Biology 5357: Chemistry & Physics of Biomolecules - Membranes & Membrane Proteins

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Lecture 3: Membrane dynamics

Required reading:

Phillips, R. (2018). **Physics of Biological Membranes** https://dx.doi.org/10.1007/978-3-030-00630-3_3

Membrane reconstitution. Lipids are solubilized in chloroform and dried down under N2 gas. Oxygen is avoided as the lipids containing double bonds can become oxidized, destabilizing the lipid bilayer. The dried lipid film is then resuspended in aqueous buffer by sonication which leads to the spontaneous formation of small unilamellar vesicles. For membrane protein reconstitution, the lipids are solubilized in a detergent with a high CMC, e.g. CHAPS and then combined with the protein, also in detergent. The detergent is removed by dialysis or bio-beads leading to the incorporation of the lipid bilayer with the membrane protein embedded within. Note, this typically leads to random incorporation of the protein orientation within the membrane, unless the protein is asymmetric. Another method of protein incorporation is by the addition of the protein in detergent to the liposomes with a small amount of additional detergent. This can allow for the incorporation of the protein directly into the membrane but is not always successful, i.e. can increase the aggregated fraction outside of the membrane.

Altering liposome area. When studying membranes, it is often important to control the form of the structure of the membrane being studied. While vesicles are stable and do not spontaneously exchange with other vesicles, there are ways of altering the sizes of these membranes. One can fuse membranes together by multiple freeze-thaw cycles of the sample, which dehydrates and destabilizes the membrane to allow breakage and fusion into larger areas leading to multi-lamellar vesicles (MLVs) that are quite large - diameters > several microns.

Giant unilamellar membranes can be formed by osmotic swelling or electroformation in which the samples are loaded onto a platinum wire, partially dehydrated and then rehydrated under an electric field which leads to GUVs with diameters that are > microns.

To make liposomes smaller, we can take MLVs or GUVs and extrude them through nucleopore membrane filters with defined pore sizes. This leads to a reproducible distribution

of smaller liposomes, typically mixtures of unilamellar and multilamellar depending on the pore size. The smaller the pores, the more uniform the size distributions and the population is more unilamellar.

Measuring the size of liposomes. To measure the size of liposomes, this can be done by cryo-electron microscopy and direct measurements of the liposome radii from which yields the histogram of liposome sizes. In addition, the population can be estimated by dynamic light scattering, which measures the auto-correlation function of scattered laser light to define the size distribution. Note, that experimentally, the size distributions can be very different than the pore size, for instance extrusion of a E. coli polar lipid membranes through a 400 nm filter yields a population that is peaked at 30 nm radii.

Supported membranes. For many studies, it is useful to work with a planar bilayer reconstitution. This can be useful for electrophysiological studies or microscopy studies. It is possible to form membranes by fusion of vesicles to surfaces.

- Black lipid membrane, formation of a lipid bilayer on a hole in the presence of some solvent like decane. Useful for single-channel electrophysiology.
- Supported lipid bilayer, spontaneous fusion of a bilayer onto a surface like a glass slide. Can be directly on the surface, with a 10 Å hydration layer, or extended further with cushioning such as with agarose or polymers.

Scaffolded membranes. For structural studies, scaffolded membranes allow for the membrane protein to be purified in a confined bilayer environment. These include nanodiscs involving apolipoproteins, saponins or styrene maleic acid copolymers (SMAs). While these provide a confined membrane area, conformational changes are often observed in these small membrane patches along with changes in membrane structure.

The fluid phases of the membrane. The fluidity of the membrane, and the nature of the lipid molecules to behave as freely diffusing solvent molecules, depends on the phase. As mentioned previously, there are several solid states of the membrane that can be adopted, which represent the various gel phases.

The liquid crystalline phase ($L\alpha$) is the fluid phase of the membrane that reflects the biological phase of the membrane. Within this phase, there can be sub-phases.

- liquid ordered lo - which is sometimes referred to as rafts enriched with saturated lipids, sphingolipids and cholesterol
- liquid disordered - mono and poly unsaturated lipids.

Phase transition temperatures of lipid bilayers. Depending on the bilayer, you may have conversion between these phases as a function of temperature. This can be measured by differential scanning calorimetry, which measures the heat capacity as a function of

temperature. In a pure lipid bilayer system, you can often measure the gel to ripple pre-transition and then the melting transition to the liquid crystalline phase. This main bilayer phase transition represents a cooperative rotameric disordering of the hydrocarbon chains.

For pure glycerophospholipid membranes with commonly used lipids in laboratory experiments, the phase transition temperatures can range from negative -60C to 80C, with the latter representing a gel phase membrane at most biological temperatures. The phase transition temperature depends on features of the lipid monomers and their ability to make interactions with the other lipids in the leaflet and bilayer.

- VDW interactions - tail length and saturation
- Head-group interactions - with each other and with water

Note the trends that are observed for saturated diacyl phospholipids. Independent of head-group, as you increase the number of carbons in the acyl chains, the transition temperature increases, meaning that that you need to add more heat to observe the gel to liquid transition. For example, Tm of DLPC (C12:0) = -2C vs. Tm of DSPC (C18:0) is 55C. This is because of the increase in VDW interactions between neighboring acyl chains leading to ordering of the lipids, stabilizing the gel state.

Introducing any amount of unsaturated bonds reduces the transition temperature, for example Tm of DSPC (C18:0) is 55C vs. Tm of DOPC (C18:1) is -17C.

Headgroups have individual dependencies, as is observed by the curves being translated up and down the temperature axis. For example, PE is a lipid that has considerably higher phase transition temperatures compared to PC lipids. For example, the Tm of DSPC (C18:0) is 55C vs. the Tm of DSPE (C18:0) is 70C. This means that it requires more heat to observe the transition from gel to liquid crystalline phase. The main reason why this occurs with PE is again because of the headgroup size. Because it is smaller it interacts with less waters and this leads to a stabilization of packing in the gel state. Note the similar relationship with PA lipids.

In biological membranes, the composition is very different from a pure phospholipid, and so we cannot expect that the transitions are similar. In reality, the phase transition profiles become much broader as soon as you introduce different mixtures of lipids. This can also depend on sub-domains, and environmental conditions such as changes in ionic conditions or pH.

Lipid dynamics. In liquid crystalline membranes, the lipids in the membrane plane are capable of different motions or dynamics. This includes:

- flip-flop between leaflets (milliseconds to seconds, even longer)
- undulatory motions of the bilayer (milliseconds to seconds)

- lateral diffusion (microseconds)
- rotation about the lipid axis (nanoseconds)
- rotation around chemical bonds (picoseconds)
- trans/gauche isomerizations (picoseconds)

Intramolecular dynamics. The dynamics of acyl chain configurations can be measured by deuterium NMR experiments as well as ESR spectroscopy using labelled lipids. With both approaches, the average orientation of the nth CH2 segment along the chain and is expressed as a classical liquid crystal order parameter

$$S_n = \frac{1}{2}(3\langle cos^2\theta\rangle - 1)$$

Theta is the angle of the CD bond relative to the membrane normal. With NMR, this can be obtained from deuterium quadraople splitting measurements.

These experiments are particularly useful for identifying whether there are different environments that the lipids sense while in the membrane. Around membrane proteins, there are 3 different environments for lipids:

- annular lipids, representing the first solvation shell around a protein, approximately 6-10 Å distance from the protein
- non-annular lipids, those lipids that are within the first solvation shell or closer, effectively binding to the protein
- bulk lipids

In addition, within the membrane, there are different phases that the lipids may form within the liquid crystalline phase:

- lo - liquid ordered, e.g. rafts, promoted by lipid-cholesterol interactions
- ld - free lipids

Different lipid mixtures are prone to form different phases depending on the environmental conditions. For example, DOPC/DPPC/cholesterol exhibits changes in the lo/ld proportions as a function of temperature. This can measured directly by deuterium NMR and quadropole splitting to map out the full phase diagram. Thus phase separation is a consequence of the lipids within the bilayer and their independent properties.

Since phase changes also accompany changes in bilayer thickness, they can also be measured in high resolution by atomic force microscopy.

Fluorescence methods of measuring phase/domain separation in membranes. However, it is common to measure changes in phase by fluorescence microscopy. Over the years, different fluorescent lipid probes have been developed that either involve modifying the head group or

lipid tails, and they will preferentially partition into ordered or disordered phases of the membrane. In addition, fluorescent proteins or molecules have been developed that target the different phases, such as cholera toxin or RAS domains.

Lateral diffusion. The diffusion of species in the membrane can be measured by fluorescence microscopy in several ways.

- Fluorescence recovery after photobleaching. This first method (developed by Dr. Elson and others) followed the experiment of Frye and Edidin and further quantified the fluid behavior of lipid bilayers. Here, an area of the membrane containing fluorescent lipids, is bleached, and the recovery of the fluorescence is quantified.
- Fluorescence correlation spectroscopy. In the second method (also developed by Dr. Elson), an autocorrelation function is calculated from the fluorescent fluctuations of diffusing particles to yield information about the diffusion coefficient.
- Single-particle tracking. Total internal reflection fluorescence microscopes can be designed to image single molecules within the lipid bilayer membrane. With this, the movement of single molecules within the membrane can be tracked and their diffusion coefficient measured by the mean square displacement. In general, the diffusion of lipids is 10^-8 cm2/s while membrane proteins are 10^-9 to 10^-10 cm2/s. In actual cell membranes, there is confined diffusion of membrane proteins.

Lipid flip-flop (transverse dynamics). Lipids can also undergo motions between the two leaflets but this is rare and far slower than the dynamics within the leaflet. Proteins such as flippases, floppases will maintain lipid gradients to overcome this.