlation time of the nitroxide moiety within the lipid bilayer. Thus, T_1^{-1} can be used as a convenient quantitative measure of membrane fluidity that reflects membrane dynamics at a certain depth in the membrane. The order parameter, which is often used as a measure of membrane fluidity, describes the amplitude of wobbling motion of alkyl chains relative to the membrane normal and does not explicitly contain time or velocity. Thus, the order parameter can be considered as ''nondynamic''. It is shown that T_1^{-1} and R_{perp} profiles reveal changes in membrane fluidity that depend on the motional properties of the lipid alkyl chain. We find that cholesterol has a rigidifying effect only to the depth occupied by the rigid steroid ring structure and a fluidizing effect at deeper locations. These effects cannot be differentiated by profiles of the order parameter. Results demonstrate that SR EPR at W-band has the potential to be a powerful tool for studying samples of small volume, ~30 nL, compared with a sample volume of ~3 μL typically required at X-band.

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Phase Boundaries in Phosphatidylcholine Membranes Saturated and Oversaturated with Cholesterol
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Conventional and saturation-recovery EPR along with differential scanning calorimetry (DSC) were used to determine the cholesterol/phosphatidylcholine (Chol/PC) mixing ratio at which cholesterol bilayer domains (CBDs) and cholesterol crystals (CR) start to form in dimyristoyl-PC (DMPC) and 1-palmitoyl-2-oleoy-PC (POPC) membranes. The Chol/PC mixing ratio was changed from

0 to 3. Lipid multilamellar dispersions investigated in this work were prepared using the rapid solvent exchange method to preserve compositional homogeneity throughout the suspension. EPR spin-labeling methods were used with spin-labeled cholesterol analogues (cholestane spin label [CSL] and androstane spin label [ASL]) as well as with a spin-labeled phospholipid (1-palmitoyl-2-(5-doxylstearoyl)phosphatidylcholine (5-PC)) to locate the phase boundary for CBD formation. DSC was used to locate the phase boundary for the formation of CRs. Results showed that in both DMPC and POPC membranes the CBD is already formed at a Chol/PC mixing ratio of 1, while CRs are formed only when the Chol/PC mixing ratio exceeds the value of 2. Previous results and data presented here indicate that when the Chol/PC mixing ratio increases, the PC bilayer becomes saturated with cholesterol first. This phospholipid bilayer, saturated with cholesterol, possesses unique physical properties. Increase in cholesterol content beyond the Chol/PC mixing ratio of 1 causes formation of the CBD, and further increase, beyond the Chol/PC mixing ratio of 2, causes formation of CRs (presumably outside of the membrane). Thus, the phase boundary at a Chol/PC mixing ratio of 1 separates the region with a single liquid-ordered phase and the region with a coexisting liquid-ordered phase and CBD. The cholesterol solubility threshold at a Chol/PC mixing ratio of 2 separates the region with a coexisting liquid-ordered phase and CBD from the region in which CRs are formed.

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Intermembrane Forces Probed by Osmotic Stress and Solid-State ^{2}H NMR Spectroscopy

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Intermembrane forces play a significant role in biological processes such as fusion, shape transformations, and lipid-protein interactions. Forces suggested to govern intermembrane interactions include van der Waals attraction, membrane undulations, hydration force, and lipid protrusions. How do the regimes of these forces overlap and how can we experimentally study them? Through use of osmolytes and dehydration we can control intermembrane spacing in liquid-crystalline DMPC-d_{54} membranes [1]. Measured order parameters from solid-state ^{2}H NMR spectroscopy allow deformations to be accessed at a molecularly resolved level [2]. Stresses from dehydration and osmotic pressure are thermodynamically equivalent, because the change in chemical potential when transferring water from the interlamellar space to the bulk water phase corresponds to an induced pressure. A unified theoretical framework predicts an ideal equation of state for the membrane system that depends inversely on the number waters per lipid as confirmed by experimental ^{2}H NMR data [1]. Non-ideal interactions (intermembrane forces) within the membrane system are treated in terms of an osmotic coefficient. Intermembrane forces have differing temperature dependences and can be separated by the temperature variation of the osmotic coefficient. At lower osmotic pressures (larger intermembrane separation) the osmotic coefficient has a linear temperature dependence, agreeing with theoretical predictions for thermal undulations. At high pressures (smaller intermembrane separation) the osmotic coefficient becomes independent of temperature, in accord with predictions for lipid protrusions. Our evidence shows that undulations dominate at intermediate intermembrane distances and protrusions dominate at short distances. We provide a new experimental method for understanding intermembrane forces. This understanding is needed for the interpretation of membrane fusion, shape transformations, and lipid-protein interactions. [1] K.J. Mallikarjunaiah *et al.* (2011) *Biophys. J.***100**, 98-107. [2] A. Leftin and M.F. Brown (2011) *BBA***1808**, 818-839.

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Synthesizing GUV from Wet Lipid Film to Achieve Better Uniformity in Lipid Composition

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Giant Unilamellar Vesicles (GUVs) are cell-sized model membrane systems that allow direct investigation of membrane-related phenomena using fluorescence microscopy. Understanding the organization and dynamics of lipid bilayers is important for understanding the processes taking place in cell membranes. Previous work showed that micron-scale domains in GUV can be observed in some ternary lipid mixtures composed of a high melting temperature lipid, a low melting temperature lipid and cholesterol. However, it is a well-known problem that GUVs synthesized by the electroformation from dry lipid films have a wide distribution of transition temperature and lipid composition. In this study, we synthesized DOPC/DSPC/cholesterol GUVs and diPhyPC/DPPC/cholesterol GUVs by the standard electroformation method from dry lipid films as well as by a modified method using wet liposomes made from the Rapid Solvent Exchange (RSE) method. We quantified the lipid composition distributions of GUVs synthesized by the two methods by measuring the transition temperature distributions of GUVs using fluorescence microscopy; since a narrower distribution of transition temperature should correspond to a more uniform distribution in GUV lipid composition. Our results show that GUVs synthesized from wet lipid film have a better uniformity in lipid composition, and the standard deviations of transition temperature are about 3 times smaller than that of GUVs prepared from dry lipid films. This improved method not only gives a better control of GUV lipid composition, but also has a potential of synthesizing GUVs from cell membranes containing native proteins without going through a dry film state.

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Behavior of Lipid Vesicles Near Solid Surfaces

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The behavior of lipid membranes near solid surfaces has a great significance both in medicine and in technology. In spite of the widespread use and study of such membrane phenomena, their theoretical analysis is rather scarce. Our main goal here is to understand the process during which membrane vesicles first adhere to solid surfaces, then rupture (or go through a series of transient ruptures) due to the mechanical tension induced by the adhesion, and finally spread along the surface forming a supported lipid bilayer. In our theoretical description we simultaneously consider the dynamics of spontaneous pore opening and closing; volume loss via leakage through the pores; and the advancement of the adhesion front. All these processes are supposed to follow an overdamped dynamics and coupled to each other through membrane tension.

Our numerical simulations uncover the nature of the entire rupture process, and make quantitative predictions about the conditions under which fast vesicle rupture and supported lipid bilayer formation is expected.

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Membrane Properties Involved in Calcium-Stimulated Microparticle Release from the Plasma Membranes of S49 Lymphoma Cells

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The mechanism of microparticle shedding from the plasma membrane of calcium-loaded cells has been investigated in erythrocytes and platelets. Recent studies have revealed the physiological and clinical importance of microparticle release from nucleated cells such as lymphocytes and endothelium. The experiments of this study were designed to address whether simple mechanisms discovered in platelets and erythrocytes also apply to the more complex nucleated cells. Three such mechanisms were addressed: potassium efflux, transbilayer phosphatidylserine migration, and membrane lipid order. The rate and amount of microparticle release in the presence of a calcium ionophore, ionomycin, was assayed by light scatter at 500 nm. To inhibit the calcium-activated potassium current, cells were exposed to 1 mM quinine or a high-potassium buffer. Both interventions substantially attenuated microparticle shedding induced by ionomycin. Microparticle release was also greatly reduced in a lymphocyte cell line deficient in the expression of scramblase, the enzyme responsible for calcium-stimulated phosphatidylserine migration to the cell surface. This result indicated that such phosphatidylserine exposure is also required for microparticle shedding. Finally, the effect of membrane physical properties was addressed by varying the experimental temperature (32-42 °C). A significant positive trend in the rate of microparticle release as a function of temperature was observed. Fluorescence experiments with merocyanine 540, trimethylammonium-diphenylhexatriene, and patman revealed significant differences in the level of apparent membrane order along that temperature range. Ionomycin treatment appeared to cause further disordering of the membrane, although the magnitude of this change was minimally temperature-sensitive. Thus, it was concluded that microparticle release depends more on the initial level of membrane order than on the change imposed by calcium uptake. In