Lecture 3 - Membrane Structure & Mechanics

Janice L. Robertson

November 20, 2023

1 Reference reading

Phillips, R. (2018). Physics of Biological Membranes

2 Self-assembly of amphiphiles

2.1 The hydrophobic effect as a major driving force for amphiphile self-assembly

Non-polar molecules are driven to self-assemble away from water due to the hydrophobic effect. At typical biological temperatures, we can estimate the change in entropy that occurs when mixing a non-polar compound with water. Water forms a network of hydrogen bonds with itself, which can be simplified into a tetrahedral network with 6 possible configurations of the central water molecule for hydrogen bonding with four neighbors. However, if one of these water molecules is converted to a non-polar compound that does not form hydrogen bonds (e.g. imagine a non-polar sphere, like Xenon), then this reduces the number of configurations to 3, just a single face of the pyramid. This loss of entropy can be calculated and amounts to 0.42 kcal/mole loss of entropy for the water position that has been removed. Addition of a methylene group adds 0.8 kcal/mole to this hydrophobic effect, and increases with extension of the carbon chains. Thus, this drives association of non-polar molecules together to reduce the water accessible surface area.

2.2 Intermolecular interactions dictate assembly structures

Beyond the hydrophobic effect, there are also intermolecular interactions that impact the stability of the self-assembled structure. For example, there are van der Waals interactions between all atoms (acyl chains and headgroups). In the headgroup region, there are also hydrogen bonds and coulombic electrostatic interactions. At the same time, these long polymer chains are maximizing their configurational entropy in this state, and so the chain adopt an ensemble of states. The balance of the overall free energy governed by

intermolecular interactions and chain entropy dictates the type of self-assembled structure that is most stable.

2.3 amphiphiles exist in equilibrium of free monomers and several assembled states

Therefore, for amphipathic molecules in water, there is an equilibrium of different self-assembled phases. Consider a solution of a single type of amphiphile in a beaker. In this environment, there is a population of free amphiphile monomers, and several self-assembled structures including a monolayer at the water air interface, micelles (single-layer or spherical amphiphile structures) and bilayers. Depending on the total amphiphile concentration, all states can exist in the solution simultaneously and are in equilibrium with one another.

2.4 The critical micelle concentration (CMC)

This defines the aggregation threshold and concentration where micelles form. Above the CMC, the concentration of free monomers remains constant, and the monolayer is saturated so the surface tension of the monolayer is constant. 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.

The CMC describes the stability, i.e. free energy of micelle formation:

$DeltaG = -RTln(CMC)$

Depending on the amphiphiles, micelles may assemble into 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 the CMC resulting in non-spherical micelle structures

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

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

which describes a conical/cylindrical molecular structure where v is the volume, a_0 is the area and l_c is the length.

- CPP lt 1/3, cone - spherical micelles
- CPP = 1/3 - 1/2, truncated cone - cylindrical micelles
- CPP = 1/2 - 1, truncated cone - flexible bilayers, vesicles
- CPP approx. 1, cylinder - planar bilayers
- CPP gt 1, inverted truncated cone or wedge - inverted micelles, hexagonal phase

2.5 Sub-classification of surfactant/amphiphile phases

2.5.1 Lamellar phases

- Subgel
- Gel
- Interdigitated Gel
- Gel, tilted chains
- Rippled gel
- Liquid crystalline

2.5.2 Micellar aggregates

- Spherical
- Cylindrical
- Disks
- Inverted micelles
- Liposomes

2.5.3 Non-lamellar liquid crystalline phases

- Hexagonal
- Inverted Hexagonal
- Inverted Micellar Cubic Phase
- Bilayer Cubic Phases

3 Detergents

Detergents or surfactants, are amphipathic molecules that typically contain a single acyl chain or short chains resulting in conical monomer shapes. They are found in biological membranes as lysolipids, but these are rare. 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 concentrating amounts to conduct protein biochemistry analyses. So, for anyone who works with purifying membrane proteins for structural or mechanistic studies, knowledge of detergents is essential. It is important to consider 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.

4 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 (C14:0/14:0), DHPC(C7:0/7:0) and CHAPSO and varying the ratio (q) leads to different structural assemblies.

5 Lipid bilayers and liposomes

For biological membranes, the prevalent structure is the liquid crystalline phase. 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
- theta - tilt angle of the hydrophobic tails

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

5.1 Experimental measurements of 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.

5.2 Lipid bilayer thickness

SAXS and SANS measurements 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.

Cholesterol is a shorter molecule that the extended biological phospholipids. However, when added to membranes, they increase the membrane thickness as the planar hydrophobic structure promotes inter-lipid packing, leading to the extension of acyl chains and increase in bilayer thickness.

5.3 Area per lipid

Lipid area follows a more complex relationship with acyl chain composition, as demonstrated by the SANS measurements.

- *Chain length, fully saturated.* As the chain length increases (DLPC - C12:0, DMPC - C14:0, DPPC - C16:0, DSPC - C18:0), area per lipid decreases. This is because neighboring VDW interactions increase, shifting the distributions towards elongated configurations.

- *Chain length, mixed saturation* As the chain length increases (POPC - C16:0,18:1, SOPC - C18:0,18:1), lipid area increases. This is because one of the chains has a fixed cis double bond which limits the neighboring VDW interactions and the chain can adopt more contracted configurations.
- *Chain length, mono-unsaturated chains* Single double bond in both chains (DMoPC - C14:1, DPoPC - C16:1, DOPC - C18:1, DEiPC - C20:1, DErPC - C22:1, DNPC - C24:1). These show two trends 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 interlipid interactions to order and elongate the chains above the double bond.

5.4 Membrane curvature

Different phospholipids have different critical packing parameters, defined by the headgroup vs. chain area. This results in different spontaneous curvatures of the membrane.

- 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

5.5 Lateral pressure

The same types of lipids that induce negative and positive curvatures induce differences in the lateral 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 inverted cone shape, with larger chain volumes relative to the headgroup packing. In addition, other small molecules that partition into the membrane can impart changes in lateral pressure profiles, which can have influences on protein packing.

5.6 Membrane mechanics

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 to the system. These properties are often described using continuum mathematical models that represent the lipid bilayer as a series of springs, or mattress. Important to note, the change in mechanical state imposes a constraint on the lipid bilayer, and as a result, the probability distributions of lipid configurations is impacted. This is another way of impacting lipid solvent properties and protein reactions.

5.6.1 Membrane bending

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. 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. On a molecular level, bending amounts to changes in lipid-lipid configurations (e.g., staggering or tilting).

5.6.2 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. On a molecular level, it relates to increases in area per lipid.

5.6.3 Membrane compression

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. On a molecular level, it corresponds to a change in the headgroup/headgroup distance across the leaflets, which could arise due to lipid tilting or change in end-to-end distance in the acyl chains.

5.6.4 Methods for measuring changes in membrane mechanics

- fluorescence microscopy
- optical tweezers/optical trapping
- microaspiration
- atomic force microscopy