Membrane-Protein Interactions

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We combine electrophysiology and small angle X-ray scattering to study interactions between biological membranes and membrane proteins.

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Membrane Proteins and Molecular Transport
(By a novice)

Membranes are ubiquitous.

Membranes compartmentalize the cell keeping the multitude of cellular machines separated into various organelles. Sugar, DNA, proteins, neurotransmitters and enzymes are efficiently stored and transported.

Amazingly, all biological membranes share the same basic structure of lipids and protein. Lipids are amphipathic molecules with a polar head that likes water and a hydrophobic tail that hates it. Two layers of lipids line up to form a 5nm thick bilayer. The 2-D layer is in a liquid state and membrane protein molecules float within the bilayer.

Without the membrane proteins nutrients, waste products, DNA, ions and other molecules could not traverse the many membranes in the cell. Macromolecular assemblies act as carriers, symporters, vesicles, fusion peptides and ion channels to transport all these chemicals across the bilayer.

Understanding how they work should have a tremendous impact on medicine, biology and even new soft-matter technology.
Ion Channels

When an ion enters solution its charge is shielded by dipole-dipole interaction. Max Born approximated the energy as,

$$U = \left( \frac{q^2}{8\pi\varepsilon_o} \times \frac{1}{\varepsilon_{solvent}r_{ion}} \right)$$

The membrane ($\varepsilon=2$) cannot screen the ion as well as water ($\varepsilon=80$) so most ions cannot penetrate the bilayer.

An ion channel is a protein-lipid-water macromolecular assembly that forms a water channel (high dielectric channel) through the bilayer.

Ion channels are amazing.

Different channels selectively filter Na$^+$, K$^+$, Ca$^{++}$ or Cl$^-$ at up to 100 million ions per second.

Channels open and close in response to voltage, chemical signals, pressure or other stimuli.

Channels are pivotal to single cellular functioning and signaling between cells.

Many diseases such as Cystic Fibrosis are the result of channel malfunctioning.
Single Molecule Kinetics using Patch-Clamping

Without ion channels the conductivity of a membrane is quite low. When a channel opens the conductivity increases by a quantized amount (typically 1-10pS).

Montal and Mueller invented a technique to deposit a planar lipid bilayer across a small hole in a teflon septum as shown in Figure Four.

If only a small number of channels are included in the bilayer on average only one will be open at any time. This permits measurement of the current through a single molecular assembly.

Figure Four: Planar Lipid Bilayer (p480, Alberts et al.)

Figure Five shows ions flowing through a single Alamethicin ion channel (as recorded by Sarah Keller) as it switches between different conductance states.

Neher and Sakmann invented the patch-clamping technique to record single ion channels in vivo. This technique uses a glass pipette to isolate the conductivity of a tiny (square micron) sized membrane patch.

Figure Five: Single Channel Conductance Record (p9, Sarah Keller’s Thesis)

Using the patch-clamping technique on planar lipid bilayers allows even finer resolution of channel conductance.
Studying Membranes with X-ray Scattering

Lipids are quite peculiar because they self-assemble into ordered structures. Merely by adding water they will form regular structures like the lamellar and hexagonal liquid crystals as sketched in Figure Six.

Liquid crystals are soft because of the delicate balance of attractive London dispersion forces and repulsive DLVO and hydration forces.

The interactions between proteins and lipids are the same interactions that control liquid crystal phase behavior.

Membrane liquid crystals are usually around 5nm in size so Small Angle X-ray Scattering is an excellent structural probe.

A single X-ray image allows the crystalline phase, lattice size, electron distribution, water content and other structural parameters to be determined. By varying sample temperature, hydration and composition the interplay of different energies can be determined.

These studies are complementary to optical, NMR, calorimetric and mechanical probes of lipid behavior.
The Project

Gramicidin is an antibiotic ion channel produced by Bacillus brevis. The monomers naturally form helical screws with water in the middle of helix. To form a channel, two monomers (shown in Red and Blue in Figure Eight) join together to form a water core through the lipid bilayer. When open, hundreds of millions of ions per second can flow through the channel.

Many antibiotic peptides (melittin, alamethicin, etc.) work in a similar fashion, although gramicidin has a unique structure.

We wish to study the response of gramicidin to different types of bilayer.

One distinct characteristic of the gramicidin channel is the channel open lifetime \(\tau\), the mean dissociation time for the dimer to split into two monomers.

The channel lifetime should depending on the membrane thickness (d), packing constraints (spontaneous curvature, r) and other properties of the membrane.

Figure Nine shows the results of some previous studies of \(\tau\) as a function of membrane thickness.

We hope to isolate the contributions of packing constraints from membrane thickness for the gramicidin channel. By comparing these results with simulations of ion channel packing we hope to gain a better idea of the interplay between the bilayer and membrane proteins.
References


Figure Ten : View Through the Gramicidin Ion Channel
(Generated using SwissPDB)