volume (8M urea minus 0M urea) as a function of voltage, we observe an exponential decay as the electric field effects override the chemical effects until finally the presence of urea does not affect the translocation properties (Protein: PDZ2 Mutants). Nanopores are a unique and powerful tool since they can accurately produce transient denaturing conditions (using electric fields) while measuring the protein's response.

Platform: Membrane Protein Function

2183-Plat
Plasticity of the Asialoglycoprotein Receptor Deciphered by Ensemble FRET and Single-Molecule Counting PALM
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The composition of many multi-subunited receptor complexes at the plasma membrane is still debated as well as the impact of changes in the receptor subunit composition. Receptor plasticity, involving changes in stoichiometry and receptor function, has been difficult to address. Here, we introduce new spectroscopic tools to dissect receptor subunit assembly of the Asialoglycoprotein Receptor. Using this highly tractable receptor model system we show that the Asialoglycoprotein Receptor can assemble into distinct oligomers at the plasma membrane and that differential subunit assembly dictates receptor specificity. With ensemble analyses of Fluorescence Resonance Energy Transfer (FRET) and analytical modeling, we quantified receptor subunit homo- and hetero-oligomerization in the living cell. Furthermore, we established single-molecule counting using Photoactivated Localization Microscopy (PALM), visualizing an asymmetric receptor subunit assembly on the single-molecule level. Our results define a probability hierarchy of oligomerization driven by different molecular motifs that entails distinct co-existent receptor subunit assemblies. The Asialoglycoprotein Receptor is involved in the clearance of thrombogenic material. The variety of potential ligands and the propensity of the subunits to form distinct oligomers may explain previous inconsistent results and underscore the importance of deciphering oligomerization in the single cell and on the single-molecule level.

2184-Plat
Signaling in the Tumor Necrosis Factor Superfamily is Driven by Formation of Receptor Networks: Unraveling the Structural and Dynamic Paradigm
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Supramolecular clustering of receptors in the tumor necrosis superfamily, including tumor necrosis factor receptor 1 (TNFR1), Fas and Death Receptor 5 (DR5) has emerged as a potentially powerful paradigm shift in the field of apoptotic signaling. The canonical view of one ligand-one receptor is giving way to a revised picture of activity-dependent receptor aggregation. However, whether clusters have a specific structural organization stabilized by specific protein-protein contacts, as opposed to non-specific aggregation, remains unknown. Owing to the difficulty of studying endogenous membrane receptors in their native states, no detailed structural information has been available to begin to address this issue. Our studies provide the first such details of macromolecular organization in ligand-induced DR5 and TNFR1 aggregates, establishing a new paradigm for transmembrane signaling that likely extends to other structurally homologous members of this important superfamily. We base our conclusions on a rigorous, innovative and multidisciplinary approach that includes cell molecular biology in human cancer and model cell lines, biophysical measurements in cellular and synthetic model systems, and state-of-the-art computational molecular modeling. Thus, we address a fundamental and open question regarding the biophysical character of supramolecular clusters in cell biology, showing that clusters should more accurately be described as highly organized networks.

2185-Plat
Entropic Tension in Crowded Membranes
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Unlike their model membrane counterparts, biological membranes are richly decorated with a heterogeneous assembly of membrane proteins. These proteins are so tightly packed that their excluded area interactions can alter the free energy landscape controlling the conformational transitions suffered by such proteins. For membrane channels, this effect can completely change the critical driving force (such as membrane tension) needed to induce the transition from the closed to the open state, and therefore influence protein function in vivo. Despite their obvious importance, crowding phenomena in membranes are much less well studied than in the cytoplasm.
Using statistical mechanics results for hard disk liquids, we show that crowding induces an entropic tension in the membrane, which influences transitions that alter the projected area and circumference of a membrane protein. As a specific case study in this effect, we consider the impact of crowding on the gating properties of bacterial mechanosensitive membrane channels, which are thought to confer osmoprotection when these cells are subjected to osmotic shock. We find that crowding can alter the gating energies by more than ≈ 2 kB T in physiological conditions, a substantial fraction of the total gating energies in some cases.
Given the ubiquity of membrane crowding, the nonspecific nature of excluded volume interactions, and the fact that the function of many membrane proteins involve significant conformational changes, this specific case study highlights a general aspect in the function of membrane proteins.

2186-Plat
Lipid-Coupled Docking of Transmembrane Substrate by the GlpG Rhomboid Protease from Escherichia Coli
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Intramembrane proteases cleave transmembrane (TM) substrates within the plane of the lipid membrane. The GlpG rhomboid protease appears to dock its TM substrates by opening laterally towards the membrane a gate formed by helix 5. A crystal structure thought to represent GlpG in a closed state indicates a lipid headgroup bound to the catalytic site. Since the lipid must unbind for the substrate to dock, the questions arise as to how fast does lipid unbinding occur, and whether there is coupling between the incoming TM substrate and the lipid bound to the active site. To address these questions we performed systematic molecular dynamics (MD) simulations of GlpG in the absence of a TM substrate, and with TM substrate at various locations relative to GlpG. We find that, indeed, the presence of the substrate causes the lipid:active site interactions to weaken rapidly, in several tens of nanoseconds. That is, the incoming substrate prepares the enzyme for docking by inducing the displacement of the active-site lipid. The structure and dynamics of the substrate docking region of GlpG depend not only on whether or not a lipid molecule is bound to the active site, but also on the presence of the TM substrate. Our results on the lipid-coupled docking of the substrate to GlpG reveal an unsuspected complexity of the lipid interactions of intramembrane proteases.
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2187-Plat
Simulation Studies of Transport of Hydrophobic Compounds through Outer Membrane Proteins of Gram Negative Bacteria
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The outer membrane of Gram negative bacteria provides an effective barrier to diffusion of both hydrophilic and hydrophobic solutes. A number of recent X-ray studies have revealed the structures of outer membrane proteins, members of the FadL family, which are responsible for transport of hydrophobic solutes. These include: FadL from Escherichia coli (PDB id 1T16), TodX from Pseudomonas putida (PDB id 3BS0) and TbuX from Ralstonia pickettii (PDB id 3BRY). These proteins family has three significant structural features: 1) an N-terminal domain which blocks the pore otherwise formed by the transmembrane β-barrel; 2) a lateral opening formed by a kink in the β-barrel; and 3) a flexible L3 loop which seems to act as entry access point into the β-barrel. Based on this, transport models have been suggested in which lateral opening provides a pathway for exit of hydrophobic solutes from the intertior of the β-barrel into the surrounding lipopolysaccharide (LPS) membrane.
To examine the barrier posed by LPS for transport of hydrophobic compounds we performed molecular dynamics potential of mean force (PMF) calculations