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

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Lecture 3: Membrane mechanics

Required reading:

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

Self-assembly of amphiphiles. Non-polar molecules will be driven to associate with one another in water due to the hydrophobic effect. This is because water forms a network of hydrogen bonds with itself, with the number hydrogen bonds described in a simplified tetrahedral network, with 6 possible configurations of the central water molecule making a hydrogen bond with a neighbor. Changing one of these water molecules to a non-polar molecule that does not form hydrogen bonds reduces the number of states to 3, just a single face of the triangle. Thus, a methylene group adds a 0.8 kcal/mole penalty due to the hydrophobic effect, which drives association of non-polar molecules to reduce the water accessible surface area.

Therefore, for amphipathic molecules in water, there is an equilibrium of different self-assembled phases. For example, consider a beaker with mixed amphiphiles in it, there would be a population of dissociated monomers, and a variety of self-assembled structures that hide the non-polar chains away from the water. These include a monolayer at the water air interface, as was the case of the oil layer in Benjamin Franklin's olive oil experiment, and assembled phases such as micelles or bilayers. There are many variants of these phases:

(I) Lamellar phases:
- Subgel
- Gel
- Interdigitated Gel
- Gel, tilted chains
- Rippled gel
- Liquid crystalline

(II) Micellar aggregates:
- Spherical
- Cylindrical
- Disks

- Inverted micelles
- Liposomes

(III) Non-lamellar liquid crystalline phases.
- Hexagonal
- Inverted Hexagonal
- Inverted Micellar Cubic Phase
- Bilayer Cubic Phases

For biological membranes, we will focus on the liquid crystalline phase.

Phase depends on amphiphile shape. Whether an amphiphile makes a micelle or bilayer depends on the critical packing parameter (CPP).

$$CPP = \frac{v}{a_0 l_c}$$

An amphiphile generally adopts an average conical/cylinder molecular structure where v is the volume, a_0 is the area and l_c is the length.

- CPP < 1/3, cone - spherical micelles
- CPP = 1/3 - 1/2, truncated cone - cylindrical micelles
- CPP = 1/2 - 1, truncated cone - flexible bilayers, vesicles
- CPP ~ 1, cylinder - planar bilayers
- CPP > 1, inverted truncated cone or wedge - inverted micelles, hexagonal phase

Detergents. Let's first consider detergent molecules that self-assemble into micelles. Detergents, or surfactants, are amphipathic molecules typically with a single acyl chains or short chains resulting in conical monomer shapes. They are not really found in biological membranes, except for the lysolipids. However, they are extremely useful for the purification of membrane proteins from membranes, enabling extraction of these proteins from their native lipid bilayer phase, and solubilization for protein biochemistry. So, for anyone who works with purifying membrane proteins for structural or mechanistic studies, knowledge of detergents are essential.

The critical micellar concentration (CMC) is the concentration at which micelles begin to form. It represents an aggregation threshold. Above the CMC, the concentration of free monomers remains constant, and the monolayer is saturated so the surface tension of the monolayer is constant. Any addition of detergent molecules leads to an increase in the number of micelles. The CMC can be determined by measuring surface tension of the monolayer in a Langmuir trough, or turbidity/absorbance measurements.

Micelles can have different shapes: spherical, prolate or oblate. These factors depend on the detergent monomers and the aggregation number (N_{agg}). In addition, the following factors affect the CMC

- **Structure of hydrophobic group:** increase in carbon chain increases micellar size, decreases CMC
- **Increase in hydrophilic head group:** increases hydrophilicity and increases CMC
- **Addition of electrolytes** for ionic surfactants decrease CMC and increase micellar size due to a reduction in head group repulsion
- **Temperature**: mainly affects nonionic surfactants, increases in temperature up to the cloud point increase micellar size and decrease CMC
- **Concentration**: aggregation number in micelle can change above CMC making non-spherical micelle structures

It is critical when working with detergents in membrane protein studies to check the CMC and work at concentrations that are at least 2-fold greater. Otherwise, the protein will be insoluble.

Bicelles. Mixing detergent and lipids leads disc-like bicelle structures which have a planar bilayer region and spherical rims. They are hybrids between micelles and bilayers and are often used for solution studies of membrane proteins. Bicelles are often made out of mixtures of DMPC, DHPC and CHAPSO and varying the ratio (q) leads to different assemblies.

Liposomes and bilayers. Assembly structures of phospholipids follow the same principles of micelle formation, except the CMC values are much lower (nM compared to mM). The exchange between free monomers in solution and the lipid bilayer is slow. The lipid bilayer structures, once formed are stable and to do not fuse with one another spontaneously.

There are several key structural properties:

A_L - surface area of lipid
D - primary lamellar repeat spacing, present in multi-lamellar structures
D_b - bilayer thickness
D_h - hydrophobic thickness
θ - tilt angle of the hydrophobic tails

These properties will be defined by the free energy minimum of the lipid molecules in the membrane, a combination of the inter-lipid interactions and configurational entropy.

Small angle scattering to measure bilayer structure. The structural features of lipid bilayers can be measured by small angle scattering approaches. X-ray scattering (SAXS) is an elastic process, where the x-rays are scattered by the electrons of the atomic shell. The electrons start oscillating, becoming a dipole and sending a spherical wave out, where the wavelength and energy do not change. It is done in solution, in a wide range of buffers and temperature. Neutron scattering (SANS) is similar to x-ray scattering, however neutrons interact through nuclear interactions, as opposed to electrostatic interactions. Neutrons have high penetration, and are point particles. Neutron detection is through nuclear reactions, rather than ionization and so the detection signal to noise is high.

Lipid bilayer thickness and lipid area. SAXS and SANS shows us how the bilayer structure changes when the lipid composition changes. Increasing the acyl chain length for PC, PG and PE shows that 1.9 Å in membrane thickness is added for every carbon in the acyl chain, for saturated chains.

Lipid area follows a more complex relationship with acyl chain composition.

- saturated chains - as the chain length increases the lipid area decreases. Possibly counterintuitive considering the chain as a free polymer, but it is not free it is in a bilayer phase forming many VDW interactions with neighboring chains. Therefore, as the chain length increases, the number of neighboring interactions is maximized as the chains become elongated, thereby ordering the lipids and decreasing the lipid area.
    - DLPC - C12:0, DMPC - C14:0, DPPC - C16:0, DSPC - C18:0
- mixed chains - as the chain length increases, the lipid area increases. One of the chains has a fixed cis double bond which limits the neighboring VDW interactions and the chain acts like a free polymer.
    - POPC - C16:0,18:1, SOPC - C18:0,18:1
- mono-unsaturated chains - single double bond in both chains. These show two phases with respect to lipid area. This depends on the position of the double bond, that it is omega-9 in all cases. Thus, as the chain length increases, the length of the saturation increases so that past 20 carbons, there are sufficient inter-lipid interactions to order and elongate the chains above the double bond.
    - DMoPC - C14:1, DPoPC - C16:1, DOPC - C18:1, DEiPC - C20:1, DErPC - C22:1, DNPC - C24:1
- mixed chains

The hydrophobic thickness of the lipid bilayer exhibits a seemingly counterintuitive relationship with cholesterol. Cholesterol is a shorter molecule in the membrane, compared to typical extended biological phospholipids. However, when added to membranes, they have an extending effect, increasing the membrane thickness as the planar hydrophobic structure promotes inter-lipid packing, promoting the extension of the acyl chains and increase in bilayer thickness.

Membrane curvature. Different phospholipids have different critical packing parameters, defined by the head-group area vs. chain area. This results in different spontaneous membrane curvatures. (Note - the shape nomenclature for lipids is flipped compared to the previous slide for detergents).

- Cylindrical: planar, e.g. PC and PS
- Conical: negative curvature, e.g. PE and PA ... tiny headgroups
- Inverted conical: positive curvature, e.g. lyso-GPLs and phosphoinositides ... large headgroups

Lateral pressure. The same types of lipids that induce negative and positive curvatures induce differences in the later pressure within the bilayer plane. For example, PE is a non-bilayer type lipid that has a higher lateral pressure in the acyl chain region due to the expansion of the chains relative to the headgroup packing. In addition, other small molecules that partition into the membrane can impart changes in lateral pressure profiles. This can have influences on protein packing.

Material properties of membranes. Even though the lipids are considered a fluid, or liquid crystalline phase within the 2D plane of the lipid bilayer, in 3D, the bilayer behaves like a macroscopic material. It can be perturbed and deformed, all with an energetic cost that can in turn affect the proteins that reside within. To understand this, the membrane is modeled as a continuum elastic material, much like a set of springs. There is a free energy minimum equilibrium structure and deviations from this minimum lead to an energetic penalty.

- ***Membrane bending.*** The free energy cost to deform a patch of membrane is described by the Helfrich-Canham-Evans free energy. The bending modulus is in units of energy, and in the range of 10-25 kT. Sterols in the membrane can increase the bending modulus up to 100 kT.

- ***Membrane stretch.*** This is the cost to change the area of the membrane from its equilibrium area. The stretch modulus is in units of energy per area, and is nearly constant over chain length and saturation.

- ***Membrane compression***, i.e. thickness changes. The cost to change the thickness of the membrane with respect to the equilibrium thickness. It is defined by a stiffness modulus which is in units of energy per area. The energetics of the membrane compression/expansion has been shown to be linked to protein conformational states by hydrophobic matching.

Experimental measurements of membrane properties.

- SANS/SAXS
- micropipette aspiration to measure membrane tension and bending rigidity
- optical trapping for bending rigidity
- atomic force microscopy - bending rigidity, membrane tension, area compressibility modulus