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

Ana Nicoleta Bondar, Stephen H. White.
University of California at Irvine, Irvine, CA, USA.

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

Lirong Chen, Lawrence J. Novicky, Mikhail Merzlyakov, **Kalina Hristova**.
Johns Hopkins University, Baltimore, MD, USA.

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

Thom Leiding[1], Alexei Polishchuk[2], William DeGrado[2], Cecilia Hägerhäll[1], Sergei Vinogradov[2], **Sindra Peterson Årsköld**[1].
[1]Center for Chemistry and Chemical Engineering, Lund, Sweden,
[2]University of Pennsylvania, Philadelphia, PA, USA.

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.

1167-Plat

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

James C. Gumbart[1], Christophe Chipot[2], Klaus Schulten[1].
[1]University of Illinois, Urbana-Champaign, Urbana, IL, USA,
[2]Nancy Université, Nancy, France.

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

Khalil R. Howard, Mohammad M. Mohammad, Liviu Movileanu.
Syracuse University, Syracuse, NY, USA.

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

Yann Gambin[1], Myriam Reffay[1], Emma Sierecki[2], François Homblé[3], Marc Genest[4], Robert S. Hodges[4], Nir Gov[5], **Nicolas Taulier**[6], Wladimir Urbach[7].
[1]Laboratoire de Physique Statistique de l'Ecole Normale Supérieure, Paris, France, [2]CNRS-Université Paris5, Paris, France, [3]Université Libre de Bruxelles, Bruxelles, Belgium, [4]University of Colorado, Denver, CO, USA, [5]The Weizmann Institute of Science, Rehovot, Israel, [6]CNRS - UMPC Paris,