Lecture Outline

1. The long path to the lipid bilayer cell membrane
2. Structures of lipid molecules
3. Lipid synthesis and composition

Reading for fun:

“Once upon a time the cell membranes: 175 years of cell boundary research” Biology direct vol. 9 32. 19 Dec. 2014, doi:10.1186/s13062-014-0032-7


“An Introduction to Biological Membranes, Composition, Structures and Function” William Stillwell, Elsevier Science 2016

In 1665, using a simple light microscope, Hooke examined tissue from a cork tree and observed a collection of compartments he termed “cells”. We know now that he was visualizing the cell wall, a rigid layer that is dense in cellulose and polysaccharides. However, this would be the first observation in understanding that compartmentalization is a necessary requirement of biology.
Soon after, in 1677, Antonie van Leeuwenhoek reported his observations of bacteria and microbes. Following up on work of others, he provided detailed reports about imaging erythrocytes, showing the compartmentalization of animal cells as well. An important observation was that animal cells and microbes did not have the distinct cell wall type compartmental structures. They were separated from the external solution but the mechanism by which they were separated was not observable.
Observation of self-contained organelles

Robert Brown named the cell nucleus during studies of orchid tissue. (b) Specimens of orchid epidermis examined successfully with the same microscope. Our experiments imaged peels of the same orchid tissue through this x2 lens. After careful adjustment, the results clearly reveal the cell nuclei. Stomata are also resolved (right), and some fine cytological details are apparent within the epidermal cells.

https://doi.org/10.1177/1079980014548963

• Jump ahead ~150 years, Robert Browne reports the observation of the nucleus in plant cells (1833), demonstrating the existence of compartmentalization within the cell as well.
<table>
<thead>
<tr>
<th>Spontaneous Generation</th>
<th>Cell Theory (1830s)</th>
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<tr>
<td>Francesco Redi's experiment (1668)</td>
<td>• All living organisms are composed of one or more cell</td>
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<td><a href="https://www.timetoast.com/timelines/spontaneous-generation">Image</a></td>
<td>• The cell is the basic unit of structure and organization in organisms</td>
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<td>• Cells arise from pre-existing cells by division (spontaneous generation does not occur)</td>
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**Modern Cell Theory**

• Cells contain hereditary information which is passed from cell to cell during division
• All cells are similar in terms of chemical composition
• All energy flow (metabolism & biochemistry) of life occurs within the cell

- Preceding this time, biological organisms were generally believed to originate spontaneously, as food and substances would be observed emerge with the existence of new life forms (e.g. bacteria, maggots) as a function of time. But experiments by Redi (1668) and Pasteur (1860-1864)
- This led to the development of Cell Theory, attributed to Lorenz Oken (1805), Matthias Schleiden (Plants - Contributions to Phytogenesis, 1837), Theodor Schwann (Animals - 1839) and Rudolph Virchow (1855)
Biology requires boundaries

- Prevent the passage of large charged molecules like proteins, DNA & RNA
- Prevent passage of small charged molecules like ATP and nutrients
- Hold ionic gradients
- Hold pH gradients
- Fluid and flexible
- Sustain diverse living conditions (temperature, acidity/alkalinity, pressure)

The implicit requirement of Cell Theory is that the cell is distinctly separated from the external world. It must be contained in some manner. The important biological molecules, like DNA, RNA and proteins must be kept in place, and salt and pH defined within optimal working ranges for proper biochemistry.

A barrier allows for the storage of information and potential energy, converting cells into batteries that fuel biological work.

Whatever the bounding mechanism may be, it must be fluid, flexible and ideally self-healing in order to sustain the complex requirements of biology.

The question is, is the barrier defined by a unique structure particular to each cell or is it something that is robust and general across biology?
The first uttering of a cellular “membrane”

- C.H. Schultz (1836) used iodine to stain the erythrocyte plasma membrane, estimating the membrane thickness to be 220 Å (22 nm)
- In Schwann’s model for animal cells, since the boundaries are not clearly visible, he introduced the existence of thin invisible membranes, necessary to separate a cell from its environment. These membranes would limit the cell volume in some way although they were invisible. Furthermore, the location of the membrane was where “fermentation”, i.e. metabolism took place.
- He was the first to use the term “membrane” in 1839, but his career essentially ended (at age 30), for having the audacity to suggest that alcohol fermentation was the result of living organisms.
Invisible boundaries must be flexible

August Johann Rösel von Rosenhof, who named his discovery "Der Kleine Proteus" ("the Little Proteus") 1755

- The early conceptualization of the cell membrane was that it was more like a cell wall that was found in plants. While it was not obviously visible, and thus not as thick, it was thought to be structured in some way and therefore rigid in animal cells like erythrocytes.
- However, there was a lot of imaging done on mobile organisms like amoebas, which could be observed to move and deform their shapes. While the amoebas clearly showed some invisible boundary, it was difficult to conceive that there was a flexible bounding structure that could allow for the amoeba's movements.
Other theories of cell boundaries

**Aggregation of the colloidal protoplasm**

A. In this vision, the cell is devoid of any membrane and all the properties of the cell are defined by the activity of the protoplasmic colloid. B. The cell is surrounded by an external layer (membrane) of which the nature is distinct to the rest of the protoplasm. Yet, in this view, the inside of the cell remains a colloid.

**Biological phase separation**

Calcium chloride and sodium alginate combine to polymerize, forming a hardened aggregate layer around a volume of solution. Shown here are the olives from Ferran Adria's restaurant elBulli.

- So, after Schwann’s mentioning of the word “membrane”, there came out several theories that argued against the idea that there was any sort of encapsulating structure at all.
- Leydig (1857) noted that in most cells the protoplasm, i.e. cellular contents, is a substance primitively approaching a sphere in shape and containing a central body called a kernel (nucleus). He recognized the existence of enclosing structures resulting from the hardening of the cell surface. In hindsight, this may sound like a complicated model, but it is not chemically unheard of. I would imagine it to exist in a fashion that was similar to something to the process of “spherification” that is being used in molecular gastronomy today. Of course, this does not explain the movements observed in amoebas.
- Max Schultze (1863) thought of cells as lumps of contractile protoplasm that were held together because of their inability to mix with the surrounding aqueous solution. This rationalized the flexibility that was observed in amoeba microscopy.
- This is something that we are learning does exist in biology, through membrane-less organelles, such as stress granules and P-bodies as products of phase separation of RNA and proteins inside of cells.
- Therefore, both of these ideas are present in chemistry and in biology, and reasonable for the time as the study of cell biology lacked quantitative studies.
Studies of plasmolysis

- Quantitative studies of cell boundaries would begin with plasmolysis experiments.
- William Hewson (1773) observed osmotic swelling and shrinking in erythrocytes and deduced the existence of a cell membrane as a structure surrounding the protoplasm. This was largely ignored in the scientific community.
Studies of plasmolysis

• These studies would be revisited, ~80 years later in plants. Karl von Nageli (1855) began the osmotic studies on plants. This was easily observable by microscope, as the inner boundary could be observed as pulling away from the cell wall.
• While qualitative, it approached a quantitative level due to the outer boundary of the cell wall as a reference.
• Sugar and salt were used to control the osmotic conditions.
• It is important to note that the process was observed to be reversible, as long as the cell did not become ruptured.
• Also, it was observed that isolated vacuoles from plant cells exhibited the same reversible behavior.
• One important thing to note that was present in all of the plasmolysis studies, is that the permeability characteristics varied quite a bit. Cells were generally permeable to water, but the rates varied and permeability varied amongst cell types and different organisms.
William Pfeffer studied Mimosa pudica and the stamen of Centaurea Jacea (Knapweed) both plants that exhibit rapid movement due to osmotic pressure. To understand osmosis better, he studied the artificial membranes of copper ferrocyanide that were being developed by Moritz Traube (1864). These membranes were thin and self-assembled, showed budding and growth behavior. He built a Pfeffer cell, which allows for the membranes to be deposited onto a clay pot to stabilize the membranes (much like the cell wall). He noticed that these thin artificial membranes exhibited the same behavior as protoplasts (cells), and postulated that cells also possess similar thin membranes around them (1877). He coined the term “plasma membrane”
### The theory of osmotic pressure

<table>
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<tr>
<th>Pure water</th>
<th>Solution</th>
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<td><img src="image1.png" alt="Diagram" /></td>
<td><img src="image2.png" alt="Diagram" /></td>
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**V** = \( n \cdot R \cdot T \)

**II** - osmotic pressure

**V** - volumes

**n** - number of solute particles

**R** - gas constant

**T** - temperature

- Jean-Antoine Nollet first documented observation of osmosis in 1748
- Van’t Hoff noticed the similarity of osmotically driven processes and developed the theory of osmotic pressure (1886) that describes how substances in dilute solutions follow the ideal gas laws
- Osmotic pressure is the minimum pressure which needs to be applied to a solution to prevent the inward flow of its pure solvent across a semi-permeable membrane.
Overton’s return to plasmolysis

- Charles E. Overton (1895-1902) returned to plasmolysis studies, quantitatively comparing over 500 substances used to control the osmotic pressure. Basically any compound that was available at the time was tested in his laboratory.
- To quantify the measurements, the measurements were conducted by visual observation, or by cell weight.
- He found that polar substances induced plasmolysis, however, aliphatic alcohols and non-polar substances have little effect.
- Thinking of van’t Hoff’s osmotic pressure law, he concluded that there is no barrier for these species. Whatever is enclosing the cell is permeable to these species selectively.
At the same time as Hans Horst Meyer (1899), Overton (1901) also found that non-polar substances that are known to act as general anesthetics in a clinical setting show an interesting behavior. Their clinical efficacy is directly correlated to the partitioning into olive oil.

Putting these results together, Overton concluded that the cell barrier was lipoidal, and likely to be made of known biological lipoidal molecules like phospholipids and cholesterol.
An interesting property about olive oil

- Pliny the Elder (1623) observed that spilling oil on the sea can still the waves.
- Benjamin Franklin (1774) conducted the following observational experiment of dropping a teaspoon of oil onto a pond and observed it spreading out to the size of the pond, about half an acre.
- With this information, he could have calculated the molecular thickness of this layer ... but alas, he did not.

"...But recollecting what I had formerly read in Pliny, I resolved to make some experiment of the effect of oil on water, when I should have opportunity...

...At length being at Clapghan where there is, on the common, a large pond, which I observed to be one day very rough with the wind, I fetched out a crust of oil, and dropped a little of it on the water. I saw it spread itself with surprising swiftness upon the surface; but the effect of smoothing the waves was not produced; for I had applied it first on the forward side of the pond, where the waves were largest, and the wind drove my oil back upon the shore. I then went to the windward side, where they began to form; and there the oil, though not more than a teaspoonful, produced an instant calm over a space several yards square, which spread amazingly, and extending itself gradually till it reached the -side, making all that quarter of the pond, perhaps half an acre, as smooth as a looking-glass.

After this, I continued to take with me, whenever I went into the country, a little oil in the upper hollow joint of my bamboo cane, with which I might repeat the experiment as opportunity should offer; and I found it constantly to succeed.

In these experiments, one circumstance struck me with particular surprise. This was the sudden, wide, and forcible spreading of a drop of oil on the face of the water, which I do not know that anybody has hitherto considered. If a drop of oil is put on a polished marble tablet, or on a looking-glass that lies horizontally: the drop remains in its place spreading very little. But when put on water it spreads instantly many feet round, becoming so thin as to produce the prismatic colours, far a considerable space and beyond them so much thinner as to be invisible, except in its effect of smoothing waves at a much greater distance..."
The first studies of surface tension of oil monolayers

**Surface Tension.**

I shall be obliged if you can find space for the accompanying translation of an interesting letter which I have received from a German lady, who with very humble appliances, has arrived at valuable results respecting the behaviour of contaminated water surfaces. The earlier part of Miss Pockels’ letter covers nearly the same ground as some of my own recent work, and in the main harmonizes with it. The later sections seem to me very suggestive, exciting, if they do not fully answer, many important questions. I hope soon to find opportunity for repeating some of Pockels’ experiments.

March 15.

_Severnale, January 12._

My LORD,—Will you kindly excuse my venturing to trouble you with a German letter on a scientific subject? Having heard of the fruitful researches carried on by you last year on the histograms of oil films, I am desirous of communicating to you some observations of my own on the subject. For various reasons I am not in a position to publish them in scientific periodicals, and I therefore adopt this means of communicating to you the most important of them.

First, I will describe a simple method, which I have employed for several years, for increasing or diminishing the surface of a liquid in any proportion, by which its property may be altered at pleasure.

A rectangular tin trough, 20 cm. long, 5 cm. wide, 8 cm. high, is filled with water to the brim, and a strip of tin sheet 4 cm. wide laid across it perpendicular to its length, so that the under side of the strip is in contact with the surface of the water, and divides it into two halves. By closing this partition to the right or the left, the surface on either side can be lengthened or shortened in any proportion, and the change can be read off on a scale fastened along the front of the trough.

No doubt this apparatus suffers, as I shall point out presently, from a certain imperfection, for the partition never completely shuts off the two separate surfaces from each other. If there is a great difference in tension between the two sides, a return current often breaks through between the partition and the edge of the trough (particularly at the time of filling). The apparatus, however, answers for obtaining any condition of tension which is as nearly possible, and in experiments with very clean surfaces there is little to be feared in the way of currents breaking through.

- In 1890, Lord Rayleigh repeated Benjamin Franklin’s experiment, measuring the area to which a given volume of oil would expand. He reduced it to a laboratory scale (0.8 mg spread out to 5500 cm^2), and measured the thickness of the layer, 1.6 nm.
- When Agnes Pockels was 19 years old (1881), she was a homemaker, taking care of her elderly and sick parents while her brother, Friedrich Pockels, studied Physics at Gottingen University. She did not have a formal education, as was customary for women at that time, but she had a passionate interest in science and particularly physics. She did however do much of the housework, and part of this included cleaning the greasy kitchen pots and pans. During this, she observed the thin films of oil and their prismatic colors. So she developed a device, made out of kitchen pans for measuring the surface tension of the thin oil film. She showed her results to the physicists at Gottingen who showed no interest. Then in 1890, she learned of Lord Rayleigh’s similar interest in this research and her brother advised her to write a letter to him.
- Rayleigh helped to communicate Pockels’ work and in 1891, her paper was published in Nature. The first of many such impactful discoveries that essentially founded this field …. She went on to measure the thickness of this layer as 1.3 nm.
- When her brother died in 1913, she stopped publishing as she no longer had access to the University and scientific community.
In 1917, Irving Langmuir publishes on the molecular orientation of oil molecules on water, building a higher resolution “Langmuir trough” to measure surface tension based on Pockels’ design, and establishing that the layer was a single-molecule 13 Å in thickness.

He is often recognized as the pioneer and the pinnacle of surface chemistry.
Following these developments in measuring surface tension, Gorter & Grendel (1925) carried out an experiment of extracting the membrane fraction from erythrocytes, and then depositing it into a Langmuir trough to measure the surface area. The oil fraction spread out to a layer with a defined area. When taking into account the number of cells, and the surface area of each cell, then determined that the area on the trough was twice the surface area of the cells. With this, they proposed that the cell membrane was a lipid bilayer.

This was a serendipitous conclusion, because their paper contained errors that cancelled out. Furthermore, when others repeated the study under different extraction conditions, they would get different conclusions. It was not a robust study, but it did put forth the idea of a lipid bilayer.

Another experiment was conducted at the same time, which quite robust but is often ignored. Fricke (1925) carried out electrical impedance/resistance measurements of erythrocyte suspensions. Assuming that the membrane is made up of oil, as Overton proposed, he used a low dielectric constant of 3, and measured the hydrophobic thickness of the membrane as 3.3 nm thick (33 Å) ... something which stands to this day.

However, he did not interpret this as a bilayer in his paper. In hindsight, given Rayleigh’s, Pockel’s and Langmuir’s measurements of 13-16 Å for the olive oil monolayer, Fricke’s data supports a lipid bilayer structure.
The Davson and Danielli model (1935) consolidated much of the results from Fricke, Gorter & Grendel into a lipid bilayer, but one that could be potentially thicker due to a lipoid core.

Danielli & Harvey (1935) found that proteins are adsorbed onto the outer surface of egg cells, and that these proteins must protect and stabilize the lipoid molecules that are not stable when in contact with the aqueous solution.

This begins to introduce the ideas of permeability in membranes. They proposed the formation of stable pores by these protein layers to allow for differential permeation (more on this in the second half of the lectures).
• J.D. Robertson, an electron microscopist used KMnO4 fixation in his preparations of the myelin sheaths of Schwann cells (1957). He observed the consistent appearance of trilaminar units - two dark bands with a light core - at all of the positions where cell membranes and barriers were expected to be. The KMnO4 was expected to have stained the protein layers that bound to the membrane surface, as was described in the Davson & Danielli model, and that these layers were separated by a light lipid core.

• Robertson present the ‘unit membrane’ model that was very similar to the Davson & Danielli model, with the exception that the membrane was formed as a true lipid bilayer.
The unit membrane hypothesis

- He observed the trilaminar structure in all preparations of cells, and for all of the organelles. Anywhere where there was expected to be a boundary.
- With this, he even went to propose that the membrane was continuous, bringing in ideas of trafficking between the cellular compartments. While this is not the case, it does highlight the fact that it is in principle, the same membrane structure shared across all of these regions, and that they are capable of exchanging under the right conditions.

Figure 4: Robertson’s now-infamous diagram of a hypothetical cell, illustrating its legend said noncomitally, “relationships of the cell membrane to various cell organelles.” In the original text relating to this figure, Robertson spoke more boldly, proposing that “it would not be unreasonable to conceive of an intracellular ‘circulatory system’ directly connected to the outside world in some places.” Given what is now known about endo- and exocytosis, as well as what is known about membrane trafficking inside the cell, such an extreme degree of membrane continuity is considered unlikely, but Robertson’s basic point was that because all cellular membranes are constructed along the same architectural principles, they are at least potentially capable of becoming continuous with each other. (From: Sci. Am., 206: 64-72, 1962)
Frye & Edidin (1970) bound mouse and human cells with fluorescein or tetramethyl-rhodamine labelled antibodies, then fused the cells together. 5 minutes after fusion, they observed separated membranes, which mixed completely over the course of 40 minutes.
The fluid mosaic model

Singer & Nicholson (1972) developed the fluid mosaic model for cell membranes.

- The membrane is a lipid bilayer, a thermodynamically stable structure comprised of phospholipids.
- The lipid bilayer is a fluid solvent, constrained in 2-dimensions.
- Proteins are embedded within the membrane and responsible for selective permeability.
- The lipid bilayer is the solvent for the membrane protein reactions. The proteins and the membrane are thermodynamically connected, i.e. the reaction is driven by the free energy of the system as a whole.
- An assumption from this model was that the membrane acts as a sea of solvent, somewhat inert, but this is mis-interpretation …
Once upon a time the cell membranes: 175 years of cell boundary research
Jonathan Lombard
A summary of membrane models over the past 200 years

- **1850s**
  - A brief history of cellular barriers. (A) The protoplasmic colloid model. The barrier is a hardened shell that forms when the dense colloidal protoplasm makes contact with the extracellular solution. Pictured here is artificial caviar made by the analogous process of spherification. Photo courtesy of J.L. Robertson.

- **1925, 1935**
  - The paucimolecular model of Davson and Danielli, where the cell barrier is modeled as a lipid bilayer with a lipoid core flanked by layers of polar and charged proteins. From Danielli and Davson (1935), Fig. 2 B is adapted with permission from the Journal of Cellular Physiology.

- **1959**
  - The unit membrane model of Robertson, indicating the train track–appearing lipid bilayer that forms a continuous membrane around the cell. From Robertson (1981), Fig. 2 C is adapted with permission from the Journal of Cell Biology.

- **1972**
  - The fluid mosaic model of Singer and Nicolson, showing a lipid bilayer with integral membrane proteins responsible for cellular permeability. From Singer and Nicolson (1972), Fig. 2 D is adapted with permission from Science.

- Our understanding of cell boundaries have gone from hardened shells to oily lipid bilayer membranes.
- The lipid bilayer is a unified mechanism of bounding biological systems. It turns out nature goes for the robust common solution than one that is particular to each organism. This seems to be a common theme in biology.
Let’s go back and reflect on this solution of an oily membrane on the physical and biological requirements of the barriers that we previously discussed.

First off, oils have a natural physical tendency to separate from water and we’ll talk about this more in the next lecture. This is something that we can observe with our own eyes when we make salad dressing. If you shake a mixture of oil and water, it makes a suspension that closely resembles the physical appearance of cells.

These layers of oil are fluid and flexible.
The physical implications of a lipid bilayer membrane

Continuum electrostatics
Born equation

- The lipid bilayer is made of oil which has a low dielectric constant (~2-3) compared to water (80).
- The dielectric constant, or relative permittivity, describes how an electric field around a charge is decreased relative to a vacuum.
- Water molecules have electric dipoles, and each individual molecule is free to rearrange to stabilize the salvation of the charge.
- However lipid molecules, and especially those constrained in a lipid bilayer, cannot rearrange, so the electrostatics are more similar to vacuum.
- The electrostatic component of the solvation free energy is described by the Born equation. This is the energy to take a charge from vacuum and place it in water.
- It is favorable to transfer into water, but unfavorable in the other direction (i.e. water to lipid).
- This means that charges, on ions or molecules, are all electrostatically prohibitive inside a stable low dielectric medium.
The physical implications of a lipid bilayer membrane

**Continuum electrostatics**

**Linearized Poisson-Boltzmann equation**

\[
\nabla \cdot [\varepsilon(r) \nabla \phi(r)] - \kappa^2(r) \phi(r) = -4\pi \rho_e(r)
\]

\[
\Delta G_{\text{elec}}(X) = \frac{1}{2} \sum \phi_x(r_j)
\]

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Fig. 6. Illustration of the important concepts in membrane electrostatics. (a) The dielectric barrier. The solid line is the electrostatic reaction-field free energy of a K⁺ ion going across a 30 Å membrane represented by a structureless continuum medium of dielectric 2. The dashed line is the electrostatic reaction-field free energy of a K⁺ ion going through a 3 Å diameter cylindrical aqueous pore of dielectric constant 80. The finite-difference calculations were done using the PBEQ module (Nina et al. 1997; Roux, 1997; Im et al. 1998) of the biomolecular simulation program CHARMM (Brooks et al. 1983). The total electrostatic potential was calculated at each point of the grid by solving the finite difference Poisson equation. No electrolyte was included in the bulk solution.

https://doi.org/10.1017/S0033583504003968

- For complex dielectrics, like a membrane slab, the linearized Poisson-Boltzmann equation can be solved using a variety of computer programs (e.g. PBEQ/CHARMM, APBS, etc …)
- Shown here is the electrostatic free energy for transferring a K⁺ ion from water to a low dielectric slab that represents the membrane. The electrostatic energy is prohibitive through the bilayer.
- Note, it is also prohibitive inside a small aqueous pore filled with high dielectric, but more on that next week.
However, membranes are not continuums and have molecular detail, as can be visualized with molecular dynamics simulations, such as in this representation.

The lipid molecules are now the solvent, and they act like polymers with their own chemical properties.
As outlined in Singer and Nicolson's paper, the membrane is the solvent for membrane embedded protein reactions. But here, the solvent has an incredible amount of complexity and strange properties that we have not thought about much when thinking about intracellular or extracellular reactions.
In the remainder of this lecture, we’ll go over the key factor leading to membrane complexity, and this is chemical composition.

While there is only one type of biological solvent that is considered important inside and outside of the cells - water, the membrane has infinite possibilities of chemical composition.

There is a range of requirements for a biological membrane. It must be within a certain range of thickness (~20-40 Å), and it must be fluid in whatever environment the cell or organism resides. It must be a barrier to protons and ions as we discussed. As long as these conditions are met, then there is an enormous range of chemical compositions that can satisfy the structure of a lipid bilayer.

This is demonstrated throughout biology. Here are the major chemical components of E. coli, humans, and an extremophile archaea that lives at pH 2 and 95 C. Notice that in these membranes, 95% of the lipids are actually attached at their acyl chains … technically forming a monolayer membrane that mimics a bilayer structure.

The question remains as to why this diversity exists, and why a particular organism has a set point of chemical composition. What happens when biology can choose the solvent in which its proteins reside?
Lipid subclasses. The blue highlighted regions show the major components of cell membranes.
There are 8 major types of lipid structures in biological membranes:

- **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

The nomenclature here is designated by the LIPID MAPS Lipidomics Gateway (http://www.lipidmaps.org), a database for lipid profiling data.
A glycerophospholipid has three major parts:
- Head group
- Fatty acid linkage
- Glycerol backbone

The glycerol backbone is chiral, and stereochemical numbering (sn) designates the attachment of the chains and phosphate.

A lysophospholipid is one in which one of the acyl chains has been removed.
The head-group substituent can range from a hydrogen in phosphatidic acid, to large groups like inositol.

Since there is a negative charge on the phosphate, a positive charge on the head-group leads to a zwitterionic species (choline & ethanolamine).

Neutral substituents lead to a negatively charged lipid: serine & glycerol (-1), inositol (-1).

cardioplin (-2) is a head group that is like a tandem of two lipids.

Inositol phosphates: PIP (-2), PIP2 (-3), PIP3 (-4). The position of the phosphate groups can be specified as PI(4,5)P2.
The ionization state depends on the pH, and electrostatics of the environment.
Glycerophospholipid structures

- The fatty acid linkage can either be ester linked or ether linked.
- Ether linked lipids are called plasmalogens, and have a tendency to form non-lamellar phases.
- Eukaryotes and bacteria typically have ester linkages, while archaea have ether linkages.
- However, ether linked lipids are found in mammals and humans, and appears higher in obese organisms and metabolic disorder.

Harayama & Riezman, 2018: https://doi.org/10.1038/nrm.2017.138
The fatty acyl chains can be of a wide variety.
• $C_{x:y}$ designates the carbon chain length and the number of double bonds, i.e. saturations
• The alpha end is next to the carboxyl group, omega is at the end of the tail
• For example, omega-3 fatty acid refers to a fatty acid with a double bond 3 carbons from the omega end
• The two acyl chains on a lipid can be different
• Acyl chains can have other modifications such as branching or cyclic groups
• Acyl chains can span the full length of the lipid bilayer membrane in tetra ether type lipids
• Generally chain lengths are between 12-22 carbons
Lipids containing a backbone of sphingoid bases
This includes ceramides, sphingomyelins, gangliosides
Form stable domains with cholesterol ("rafts")
• amphipathic, but small head group hydroxyl
• Significant effects on membrane structure and properties. Cholesterol acts as a buffer in maintaining membrane fluidity at low and high temperatures, and increases membrane thickness
• Shorter than typical membrane thickness

Harayama & Riezman, 2018; https://doi.org/10.1038/nrm.2017.138
Lipid biosynthesis. Schematic overview of the pathways involved in the synthesis of fatty acids (FAs), cholesterol, phosphoglycerides, eicosanoids and sphingolipids. The enzymes involved in catalysing steps in lipid biosynthetic pathways are indicated in red. (a) Glucose- or glutamine-derived citrate is first converted to acetyl-CoA by ACLY. (b) For FA biosynthesis, acetyl-CoA is converted into malonyl-CoA. The repeated condensation of acetyl-CoA and malonyl-CoA by the multifunctional enzyme FASN leads to the generation of palmitic acid, a fully saturated 16-carbon FA. The introduction of a double bond in the Δ9 position of the acyl chain by SCD generates mono-unsaturated FAs. (c) Subsequent elongation and further desaturation produces the repertoire of FAs with different saturation levels. (d) Essential FAs (ω3 and ω6 FAs) cannot be synthesised by human cells and need to be provided from dietary sources. (f) Phospholipids contain acyl chains and polar head groups derived from serine, phosphocholine or phosphoethanolamine. (i) Cholesterol biosynthesis is initiated by the conversion of acetyl-CoA to acetoacetyl-CoA. Addition of another acyl group by HMGCS produces 3-methylglutaryl-3-hydroxy-CoA, which is converted to mevalonate by HMGCR. Subsequent reactions result in the production of farnesoyporphosphate, an essential intermediate for protein prenylation. Cholesterol also forms the structural backbone for sterol hormones (cholesterol). Enzyme abbreviations: ACAT, acetyl-CoA acetyltransferase; ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; AGPAT, 1-acylglycerol-3-phosphate O-acyltransferase; COX1/2, prostaglandin-endoperoxide synthase (PTGS); DGAT, diacylglycerol O-acyltransferase; ELOVL, fatty acid elongase; FADS, fatty acid desaturases; FASN, fatty acid synthase; GPAT, glycerol-3-phosphate acyltransferase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; HMGCS, 3-hydroxy-3-methylglutaryl-CoA synthase; MTP, phosphatidylcholine:cholesterol acyltransferase; SPHK, sphingosine-1-kinase. Metabolite abbreviations: α-KG, α-ketoglutarate; CDP-DAG, cytidine diphosphate-diacylglycerol; CER, ceramide; DAG, diacylglycerol; FA, fatty acid; LPA, lysophosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIPx, phosphatidylinositol phosphate; PS, phosphatidylserine; S1P, sphingosine-1-phosphate; SPH, sphingosine; TAG, triacylglyceride.

- Key substrate: Fatty acids derived from synthesis from Acetyl-CoA from TCA cycle, and from diet
- Key substrate: Glycerol-3-phosphate from TCA cycle
Locations of lipid synthesis

- Free fatty acids
- Sterols are synthesized in the cytosol and ER
- However, phospholipids and other lipid types are synthesized in the membranes of different organelles, such as the ER, mitochondria, Golgi and peroxisomes
- Lipids are transferred to the plasma membrane and to different organelles by lipid transfer proteins (LTP)
Lipid synthesis and degradation

Harayama & Riezman, 2018; https://doi.org/10.1038/nrm.2017.138
Lipids are extracted with solvent and then analyzed by TLC, HPLC and mass-spectrometry.

Commonly used extraction procedures in lipidomics and the lipid classes they cover. Published methods include chloroform and methyl tert-butyl ether (MTBE) based extractions [23, 24, 28, 29, 30, 31, 32], butanol and butanol-methanol (BUME) extraction procedures [17, 25, 33, 34, 35], and an extraction procedure using acetic acid (AcOH) with isopropanol and hexane [9-11]. (L)PC, (lyso)phosphatidylcholine; (L)PE, (lyso)phosphatidylethanolamine; (L)PG, (lyso)phosphatidylglycerol; (L)PI, (lyso)phosphatidylinositol; (L)PS, (lyso)phosphatidylserine; AcCN, acyl-carnitine; FAHFA, branched fatty acid esters of hydroxy fatty acid; Cer, ceramide; So, sphingosine; (L)SM, (lyso)sphingomyelin; DG, diglyceride; TG, triglyceride; CE, cholesterol ester; (L)PA, cyclic phosphatidic acid; CL, cardiolipin; CerP, ceramide-phosphate; S(1)P, sphingosine-1-phosphate; BMP, bis(monoglyceride)phosphate; MG, monoglyceride; GluCer, glucosyl-ceramide; LacCer, lactosyl-ceramide; FA, fatty acid; oxPL, oxidized phospholipids; GL, glycerides; PL, phospholipids; PK, polyketides; PR, prenols; SL, sphingolipids.
Analyzing lipid composition - Thin Layer Chromatography

- Stationary phase (solid, or liquid supported solid). Typically silica gel or alumina
- Mobile phase (liquid or gas)
- $R_f = \text{distance travelled by component/distance travelled by solvent}$
- Separates molecules by polarity due to hydrogen bonding with the silica stationary phase
- A first step before moving to HPLC and mass spectrometry

Fig. 2. Chromatogram showing the separation of different lipid classes by TLC. First the plates were run to 5 cm from the bottom in chloroform–methanol–acetic acid (90:10:1, v/v/v). After drying the plates were run in hexane–diethyl ether–acetone (60:40:5, v/v/v) to 16 cm. Again, the plates were dried and then run in hexane–diethyl ether (97:3, v/v) to 19 cm. Reprinted with modification and permission from [52].

Fuchs et al., 2011; https://doi.org/10.1016/j.chroma.2010.11.066
**Lipid Composition**

**Figure 2**: Lipidomic survey of budding yeast. The top figure shows the relative proportions of different lipid types as a function of the physiological state of the cells as determined by where they are along the growth curve (inset). The lower panel illustrates that for each lipid type shown in the top panel, there is an incredible diversity of chemically related lipids that differ in tail length and degree of saturation. CL: cardiolipin; Erg: Ergosterol; IPC: inositolphosphorylceramide; MIPC: mannosyl-inositol phosphorylceramide; MIP2C: mannosyl-di-inositolphosphorylceramide; PA: phosphatidic acid; PC: phosphatidylcholine; PE: phosphatidyl-ethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: Tricaprylin; DAG: diacylglycerol; LPC: Lysophosphatidylcholine (Top panel adapted from C. Klose et al., PLoS One, 7:e35063, 2012; lower panel adapted from C. S. Ejsing et al., Proc. Nat. Acad. Sci., 106:2136, 2009.).

- Regarding acyl chains, you can see that the chains range from 12-20, but that 16/18 are the most common lengths.
- Furthermore, it is common to have one double bond in the chain. Unsaturated groups introduced “kinks” into the chains, decreasing lipid order.

Lipid Composition

Note, most bacteria do not make PC (but some do)

While we do not understand many of the reasons why there are differences in composition, these differences are generally important for cellular function, or protein function.

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**Table 10-1  Approximate Lipid Compositions of Different Cell Membranes**

<table>
<thead>
<tr>
<th>LIPID</th>
<th>LIVER CELL PLASMA MEMBRANE</th>
<th>RED BLOOD CELL PLASMA MEMBRANE</th>
<th>MYELIN</th>
<th>MITOCHONDRION (INNER AND OUTER MEMBRANES)</th>
<th>ENDOPLASMIC RETICULUM</th>
<th>E. COLI BACTERIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>17</td>
<td>23</td>
<td>22</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>7</td>
<td>18</td>
<td>15</td>
<td>25</td>
<td>17</td>
<td>70</td>
</tr>
<tr>
<td>Phosphatidylerine</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td>trace</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>24</td>
<td>17</td>
<td>10</td>
<td>39</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>19</td>
<td>18</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Glycolipids</td>
<td>7</td>
<td>3</td>
<td>28</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Others</td>
<td>22</td>
<td>13</td>
<td>8</td>
<td>21</td>
<td>27</td>
<td>30</td>
</tr>
</tbody>
</table>

From: The Lipid Bilayer

New York: Garland Science; 2002


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Included text: "Figure 3: Lipid synthesis and steady-state composition of cell membranes. Lipid production is spread across several organelles. The top panel shows the site of synthesis for the major lipid. The main organelle for lipid biosynthesis is the endoplasmic reticulum (ER), which produces the bulk of the structural phospholipids and cholesterol. The lipid composition of different membranes also varies throughout the cell. The bottom graphs show the composition out of the total phospholipid for each membrane type in a mammalian cell. As a measure of sterol content, the molar ratio of cholesterol to phospholipid is indicated. SM: sphingomyelin; R: remaining lipids. For more detailed notation see previous figure caption (Adapted from G. van Meer et al., Nature Mol. Cell Biol., 9:112, 2008.)


- Note that cholesterol is significantly higher in the plasma membrane
Distribution of lipids across the bilayer

• These are results from the plasma membrane of erythrocytes
• There is a slight preference for negatively charged lipids (PS & PI) in the lower leaflet, which follows the positive inside rule of proteins

Figure 10-5 The distribution of specific erythrocyte membrane lipids between the inner and outer face is asymmetric.
Lipid composition is tunable

Phospholipid compositions observed for various strains of E. coli, as measured at various growth phases or following culturing in unusual media. Example 1 is wild type strain SD12 during exponential growth. Example 2 contains an interrupted allele of PS synthase in strain AH930 during exponential growth. Example 3 is strain SD10, which contains a temperature sensitive PS synthase, during exponential growth. Example 4 is strain SD10 grown at stationary phase. Example 5 is a double mutant of strain SD312 containing a mutated phosphatidylglycerophosphate synthase and a defective CL synthase during exponential growth. Example 6 is strain CB64-CLI with a knockout of CL synthase during exponential growth. Example 7 is strain SD12 containing a temperature sensitive PS synthase and a defective CL synthase during exponential growth. Example 8 is strain SD12 grown under high D-mannitol conditions during exponential growth. Example 9 is strain SD10 grown under high mannitol conditions during stationary phase. Abbreviations: phosphatidylethanolamine (PE), phosphatidylglycerol (PG), cardiolipin (CL), phosphatidylmannitol (PM), diphosphatidylmannitol (DPM), phosphatidylserine (PS). This figure was adapted from (21).

- These results show the composition of E. Coli membranes under different forms of genetic control, or extreme growth media
- E. Coli has also been observed to change its composition with temperature, favoring shorter and unsaturated acyl chains at colder temperatures. However, the change is small, on the order of 2-3% of the total membrane lipids
We already know that eukaryotes and prokaryotes have some difference in their membranes. Prokaryotes do not typically make PC for instance.

There is another major divide in membrane composition that occurs between Archaea compared to eukaryotes and prokaryotes, and this happens at the initial point of lipid synthesis.
The last universal common ancestor (LUCA)

Archaea have distinctly different membranes than eukaryotes and bacteria. They contain plasmologens, ether linked lipids, as opposed to ester linked that is present in all other organisms.

It was previously thought that ether linked and ester linked lipids are not stably miscible (although this evidence is unclear).

Yet, both organisms contain homologous membrane proteins, like the FO/F1 ATPase pump exists in both types of organisms.

Therefore, one theory proposes that the last universal common ancestor (LUCA) must have contained a bilayer that was unlike either archaea or eubacterial membranes. It is postulated that a fatty acid membrane may have existed during the time when membrane proteins evolved.
This may go along with other ideas of early life and the origins of membranes. It has been proposed that life originated in an environment like the deep sea thermal vents. Here, porous mineral structures may have provided scaffolds to primitive fatty acid membranes, much like was designed in the Pfeffer cells.

However, formation of stable membranes must have occurred for these separate kingdoms of life to leave this environment and flourish on earth.
Converting *Escherichia coli* into an archaeabacterium with a hybrid heterochiral membrane

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Edited by Eugene V. Koonin, National Institutes of Health, Bethesda, MD, and approved February 27, 2018 (received for review December 12, 2017)

[Link](https://doi.org/10.1073/pnas.1721604115)