microtubule towards the periphery with a nearly stationary tip. Interestingly, the curvature distribution of microtubules in an in vitro kinesin-microtubule gliding assay is also exponential. These experimental results are compared with computer simulations of microtubules in an explicit solvent with molecular motors. The primary conclusion of this work is that many of the known mechanisms of microtubule deformation do not play a significant role in mediating microtubule bending in LLC-PK1 cells; rather, molecular motors appear to generate most of the strain energy stored in the microtubule lattice.

1876-Plat Microtubule-driven Multimerization Recruits Ase1 onto Overlapping Microtubules

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Polarity-specific microtubule organization is important both during cell division and interphase. Bipolar microtubule-dependent motor proteins such as Eg5 as well as passive microtubule bundling proteins such as Ase1 play important roles in these ordering processes. Ase1 preferentially crosslinks anti-parallel microtubules and localizes to the zones where microtubules overlap with remarkable prevalence. Here we show that this localization to the overlap zone depends on the capability of Ase1 dimers to form multimers on the microtubule lattice. We find that single dimers diffuse along the microtubule lattice, and can form multimers when concentrated enough. At intermediate concentrations, however, Ase1 multimerization is restricted to regions of microtubule overlap. These findings reveal an intriguing cooperative mechanism that controls targeting of Ase1.

Platform AW: Membrane Fusion

1877-Plat Energetics and Dynamics of SNAREpin Folding Across Lipid Bilayers Invesitgated by Direct Force Measurements

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The core principle of cellular membrane fusion consists of the assembly of cognate SNARE proteins initially residing in opposing membranes to yield a stable, bridging complex (the SNARE-pin) that triggers bilayers to merge. However, the energy released upon SNARE-pin formation, the kinetics of SNARE association and the extent of SNARE-pin assembly prior to fusion remain unknown.

To address these questions, we have used the Surface Force Apparatus to measure in real time the interaction energy versus distance profiles between assemblies of neuronal cognate v- and t-SNARE proteins anchored to lipid bilayers. The energetics and dynamics of SNAREpins formation and the different intermediate structures sampled by cognate SNAREs in the course of their assembly have been determined. The interaction energy versus distance profiles of assembling SNAREpins reveal that SNARE motifs begin to interact when the membranes are 8 nm apart and SNARE-pin formation across lipid bilayers occurs rapidly (less than 1 minute) when the bilayers reach such distance. Even after very close approach of the bilayers (~ 2–4 nm), the SNAREpins remain partly unstructured in their membrane-proximal region. The energy stabilizing a single SNAREpin in this configuration was deduced from these direct force measurements and was found to be about 35 k_BT, which corresponds closely with the energy needed to fuse outer but not inner leaflets (hemifusion) of pure lipid bilayers (40–50 k_BT).

In the presence of complexin, the interaction energy versus distance profiles show striking different features, which reveals its role in the membrane fusion process. How complexin affects the formation of SNAREpins will also be addressed.

1878-Plat Effect of Spontaneous Curvature on the Adsorption of Lipids to the Air/Water Interface

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Prior studies indicate that lipids in the H_{II}-phase mimic the rapid adsorption of pulmonary surfactant in the lung. Structural analysis, however, using small angle X-ray scattering (SAXS) and ^{31}P nuclear magnetic resonance shows that, irrespective of temperature, calf lung surfactant extract (CLSE) forms only lamellar structures. The studies reported here seek to determine the effect of spontaneous curvature on the adsorption of lamellar vesicles. We used SAXS to monitor the structures present, and compared the adsorption of dielaidoyl phosphatidylethanolamine (DEPE), which forms $L_{\beta'}$, L_α, and H_{II} phases at accessible temperatures, with dipalmitoyl phosphatidylcholine (DPPC), which forms only lamellar structures. Below the $L_{\beta'}$-L_α transition temperature, adsorption of DEPE and DPPC failed to lower surface tension below 60mN/m. Vesicles in the L_α phase at 42°C adsorbed faster for both compounds. For DPPC, the increase in rate was limited, and surface tension fell only to ~50 mN/m. DEPE instead adsorbed rapidly to ~25 mN/m. Our results suggest that an equilibrium tightly-curved structure is unnecessary for rapid adsorption. The presence of spontaneous curvature, indicated by the ability to form H_{II} structures at higher temperatures, although unexpressed in the lamellar vesicles, favors formation of curved structure in the presence of an air/water interface, and is sufficient to produce rapid adsorption.

1879-Plat Membrane Hemifusion: Energetics and Growth Kinetics

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This work presents a quantitative model of the kinetics of hemifusion diaphragm development during fusion which predicts time-dependent diaphragm growth. Membrane fusion is a crucial step in many cell functions, including exocytosis and intracellular trafficking. Considerable experimental evidence suggests an important intermediate on the fusion pathway is the hemifused state, in which contacting membrane leaflets are merged but distal leaflets remain separate. Micron-scale growing hemifusion diaphragms have been observed *in vitro* [Chernomordik et al, *BBA,* 1987], while *in vivo* nano-scale diaphragms connecting synaptic vesicles with the plasma membrane have been detected using electron microscopy [Zamphigi et al, *Biophys J*, 2006]. Diaphragm growth may be important in increasing fusion probability, both by increasing the area where a fusion pore may form and by enlarging the rim where lipids are highly strained and fusion pores may preferentially develop. We begin by analyzing the diaphragm rim strains and energies with scaling analysis which proves to be consistent with previous theoretical work [Kozlovsky et al, *Biophys J*, 2002]; key concepts are a line bending energy and an effective line tension opposing growth due to rim stresses. Protein machinery, such as SNAREs, may function to nucleate hemifusion and to drive diaphragm growth to a critical size, beyond which membrane tension can overcome line tension and drive growth. For cellular scale membranes we find growth is limited by lipid area transfer required for hemifusion to proceed. In a second scenario, line-active species grow the diaphragm by reducing its line tension to an effectively negative value with sub-linear area time dependence. Hemifusion growth kinetics exhibit different time dependencies in the two cases and depend on membrane and line tension, lipid dynamics and total membrane area.

1880-Plat Calculation of Free Energy Barriers to the Fusion of Small Vesicles

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The fusion of small vesicles, either with one another or with a planar bilayer, is studied by means of a microscopic model in which the bilayers are composed of hexagonal- and lamellar-forming amphiphiles. Using the self-consistent-field approximation, we estimate the free energy of fusion intermediates as a function of the compositions of hexagonal and lamellar formers and of the vesicle radius. We find the initial barrier to form the stalk hardly varies with either composition or vesicle radius. However these variables do affect the second barrier, that to expansion of the hemifusion diaphragm and formation of the fusion pore. In particular, for a fixed vesicle radius, the energy of the second barrier decreases more or less linearly as the concentration of hexagonal formers increases. For a fixed composition, the energy of the second barrier decreases rapidly as the vesicle radius decreases. As a consequence, once the initial barrier to stalk formation is overcome, fusion involving small vesicles should proceed with little or no further input of energy.

1881-Plat Toward A High-resolution Structure Of The HIV gp41 Protein In Membranes

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Merging of membrane-bound compartments is coordinated by proteins and many details of this process are not clear. Membrane-sheathed enveloped viruses like HIV and influenza infect target cells using their own viral fusion proteins. In this study, the membrane-associated structure of the HIV gp41 fusion protein was probed with solid-state nuclear magnetic resonance (SS-NMR) as well as other biophysical techniques. Three constructs were studied:

(i) N-terminal 34-residues (FP34),
(ii) N-terminal 70-residues (N70), and
(iii) the folded ectodomain with the native loop replaced by a short linker (Fgp41).

All constructs included the functionally important ~20-residue N-terminal "fusion peptide" (FP) domain of gp41 which binds to target cell membranes. N70 models part of the pre-hairpin conformation of gp41 which is an early structure in fusion and a key drug target for fusion inhibitors. Fgp41 models part of the low energy hairpin conformation of gp41 which is a late-stage folded structure in fusion. Local secondary structure in these constructs was probed with SSNMR chemical shift measurements of selective ^{13}C sites in the FP region. The FP adopted primarily beta strand conformation in either neutral or negatively charged membranes whose cholesterol content was comparable to that found in membranes of host cells of HIV. Distinct populations of helical and beta strand FP conformations were detected in membranes which lacked cholesterol. In cholesterol-containing membranes, intermolecular ^{13}C-^{13}C distance measurements revealed intermolecular strand association and could be fitted with a significant population of parallel beta sheet structure of the FP region. Biophysical characterization of Fgp41 in solution revealed approximately 80% helical structure with high thermostability. A high-resolution structure of the FP domain of membrane-associated Fgp41 will help resolve central questions in viral membrane fusion as well as reveal potential new therapeutic targets to inhibit the process.

1882-Plat Simulating Vesicle Fusion: Effects Of Membrane Composition And Curvature

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One challenge in understanding membrane fusion is how changes in physical properties of lipid membranes affect fusion and how they may be employed in physiological regulation of fusion events. Cellular and viral proteins involved in fusion are thought to alter the curvature and local lipid composition of membranes and perhaps to induce local lipid disorder. We have used ensemble molecular dynamics simulation to develop physical models for each of these mechanisms and its effects on the fusion process. By analyzing thousands of such simulations, we can derive a more general model

for how lipid composition and curvature control fusion kinetics. Here we report simulations where both the composition and the curvature of lipid membranes were varied, with curvature regimes ranging from small 15-nm vesicles to larger vesicles to planar bilayers. We also consider the effects of curvature fluctuations in bilayers. We find a pronounced effect of curvature on fusion rates; in addition, the effects of lipid composition remain relatively independent of curvature over the systems simulated. We then apply these models to interpret the effects of fusion proteins on membrane curvature.

1883-Plat SNAP-25: Palmitoylation and membrane interactions

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Understanding neurotransmitter release requires defining the cellular machinery and environmental factors essential for synaptic vesicle exocytosis. We have studied SNARE-induced fusion using a model system based on planar lipid bilayers (BLMs) and native secretory vesicles. In this system, SNAP-25 can function as a v-SNARE and the loss of SNAP-25 from vesicles reduces fusion rates. To further define the role of SNAP-25 in fusion, we examined its interaction with other SNARE proteins and with membranes subsequent to altering SNAP in several ways. Using an environment sensitive tryptophan fluorescence assay, we observe a distinct change in SNAP-25's interaction with membranes following palmitoylation or oxidation of the cysteines in the membrane-associating region of SNAP25's linker region. Palmitoylation was performed in the presence of the palmitoyl transferase HIP14 (huntingtin interacting protein 14). Palmitoylation was confirmed by using a modification of Green's assay, which includes removal of palmitic acid with hydroxylamine followed by biotinylation and detection with streptavidin-HRP (Drisdel and Green, BioTechniques *36*:276–285. 2004). Exact quantification of palmitoylation is difficult because the cysteines are close together and exactly one streptavidin (with four binding sites) can bind multiple biotinylated cysteines. This ambiguity can be reduced by pre-incubating streptavidin with biotin (1:3.5), thus producing a streptavidin with just one binding site. Palmitoylation of SNAP-25 may be important not only for targeting of SNAP-25 to the membrane, but also in allowing SNAP-25 to modify or be modified by the fusing membranes.

1884-Plat Effect Of Sphingomyelinase-Mediated Generation of Ceramide on Aggregation of Low Density Lipoproteins

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The Response-To-Retention hypothesis states that retention of aggregated LDL within the intima is the single event necessary and sufficient to provoke atherosclerosis. We became interested in this hypothesis and an observation reported ex vivo that LDL aggregates tend to be nominally 100 nm diameter, or "64-mers." We demonstrated in earlier studies that LDL aggregation follows principles of colloid science and is well-described by a mass action model. In the case of sphingomyelinase(Smase)-induced aggregation, we demonstrated that the relative number of LDL and enzyme molecules, rather than individual concentrations, determines the final aggregate size. While a 100 nm aggregate size can sometimes result in enhanced uptake by macrophages, there does not appear to be anything special about 100 nm per se. In the current study, we extend these ideas and perform a careful measurement of ceramide generation during LDL aggregation. Ceramide is assayed by resorufin fluorescence for magnesium (Mg2+)-dependent Smase in the enzyme concentration range 0.00–0.22 units/mL at a substrate concentration of 0.33 mg LDL/mL. All reaction profiles were fit with a single model involving a unique set of five kinetic rate constants (traditional Michaelis-Menten parameters, plus irreversible binding between ceramide and Smase, an enzyme diffusion time lag, and binding of enzyme to substrate). Identical conditions were used in complementary LDL aggregation studies involving dynamic light scattering (cumulant fits to determine light-scattering-intensity-weighted average diffusion coefficients and effective aggregate diameters via Stokes-Einstein equation). Whereas temporal profiles of both aggregation and ceramide exhibit expected Smase concentration dependencies, a non-obvious result ensues when aggregation is plotted versus ceramide generation; all data fall onto a single, straight line. This reduction of data onto a single master curve reinforces the notion that fundamental principles can and do apply to the study of biologically-relevant phenomena.

Heme Proteins

1885-Pos Effects of Distal Pocket and Subunit Interface Mutations on Ligand Binding in Hemoglobin

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Structural and functional studies have been performed to investigate the effects of αL29F, αL29W, αV96W, βN108K, αV96W/βN108K, αV96W/αL29F/βN108K and αV96W/αL29W/βN108K substitutions on the ligand binding properties of human hemoglobin. The αL29F and αL29W mutations, which are located in the ligand binding pocket, exhibit a slow phase for ligand association after complete and partial photolysis, which reflects a change in the intrinsic ligand-binding properties of the mutant subunit itself. This has been investigated further by examining ^{1}H-NMR spectra of partially saturated samples. The βN108K mutation, located in the $\alpha_1\beta_1$ interface also exhibits a slow phase after complete photolysis, but in this case, the amplitude and rate constants suggest the presence of a population of 'T'-state tetramers rather than a change in the intrinsic ligand-binding properties of either subunit. The αV96W substitution is known to introduce a novel hydrogen bond