of membrane tension, composition and curvature on KvAP activity and distribution are currently underway.

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1164-Plat

Lipid Membrane Composition has A Dramatic Effect on the Dynamics of the GlpG Rhomboid Protease from Escherichia Coli

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Intramembrane proteases cleave transmembrane substrates to liberate physiologically important molecules. The proteolytic activity of these enzymes can be significantly influenced by the composition of the lipid membrane. Here, we find that the composition of the lipid membrane has a dramatic effect on the motions of the GlpG rhomboid serine protease from Escherichia coli.

In a 1-palmytoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) lipid bilayer, conformational changes of two critical structural elements of the protease, the cap loop close to the active site and the regulatory loop L1, occur within 40ns of unconstrained molecular dynamics simulations. In contrast, in a 1-palmytoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine (POPE) lipid bilayer, a conformational transition of the cap loop is observed only after ~80ns, ~20ns after that of loop L1. This sensitivity of the enzyme motions on the lipid membrane composition is explained by differences in how POPC and POPE lipid headgroups hydrogen bond among themselves, and with protein amino acids. Tight interactions between a lipid headgroup and the active site restrict the dynamics of the cap loop.

An atomistic description of the structure and dynamics of the protease:substrate complex is a critical first step towards understanding how the protease works. Molecular dynamics simulations of GlpG together with the Spitz model substrate reveal that docking of the substrate to the enzyme involves a complex interplay of changes in the structure and dynamics of the substrate, the protease, and the surrounding lipid molecules.

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1165-Plat

Energetics of Glycophorin A Dimerization in Mammalian Plasma Membranes

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Quantitative measurements of protein interaction strengths are crucial for describing signaling networks and predicting cellular responses to environmental stimuli. Of all interactions between biological macromolecules, interactions between membrane proteins are the least characterized, and their strength is often measured in model detergent and lipid systems that poorly mimic the complex biological membrane. Measurements in models systems, however, are not likely to yield accurate predictions, because interactions in the native cellular environment occur within the context of a crowded system.

Here we explore the utility of plasma membrane-derived vesicles as a model crowded environment for quantitative characterization of membrane protein interactions in mammalian membranes. In particular, we study the dimerization energetics of Glycophorin A (GpA), the primary sialoglycoprotein of human erythrocyte membranes, using the "quantitative imaging Föster resonance energy transfer (QI-FRET)" method. We determine the FRET efficiency, and the donor and the acceptor concentrations in single plasma membrane-derived vesicles loaded with GpA. These measurements yield, for the first time, the free energy of GpA dimerization in mammalian membranes. Supported by NSF MCB-0718841.

Li E, Placone J, Merzlyakov M, Hristova K (2008) Quantitative measurements of protein interactions in a crowded cellular environment. *Anal Chem* 80: 5976-5985

1166-Plat

Monitoring Proton Flux Quantitatively; Influenza Proton Channel A/M2

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An improved methodology for monitoring proton translocation across membranes is presented, along with results from the Influenza A virus proton channel A/M2.

We have constructed a liposome-production instrument which creates proteoliposomes from a lipid/detergent/protein mixture by gradually adding hydrophobic beads while continuously monitoring sample turbidity. The method is fast and reproducible, and facilitates enhanced control of key protein reconstitution parameters. It also enables synchronous measurements of protein-mediated ion flow and passive permeability across the bilayer. Two novel pH-sensors are presented: Glu3 and TCHP. These porphyrin-based probes are membrane-impermeable, do not interact with biological complexes, have physiologically appropriate pK, and display high extinction coefficients. Glu3 is also ratiometric in emission.

H+, K+ and Na+ permeabilities were determined in liposomes of different lipid and cholesterol composition. The effect of detergent/lipid and lipid/protein ratios on ion permeability was systematically investigated. The proton channel A/M2, key to Influenza A virus propagation and an antiviral drug target, was successfully reconstituted. The proton translocation rate was determined to 8.3 protons per second and A/M2 tetramer. We also found that the presence of protein in the bilayer enhanced the passive ion permeability. Lowering the protein/lipid ratio minimized this effect, and prolonged the measurement window of proton movements to several minutes, and thus resulted in the most reliable data.

Preliminary data from membrane-spanning subunits of respiratory Complex I are also presented.

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The Role of the Protein-Conducting Channel in the Membrane Insertion of Transmembrane Segments

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In all domains of life, the majority of membrane proteins are inserted into the membrane via a protein-conducting channel, also known as the SecY or Sec61 complex. In addition to a translocation pathway across the membrane, this channel features a unique lateral gate, which can open toward the membrane, permitting the sequential insertion of transmembrane segments (TMs). How this insertion occurs is still unclear, although a thermodynamic partioning between channel and membrane environments has been proposed. However, experiment- and simulation-based scales for the free-energy insertion cost of various amino acids differ, sometimes significantly as in the case of arginine (2-3 kcal/mol in experiment compared to 17 kcal/mol in simulation). Using free energy perturbation (FEP) simulations, we have calculated the insertion cost for an arginine located on a background poly-leucine helix, both in the center of a pure bilayer and in the center of a model of SecY featuring an open lateral gate. We find that the presence of SecY greatly reduces the membrane insertion cost for arginine, in agreement with prior simulations. We also consider the free energy cost for the insertion of the background helix from SecY to the membrane, which had been neglected previously.

1168-Plat

Nature as A Scaffold: The Rational Redesign of a Protein Pore

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Our major goal is to engineer a transmembrane protein pore that acts as a single-molecule nanopore probe for sensing double-stranded DNA (dsDNA), folded proteins and their complexes with interacting agents. The protein of choice is Ferric hydroxamate uptake protein component A (FhuA), a multifunctional outer membrane protein found in E. coli., which facilitates the uptake of Fe3+, along with phage binding and the translocation of small peptides. Using standard protein engineering, we modified the FhuA protein by removing the N-terminal 160 residue-long cork and by deleting several large extracellular loops to form an open protein pore with an elliptical cross section of ~ 49 x 36 Å. This engineered protein nanopore exhibits a stable open-channel activity for long periods of time, with a unitary conductance of ~5 nS. We show evidence that this engineered FhuA-based nanopore acts as a stochastic sensing element for detecting small folded proteins at single-molecule resolution.

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1169-Plat

Modulation of the Lateral Mobility of Transmembrane Peptides with Hydrophobic Mismatch

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Paris, France, [7]Laboratoire de Physique Statistique de l'Ecole Normale Supérieure, Paris, France.
An hydrophobic mismatch between protein length and membrane thickness can lead to a modification of protein conformation, function, and oligomerization. To study the role of hydrophobic mismatch, we have studied the change in mobility of transmembrane peptides in model bilayers. The studied peptides possess an hydrophobic helix of various length $d\pi$, and the hydrophobic thickness, h, of the bilayers can be tuned at will. For each mismatch value, using Fluorescence Recovery After Pattern Photobleaching (FRAPP), we precisely measured the diffusion coefficient D of the embedded objects and gained access to their apparent size. This enables us to observe the orientation or oligomerization state of the peptides versus their concentration, and discover that the effects of positive and negative mismatches on diffusion are highly asymmetric. For bilayers thinner than $d\pi$, the diffusion coefficient decreases and the derived characteristic sizes are larger than the peptide radii. As suggested by previous studies, the peptides should accommodate by tilting, and this scenario was confirmed by ATR-FTIR spectroscopy. As the membrane thickness increases, the value of the diffusion coefficient increases: the peptides raise (i.e. their tilt is reduced) and reach an upright position and a maximal mobility for $h \approx d\pi$. Using accessibility measurements, we show that when the membrane becomes too thick, the peptide polar heads sink into the interfacial region. Surprisingly, this "pinching" behavior does not hinder the lateral diffusion of the transmembrane peptides. But it creates interactions between the embedded peptides, and collective behaviors emerge. For low peptide concentration, the transmembrane anchorage of the peptide is broken as the bilayer is swollen. For intermediate concentrations, we observed the arrangement of small monodisperse clusters, while polydisperse macro-domains are formed at higher peptide density, leading to spontaneous and reversible formation of "vesicles".

1170-Plat
The Gating Mechanism of Yeast Aquaporin Studied by Molecular Dynamics Simulations
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Aquaporins are membrane proteins responsible for the permeation of water and other solutes through the cell membrane. They arrange in a tetrameric conformation, where each monomer acts as a highly efficient single-file water channel. The x-ray structure of the yeast aquaporin (Aqy1), recently found at a very high resolution (1.15 Angstrom), revealed the conformation of the extended N-terminus - an unusual feature within the family of aquaporins - occluding the water pore. In contrast, functional assays with spheroplast of P. pastoris showed a substantial increase in the water transport activity when Aqy1 was present compared to an assay where Aqy1 was deleted, indicating that Aqy1 is a gated water channel. Here we address the question of a putative gating mechanism of Aqy1 by using molecular dynamics simulations. Our findings suggest that Aqy1 may be regulated by both phosphorylation of a serine residue (Ser107) or membrane-mediated mechanical stress. Both possibilities lead to similar opening transitions after a local rearrangement of the residues Tyr31, Leu189, Ala190 and Val191, located in the gate of the pore. We observed that there is a principal collective motion causally involved in these gating transitions, and that is possible to attain reproducible opening events along this collective coordinate. The simulation results are therefore consistent with a mechanism in which both phosphorylation and mechanosensitive gating can trigger the channel opening. Aqy1 regulation may help yeast to survive rapid freezing and thawing, and sudden osmotic changes.

Platform Y: Protein Dynamics I

1171-Plat
Protein Similarity Derived Solely from Molecular Dynamics
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The dynamic motions of many proteins are central to their function. It therefore follows that the dynamic requirements of a protein are evolutionary constrained. In order to assess and quantify this, one needs to compare the dynamic motions of different proteins. Comparing the dynamics of distinct proteins may also provide insight into how protein motions are modified by variations in sequence and, consequently, by structure. The optimal way of comparing complex molecular motions is, however, far from trivial. The majority of comparative molecular dynamics studies performed to date relied upon prior sequence or structural alignment to define which residues were equivalent in 3-dimensional space. Here we discuss an alternative methodology for comparative molecular dynamics that does not require any prior alignment information. We show it is possible to align proteins based solely on their dynamics and that we can use these dynamics-based alignments to quantify the dynamic similarity of proteins. Our method was tested on 10 representative members of the PDZ domain family. As a result of creating pairwise dynamics-based alignments of PDZ domains, we have found evolutionarily conserved patterns in their backbone dynamics. We compare the results to other recently developed methods.

1172-Plat
Structure Fluctuations in Proteins and their Relationship to Amino Acid Propensities
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The spectrum and scale of fluctuations in protein structures affect the range of cell phenomena, including stability of protein structures or their fragments, allosteric transitions and energy transfer. The study presents a statistical-thermodynamic analysis of relationship between the sequence composition and the distribution of residue fluctuations in protein-protein complexes [1]. A one-node-per-residue elastic network model accounting for the nonhomogeneous protein mass distribution and the inter-atomic interactions through the renormalized inter-residue potential is developed. Two factors, a protein mass distribution and a residue environment, were found to determine the scale of residue fluctuations. Surface residues undergo larger fluctuations than core residues, showing agreement with experimental observations. Ranking residues over the normalized scale of fluctuations yields a distinct classification of amino acids into three groups: (i) highly fluctuating - Gly, Ala, Ser, Pro and Asp, (ii) moderately fluctuating - Thr, Asn, Gln, Lys, Glu, Arg, Val and Cys (iii) weakly fluctuating - Ile, Leu, Met, Phe, Tyr, Trp and His. The structural instability in proteins possibly relates to the high content of the highly fluctuating residues and a deficiency of the weakly fluctuating residues in irregular secondary structure elements (loops), chameleon sequences and disordered proteins. Strong correlation between residue fluctuations and the sequence composition of protein loops supports this hypothesis. Comparing fluctuations of binding site residues (interface residues) with other surface residues shows that, on average, the interface is more rigid than the rest of the protein surface and Gly, Ala, Ser, Cys, Leu and Trp have a propensity to form more stable docking patches on the interface. The findings have broad implications for understanding mechanisms of protein association and stability of protein structures.
1. A.M. Ruvinsky and I.A. Vakser. arXiv:0907.5021v1

1173-Plat
Direct Observation of Ligand Dynamics in Cytochrome *C* Using Time-Resolved FTIR Spectroscopy
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Horse heart cytochrome *c* (cyt c) has emerged as a paradigm for the study of protein folding. The folding of reduced cyt *c* induced by photodissociation of CO from the CO-bound unfolded protein has been studied extensively. Following a nanosecond light pulse, four transitions have been resolved with time constants of approximately 1-5, 50-100, 200-500, and 1000-10,000 μs. While originally thought to be associated with CO rebinding to two different partially folded states of cyt *c*, the two slower process are now understood to reflect the bimolecular reassociation of CO followed by religation of the His18, which by the base elimination mechanism is induced to dissociate after CO photolysis. Thus, the two slower time constants turn out not to report on protein folding, but instead reflect the complexity of heme ligation. The two faster time constants have been attributed to ligation at the heme center by protein side chains. Here, to unambiguously determine the post-photodissociation steps involving CO, we monitored the CO vibration following photodissociation with step-scan FT IR spectroscopy. We find that like the slower timescale processes, the 50-100 μs timescale process is associated not with protein dynamics, but with CO ligand dynamics. The data clearly demonstrate that whatever the origins of the spectral changes, they clearly involve CO rebinding or changes in the environment of an already bound CO ligand. In addition to these fast dynamics, we also find multi-phasic CO rebinding on timescales of 1-100 s. The dependence of the associated amplitudes on denaturant concentration suggests that a unique species exists at intermediate denaturant concentrations, consistent with a folding-unfolding process of the protein driven by CO dissociation. This may