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

Lecturer: Prof. Janice L. Robertson, Dept. of Biochemistry & Molecular Biophysics
Office: MCD 223A (in Lab 225)
Email: janice.robertson@wustl.edu

Lecture 2: The chemical composition of cell membranes.

Required reading:

Meer, G., Voelker, D., Feigenson, G. (2008). **Membrane lipids: where they are and how they behave.** Nature reviews. Molecular cell biology 9(2), 112-24.
https://dx.doi.org/10.1038/nrm2330

Harayama, T., Riezman, H. (2018). **Understanding the diversity of membrane lipid composition.** Nature Reviews Molecular Cell Biology 19(5), 281-296.
https://dx.doi.org/10.1038/nrm.2017.138

Cellular membranes are made up of lipid bilayers. All cellular membranes throughout biology are made up of lipid bilayers with ≈ 30 Å hydrophobic thickness. There are a few exceptions to this rule, but this is generally the case. The headgroups are polar and charged and add an additional ≈ 10 Å to the total membrane thickness. Thus, the lipid bilayer structure arises due to the fact that the molecules that comprise it have an amphipathic structure - polar head groups and non-polar chains. This results in two leaflets that are driven to come together by the hydrophobic effect, a shielding of the non-polar acyl chains from water. Note, because the lipid bilayers are stabilized by the hydrophobic effect, rather than interleaflet interactions, allows the lipids within the lipid bilayer to behave as a fluid.

Note that this yields a unique type of environment for the biomolecules and proteins that are embedded within the membrane. Macroscopically, the lipid bilayer membrane possesses its own stability and behaves like an elastic material when distorted in 3D, perpendicular to the membrane plane. However, within the 2D plane of the membrane, the individual lipid molecules act like a fluid or solvent, under typical biological conditions. With this, the proteins that are embedded within the membrane are dependent on both molecular and macroscopic driving forces that come from the membrane itself. Consider that ≈ 25% of protein encoding genes are for membrane proteins. This means that this significant fraction of biologically essential proteins are in a completely foreign solvent environment compared to what we have typically learned about in our past studies of protein biophysics and biochemistry.

Chemical diversity in the biological membranes. Since the drive to assemble the lipid bilayer comes mainly from the amphipathic structure of the lipids and the surrounding water, as opposed to specific intermolecular interactions, this means that biology can obtain a lipid bilayer structure with many different types of lipid molecules. The implication of this is that there is an enormous difference in the solvent space for proteins that reside in the membrane compared to proteins that reside in intracellular or extracellular spaces. As far as we know of

terrestrial biology, there is only one type of solvent for these reactions - water. We also know that water dictates a large component of the stability of proteins and their reactions. However, in cellular membranes, it turns out that there are infinite possibilities of the chemical composition of solvent. Here are a just three examples of the major lipid components in the inner membrane of *E. coli*, brain, and the archaea *S. sulfolubus*. This last example highlights one of the exceptions to the lipid bilayer. *S. sulfolubus* is an extremophile that thrives at acidic pH (pH 2-3) and high temperatures (75-80 °C). Keep in mind that there are similar protein homologues in each of these organisms and these conditions are typically denaturing for proteins, even membrane proteins. As a result, it appears as though the membrane composition has evolved to include a majority of tetraether type lipids, which are single molecules that mimic two phospholipids attached at the ends of their tails. With this, they introduce a covalent attachment between the two leaflets, and thus can yield a mimic of a lipid bilayer type of structure while actually being one single layer. This is a way in which the membrane can maintain stability while remaining fluid.

In reality, each cell may have 100s-1000s of different types of lipid molecules and these compositions differ between the different membrane compartments. This slide shows an overview of the different types of biological lipid molecules.

There are 8 categories of unique lipid structures in biological membranes. The nomenclature here is designated by the LIPID MAPS Lipidomics Gateway (http://www.lipidmaps.org), a database for lipid profiling data:

1. Fatty Acyls - eicosanoids derived from arachidonic acid, e.g. prostaglandins, leukotrienes
2. Glycerolipids - e.g. triglycerides
3. Glycerophospholipids - i.e. phospholipids. These are the major components of membranes.
4. Sphingolipids - e.g. sphingomyelins, gangliosides
5. Sterol lipids - e.g. cholesterol, steroids
6. Prenol lipids - e.g. carotenoids, retinol
7. Saccharolipids - lipid A, an important component of prokaryotic outer membranes and a factor in the development of antibiotic resistance
8. Polyketides - secondary metabolites and antibiotics

Glycerophospholipids. In general, glycerophospholipids are one of the most prevalent types of lipids in membranes. They are composed of (from right to left on figure):

- an alcohol (green)
- a phosphoric acid unit (green)
- a glycerol (red)
- 2 fatty acids (blue)

These are divided amongst three main sections: the head group, glycerol backbone and acyl chains. This structure leads to an amphipathic molecule in which approximately half of the molecular volume is non-polar, and the other half is polar.

Fatty acid-glycerol linkages. Typically, the fatty acids are esterified to the glycerol at carbon position C-1 and C-2. The glycerol backbone is chiral and this reflects the sn-1 and sn-2 positions respectively. However, there are other variants of linkage chemistry. It is possible to have ether and vinyl-ether linkages of lipids. The ether linked lipids are commonly referred to as plasmologens and they are prevalent in archaeal membranes, however they are also found in bacteria and eukaryotes, though less prevalent than the ester linked lipids. Note, these changes in chemistry can have structural and physical implications. Ether linked lipids have a greater tendency to form non-lamellar phases and are hypothesized to be important for processes that require disruption of membranes, such as fusion.

Headgroup. The head-group substituent on a glycerophospholipid can range from a hydrogen in the case of phosphatidic acid, to larger groups like inositol.

In the case of cardiolipin, or diphosphatidylglycerol, the substituent is a phosphatidyl-glycerol, connecting two lipids together by the head group to yield a larger lipid with two phosphates, and four acyl chains.

All head groups are ionizable. They are either overall charged or zwitterionic depending on the pKa and pH or other ionic conditions. There is a negative charge on the phosphate group, and either positive, neutral or negative charges on the substituent. Under typical biological conditions at pH 7.0: PA(-1), PS (-1), PG (-1), cardiolipin(-2) & PIPs are negative (PIP (-2), PIP_2 (-3), PIP_3 (-4)); PC & PE are neutral.

The overall charge is dependent on the environment and the individual pKas of the ionizable groups. Here is a plot of phosphatidylserine, with the ionization curves for the phosphoric acid group, and the carboxyl and amino groups on the serine substituent. Thus, under a physiological range of pH, we can see that this lipid should have a net charge of (-1) + (-1) + (+1) = -1, but we can also see how this may change if the pKas of any of these chemical groups becomes changed, or the pH of the reaction environment is altered.

Fatty acyl chain modifications. There is a lot of chemical diversity in the fatty acyl chains. $C_{X:Y}$ designates the carbon chain length (X) and the number of double bonds in that chain (Y). A lysophospholipid is one in which one of the acyl chains has been removed.

In terms of acyl chain length, these are generally between 12-22 carbons, with 16-18 being the most common chain length across all kingdoms of biology. In the case of the tetraether lipids, the chains are twice as long and span the full length of the lipid bilayer membrane.

A double bond is called an unsaturated bond due to the loss of hydrogenation at that segment. The position of the double bonds are described by designating the first bond next to the fatty acid linkage as the "alpha" position, whereas the "omega" position is at the end of the acyl tail.

For example, omega-3 fatty acid refers to a fatty acid with a double bond 3 carbons from the omega end. Note, an omega-3 fatty acid may have more than one unsaturated bond, but the first one from the tail is at position omega-3. In mammals, it is typical that the sn-1 chain is saturated and the sn-2 chain unsaturated.

Acyl chains can have other modifications such as branching or cyclic groups. This is common in bacteria and archaea.

Sphingolipids. Sphingolipids have a head group derived from an amino alcohol, a phosphate, one variable fatty acid chain, and one parent C-18 amino alcohol sphingosine chain - this is the same chain in all sphingolipids. The linkage is via a nitrogen ester - not oxygen ester as is in the case of glycerophospholipids. They are similarly ampipathic There are more than 60 different types of sphingolipids present in human membranes comprised by different attachments to the C-1 headgroup alcohol. One common modification is glycosylation. Note, sphingolipids are rare in plants and bacteria. A cerebroside, is a variant of a sphingolipid that does not have the phosphate.

Sterols. A major component in many plant and animal membranes, but not present in prokaryotes. In animals, the prevalent sterol is cholesterol. In yeast, it is ergosterol and plants have beta-sitosterol. Prokaryotes have similar compounds to sterols called hopanoids.

Sterols are also amphipathic, but they have a very different structure to phospholipids and sphingolipids. They are mainly comprised of a fused set of planar rings which has a hydroxyl group on one end that constitutes the polar end of the molecule. Then, on the other end there is a flexible hydrocarbon tail.

Sterols have a significant effect on membrane structure and properties of the lipid bilayer. They are typically shorter than the extended length of the neighboring phospholipids, but they act to increase membrane thickness, and maintain membrane fluidity at low temperatures, due to inter-lipid interactions. They also are proposed to interact with membrane proteins, and enrich in lipid rafts with sphingolipids. Cholesterol is also the precursor for all steroid hormones.

The sterols can be esterified to various fatty acids and proteins. In the outer membrane of bacterial, the hopanoids can undergo similar modifications with lipid A, a saccharolipid, to form the critical rigidity of the membrane, and this is tied into the mechanism of antibiotic resistance.

Membrane Lipid Distribution. These distributions vary widely amongst different cell types and different organisms. It can change with diet, environmental conditions, age. Main facts about mammalian cell membrane composition:

- The major lipids are PC > PE. Note, bacteria cannot make PC so PE is the primary lipid.
- There are no cationic lipids
- Anionic lipids (PA, PS, PI, PG, CL) are found a lower levels than the main structural lipids PC & PE
- PC is a truly zwitterionic lipid with no net charge. PE is zwitterionic but the pKa of the primary amine is 8.5, so there is a slight negative net charge on PE at neutral pH.
- cholesterol goes from trace amounts (e.g. inner mitochondrial membrane) to the most predominant lipid (e.g. plasma membrane)
- Cardiolipin is almost exclusively in the mitochondrial inner membrane. It's also prevalent in bacteria.
- fatty acids, and lysolipids are in low amounts. They are practically detergents and will dissolve membranes in high quantities.
- sn-1 chains are typically 16:0 or 18:0, while sn-2 chains are mainly unsaturated (containing double bonds) and vary from 18-22 carbons long. However, lipids from C12-C22 are generally observed.
- The Golgi membrane is mainly comprised of shorter chain lipids, and it appears as though Golgi specific membrane proteins have evolved to be shorter also.

Lipid synthesis and modifications. There are two major ways in which that fatty acyl chains can be incorporated into phospholipids. The first is acylating glycerol-3-phosphate during biosynthesis of phospholipids. The second is remodeling existing phospholipids by acyltransferases and transacylases. There are also headgroup modifying enzymes, such as phosphatidylethanolamine methyltransferase that will convert PE to PC. These membrane asociated enzymes have preferences for certain substrates, and their overall expression as well as substrate bias leads to some of the compositional differences observed in different tissues.

Overall, the biogenesis and modulation of lipids is highly complex. I will not ask you to describe any of these reactions here. But I show an example of the different reactions for reference, and to grasp the different reactions that lead to the diversity of membrane compositions in cell membranes. Please see the required reading for more information.

The location of the synthesis of these lipids are specific. For instance, cardiolipin is synthesized in the mitochondrial membrane and is largely kept there. Cholesterol on the other hand is synthesized in the ER membrane, and is in low levels there. In addition to lipid synthesis, there are also lipid transport mechanisms within the cell. For cholesterol, it can move by vesicular transport, diffusion while bound to a carrier protein, or transfer by membrane contacts.

Membrane asymmetry. In addition to differences in membrane compositions, there are differences in the distribution across the two leaflets, especially in the plasma membrane. The

negatively charged lipids are predominantly in the inner leaflet facing the cytoplasm. PS is one of these and the appearance of PS on the outer leaflet is a signal for apoptotic stress. Glycolipids are selectively on the outer leaflet. There are membrane embedded enzymes called flippases, floppases and scramblases that regulate these distributions.

Lipidomics. The study of lipid profiling is called lipidomics. Membranes can be extracted from cellular samples by carrying out a solvent extraction, typically using a mixture of chloroform, methanol and water (e.g. Bligh and Dyer, or Folch). The lipids are collected in the organic phase and the solvent evaporated and then analyzed. Previously, lipids could be assessed by thin layer chromatography. Now, laboratories will use mass spectrometry to obtain a complete analysis of the entire lipid profile including headgroup and chain modifications.

With this, studies can be conducted to examine the change in lipid profiles under different conditions such as in disease. For example, E. coli was grown in the presence of different stress inducing agents, and the change in lipid profile measured. In a similar study, E. coli grown at colder temperatures were found to increase chain unsaturation and decrease length. The results demonstrate that lipid composition is a biological variable that is dramatically altered under different conditions. Still, the exact meaning and implications of these chemical changes on the function of the membrane and the proteins within remain an important question in biology.