

Lecture 2 - Chemical composition of the cellular membrane

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1 Reference reading

Harayama, T., Riezman, H. (2018). Understanding the diversity of membrane lipid composition. *Nature Reviews Molecular Cell Biology* 19(5), 281-296.

2 Homeoviscous adaptation

As a reminder, all biological membranes are structured as lipid bilayers with 30 Å hydrophobic thickness formed by hydrocarbon chains. The headgroups are polar or charged and add an additional 10 Å to the total thickness. The lipid bilayer self-assembles into a stable structure due to the amphipathic properties of the molecules that comprise it.

While the lipid bilayer structure is adopted for all cell membranes across biology (as we know it), we also know that a major biological requirement for the membrane is that it acts as a barrier, but it remains fluid. This allows for important biological reactions to occur within, such as binding, protein folding and conformational changes to occur. Yet, organisms like bacteria and archaea exist in a wide variety of external environments, including different temperatures. But, we also know that the lipids undergo a main phase transition, converting to the gel phase below the transition temperature (think about bacon grease, which is mainly oleic acid and cholesterol). Consider organisms like psychophiles, that thrive below freezing temperatures. How do they keep their membranes from turning to gels?

It turns out that there is a biological phenomenon called homeoviscous adaptation. In an experiment conducted in 1974, *E. coli* were grown at different temperatures, and the lipids were extracted and the phase transition temperature measured¹. It was found that this temperature shifted along with the growth temperature. The reason for this is that

¹Sinensky M. Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proc Natl Acad Sci U S A*. 1974 Feb;71(2):522-5. doi: 10.1073/pnas.71.2.522. PMID: 4360948; PMCID: PMC388039.

the cells alter the lipid composition in their membranes to provide on the fly adaptation to the surrounding temperature, and thus maintain a certain viscosity.

2.1 Chemical diversity in the biological membranes

The driving force for the lipid bilayer to self-assemble is robust and depends on burial of non-polar molecules away from water, i.e., the hydrophobic effect. It depends less so on the specific molecular details of the amphipathic molecules (within reason) and this means that biology can faithfully form lipid bilayers with many different types of lipid molecules. The implication of this is that there is enormous solvent variability with cellular membranes, whereas proteins that reside outside of membranes have evolved in only one solvent environment - water. Since we know that the physical relationship of proteins with water is a large driving force for soluble protein reactions (also due to the hydrophobic effect), this raises interesting questions about the relationship of lipid solvent and membrane protein reactions.

To get a sense of the variability of membrane compositions, examine the major lipid components of *E. coli*, brain, and the archaea *S. sulfolubus*. *S. sulfolubus* is an extremophile that thrives at acidic pH (pH 2-3) and high temperatures (75-80 °C). Keep in mind that all of these organisms have homologous membrane proteins of similar structure and function. How can the proteins even exist in a stable form when they are subjected to what we know are typically denaturing for proteins, even for membrane proteins. We can see that the major changes are the lipid composition, where the archaea has a membrane that is predominantly comprised of tetraether type lipids, where there is actually a lipid monolayer that mimics a bilayer structure. Here, the lipids are phospholipids, but with the acyl chains at the ends of their tails. This introduces a covalent attachment between the two leaflets, which presumably stabilizes the bilayer structure. The question that remains, is whether the changes in lipid composition also impact the behavior of the proteins that reside within, or are these striking differences in lipid composition implying that lipids are inert solvent species?

2.2 8 categories of biological lipodal molecules in membranes

In reality, each cell may have 100-1000s of different types of lipid molecules and these compositions differ across membrane compartments, and vary with lifetime. 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:

- Fatty Acyls - eicosanoids derived from arachidonic acid, e.g. prostaglandins, leukotrienes
- Glycerolipids - e.g. triglycerides

- Glycerophospholipids - i.e. phospholipids. These are the major components of membranes.
- Sphingolipids - e.g. sphingomyelins, gangliosides
- Sterol lipids - e.g. cholesterol, steroids
- Prenol lipids - e.g. carotenoids, retinol
- Saccharolipids - lipid A, an important component of prokaryotic outer membranes and a factor in the development of antibiotic resistance
- Polyketides - secondary metabolites and antibiotics

2.3 Fatty acyls

These are carboxylic acids with a single aliphatic chain. They are prevalent in dietary sources and precursors for other types of lipid synthesis. The chains within other lipid types, i.e. phospholipids, are also called fatty acid chains. Found in various chain lengths and with chain modifications (cis/trans unsaturation, i.e. double bonds, cyclo additions, iso/aneiso branching) that are known to modulate membrane fluidity, as well as other membrane properties (more on this next lecture).

2.4 Glycerolipids

These are diglycerides (e.g. diacylglycerol, DAG) and triglycerides (e.g. significant component of olive oil). Constructed on an sn-glycerol-3-phosphate backbone, ester linked to fatty acyl chains. There is no phosphate or head group, and so these lipids are very hydrophobic and are often found inside of lipid droplets or lipoprotein particles (e.g. HDL, LDL). DAG is a second messenger signaling lipid produced by the hydrolysis of PIP2 by phospholipase C, stimulating calcium release from IP3.

2.5 Glycerophospholipids

Glycerophospholipids (i.e. phospholipids) are the most prevalent lipid type in biological membranes. They have the following structure:

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

This structure yields a molecule that is amphipathic, where approximately half of the molecular volume is non-polar, and the other half is polar.

2.6 Fatty acid-glycerol linkages

Typically, fatty acids are esterified to the glycerol at carbon positions C-1 and C-2. The glycerol backbone is chiral and this corresponds to the sn-1 and sn-2 positions respectively. However, there are variations of the linker chemistry - ether and vinyl-ether linked lipids. These lipids are called plasmalogens and they are prevalent in archaeal membranes. They are also found in bacteria and eukaryotes, though less common than the ester linked lipids (20 mol % of total phospholipid mass in humans^a). Yet, some tissues, like the heart and mitochondria within, have an abundance of plasmalogen lipids. Note, these changes in chemistry can have structural and physical implications. Ether linked lipids are more susceptible to oxidation. They also 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.

^aBraverman NE, Moser AB. Functions of plasmalogen lipids in health and disease. *Biochim Biophys Acta*. 2012 Sep;1822(9):1442-52. doi: 10.1016/j.bbadis.2012.05.008. Epub 2012 May 22. PMID: 22627108.

2.7 Variations in fatty acyl chain chemistry

Much of the chemical diversity of membranes comes from the fatty acyl chains, which can vary with the following structures:

2.7.1 Chain length (X)

This indicates the number of carbons in the the acyl chain. The nomenclature describes this as CX:Y, where X is the number of carbons. Typically, biological acyl chains are 12-22 carbons long, with 16-18 being the common chain length across all kingdoms of biology.

2.7.2 Chain number

Lipid molecules can have one (fatty acids, lysophospholipids, sphingosine), two (glycerophospholipids, sphingolipids, diglycerides), three (triglycerides) or even four chains (cardiolipin, i.e., diphosphatidylglycerol). There are tetraether lipids, and while the name implies that there are four ether linkages, there may be only two chains as these molecules mimic two lipids attached end-to-end by their chains. These lipids are common in archaea and span the full membrane to provide a monolayer that mimics a lipid bilayer. Lipid A contains six chains!

2.7.3 Saturation (Y)

This describes the number of double bonds, or unsaturation, in an acyl chain ("hydrogenated oils" are fully saturated). The nomenclature indicates the number of double bonds in the chain, i.e., Y in CX:Y. Note, these double bonds are susceptible to oxidation. A double bond can be cis (same side) or trans (opposite sides), with trans bonds decreasing membrane fluidity (think "trans fats").

2.7.4 Position of saturation

Where the double bonds are located also makes a difference in the behavior of the lipid in the membrane. There is additional nomenclature for this, where the first bond next to the fatty acid linkage is the "alpha" position, and the last bond in the chain is called the "omega" position. For example, omega-3 fatty acid refers to an acyl chain with a double bond at the third last bond in the chain. 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.

2.7.5 Chain modifications

Acyl chains can have other modifications such as branching or cyclic groups. This is common in bacteria and archaea. The main effect of these modifications is on the fluidity of the membrane, with moieties that increase neighboring chain interactions decreasing fluidity, and chain kinks introduced by cis double bonds increasing fluidity.

2.8 Headgroup chemistry

2.8.1 Headgroup moieties

- hydrogen - phosphatidic acid, PA, negative
- serine - phosphatidyl-serine, PS, negative
- glycerol - phosphatidyl-glycerol, PG, negative
- choline - phosphatidyl-choline, PC, neutral, zwitterionic
- ethanolamine - phosphatidyl-ethanolamine, PE, neutral, zwitterionic
- disphosphatidylglycerol, i.e., cardiolipin, CL, negative -2
- inositol, phosphatidyl-inositol, PI, negative
- phosphoinositides, PIP (-2), PIP₂ (-3, position matters, e.g., PI(4,5)P₂), PIP₃ (-4, position matters)
- Cationic lipids can be synthesized (EPC) but not biological (?)

2.8.2 Ionization

Phospholipid headgroups are charged as the phosphoric acid group is ionizable. Even if the headgroup is overall neutral, it is zwitterionic with local charge on different chemical groups. Other groups in the alcohol moiety are ionizable also, and so the charge is dependent on these pK_as (subject to the environmental conditions and structure) and the pH. For example, examine a titration 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 pK_as of any of these chemical groups becomes changed, or the pH of the reaction environment is altered. The charges listed above are most probable at pH 7.0.

2.9 Sphingolipids

Sphingolipids are major constituents of eukaryotic cell membranes, but rare in plants and bacteria. Common in myelin, nerves and brain tissues. They are similarly amphipathic to glycerophospholipids, but have distinctly different chemical structures:

- headgroup derived from an amino alcohol, and can vary due to different attachments
- phosphate (except for cerebroside)

- nitrogen ester linkage (not oxygen ester as in the case of glycerophospholipids)
- parent C-18 amino alcohol sphingosine chain (same one in all sphingolipids)
- one variable fatty acid chain

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, and these types of lipids are referred to as glycolipids. A cerebroside, is a variant of a sphingolipid that does not have the phosphate. In general, sphingolipids are found to form segregated domains with sterols and are substantial in plasma membranes.

2.10 Saccharolipids

Note, these are different from glycolipids. Glycolipids are defined as having a sugar bound by a single glycosidic linkage to a fatty acid, whereas saccharolipids are fatty acids that are directly linked to the sugar backbone. Lipid A is an example that is prevalent in outer membranes of bacteria, and is an essential component of antibiotic resistance mechanisms.

2.11 Polyketides

Derived from polyketides, i.e., multiple ketone unit intermediates. Synthesized from acetyl-coA by polyketide synthases. These are natural products and are secondary metabolites, antibiotics, antifungals, amongst other strange biologics. Examples - doxycycline, erythromycin, cholesterol lowering drug lovastatin, flavonoids like curcumin.

2.12 Prenol lipids

Formed from terpene units. Synthesized from the 5-carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate. Includes carotenoids, retinol, quinones, vitamin E and K. These are membrane embedded antioxidants.

2.13 Sterols

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

Sterols are amphipathic, but less so. They are mainly comprised of a fused set of planar rings which has a small hydroxyl group on one end that constitutes the polar end of the molecule. On the other end, there is a flexible hydrocarbon tail.

Sterols have a significant effect on membrane structure and lipid bilayer properties. They are shorter than the extended length of neighboring phospholipids (e.g., with C16:0/C18:1

chains), but counterintuitively, they act to increase membrane thickness. This is because their rigid planar structure encourages neighboring lipids to adopt more extended configurations to maximize neighbor van der Waals contacts. In addition, cholesterol helps to maintain membrane fluidity at low temperatures, which is because it prevents lipids from maximizing neighboring interactions to form the gel phase (what would you guess happens at higher temperatures?). Therefore, cholesterol that mixes with the neighboring lipids appears to have a buffering action on the membrane to help maintain the proper fluid membrane conditions across a wider range of conditions. However, there is another behavior of cholesterol/phospholipid mixtures, which is the self-assembly of cholesterol rich domains. Historically, these have been called "rafts" but more correctly described as liquid-liquid phase separation. In mammalian plasma membranes, cholesterol is present at 50% of the lipid components tends to associate with sphingolipids.

Note, cholesterol is the precursor for all steroid hormones. Sterols can be esterified to various fatty acids and proteins. In the outer membrane of bacteria, hopanoids can undergo similar modifications with lipid A, a saccharolipid, to form the critical rigidity of the membrane tied into the mechanism of antibiotic resistance.

2.14 Lipid compositions in cells

. These distributions vary widely amongst different cell types, organelles and different organisms. It can change with diet, environmental conditions, age. These have been measured in the past by thin layer chromatography, but now is carried out more accurately with mass spectrometry lipidomics. Main findings about mammalian cell membrane composition:

- The major lipids are PC, then PE. Note, bacteria cannot make PC so PE is the primary lipid.
- 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)
- chain length varies 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.

Keep in mind, membranes composition changes due to different conditions such as environmental stress and disease. For example, *E. coli* was grown in the presence of different stress inducing agents, and the change in lipid profile measured, yielding a wide variety of changes. In a similar study, *E. coli* grown at colder temperatures were found to increase chain unsaturation primarily and also decrease chain length. The results demonstrate that lipid composition is an important biological variable that can be dramatically altered under different conditions. The exact implications of these chemical changes on the function of the membrane and the proteins remain an important question in biology.

2.15 Membrane asymmetry

. In addition to differences in membrane composition, 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.

2.16 The location of the synthesis of lipids is 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, but 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.

3 Summary

The chemical compositions of cellular membranes is complex and dynamic. While the primary constraints are maintaining a barrier while achieving proper fluidity, we really

don't understand what are the roles of many other lipoidal molecules. There is immense potential to uncover new biological concepts, especially in regulation and signaling by lipid species in cell membranes.