MEMBRANE FUNCTION, Part 1.

The Science and History of Our Understanding of Membrane Structure[1]

{Membranes are described in chapter 6 of Sadava. Note, though that the history of our views of membrane structure is not covered in this text. The current idea of "proteins floating in a sea of lipids" was not the first model to explain membrane structure – this notes packet gives a bit of background on earlier models.}

The cell theory established the central importance of the cell as the basic structural unit of independently existing life. Very few cells can be observed with the naked eye. Even with the light microscope details of cell structure escape observation unless the specimen being examined is properly treated. The secrets of the cell were only revealed when instruments and preparation techniques were developed which enabled investigators to see inside the cell.

Cytology, the study of cells, had two periods of rapid growth, each associated with advances in microscopy and microscopic technique. The light microscope did lead to the development of the cell theory, but the details of cell structure could not be appreciated until differential staining techniques were invented. Stains that selectively reacted with certain cell components revealed that the cell was more than a homogeneous blob of protoplasm separated into a nucleus and cytoplasm. In the late 1800s the development of staining technique suggested that the cell was more structurally complex than "a slice of banana (the nucleus) embedded in a mass of Jell-O (the cytoplasm)", but the remarkable level of cell structural complexity was not appreciated until transmission electron microscopy was developed. Its invention and development as a tool for examining the fine details of cell structure (starting in the 1940s) laid the foundation for our current understanding of both cell structure and function.

There is no way that we can cover the details of cytology in this course, but the principle of structure-function correlation at the sub-cellular level can be illustrated by a detailed examination of one structure - the cell or **plasma membrane**. The reason we will single out this structure is that many of the important activities of the cell are associated with membranes. Thus, knowledge of membrane structure and function is essential for grasping the principles of cell biology.

Use of models in science.

Models are **conceptual plans that relate component parts to each other.** A good model incorporates what is known about some entity and adds in the **best guesses one has about missing parts and/or the ways the parts inter-relate**. Models can be quite useful when the subject under study (the membrane in this case) cannot be observed directly. Models, like hypotheses, may turn out to be wrong. However, they serve a valuable purpose if they stimulate experiments and the search for confirmatory evidence. A good model will, like a good hypothesis, **generate testable predictions about the physical characteristics and performance of the system under study.** Don't forget though, that models are nevertheless hypotheses and should never be taken for more than what they are – they are not reality and should not be consulted to obtain the final word on some subject. Thus, as useful as they are, they **cannot substitute for measurement and experiment on the real system**.

Membrane Structure – Early Observations and Models

Although it could not be observed under the light microscope, early cell biologists quickly grasped that something must exist that effectively separates the inside of the cell from its external environment. They also realized that this structure would be more than a simple barrier since it obviously let some substances pass while it blocked others. Moreover, the rate at which it let materials pass often varied over time.

The first glimpse of the cell membrane's structure came in 1925 when **Görter & Grendel** found a way to release the contents of red blood cell, thus leaving only the membranes called erythrocyte ghosts. Analysis of these ghosts revealed the presence of lipids. Moreover, a calculation showed that there was enough lipid to encircle a red blood cell two times. Thus, Gorter & Grendell demonstrated that the plasma membrane quite likely consisted of two layers of lipid -- it was a **bimolecular layer**.

Here is a straightforward example of the marriage of quantitative chemical analysis (total lipid in erythrocyte ghosts) and mathematics to yield a very important result – that membranes must consist in part of a double, not single, layer of lipids.

A little later in the mid-1930s, **Schmidt** found that the rotation of polarized light by myelin sheaths (concentric arrangements of membranes around neurons) suggested the **presence of protein** in membranes.

In part, the work just cited lead **Davson and Danielli** to develop a model of membrane structure consistent with these two observations. Their model is as follows: The membrane is a bimolecular lipid layer, capped on the inside and outside by protein. To account for the easy movement through membranes by small molecules, they also postulated the presence of **protein-lined pores**.

The **Davson-Danielli model** can be thought of as a sandwich where the bread represents the outer and inner layers of protein and the sandwich filling the lipid component (however, this sandwich model is inadequate since it has no protein pores!).

Other Models in this course: We will see a number of other models; some are explicitly mathematical. Examples include the various equations of **population ecology** such as **exponential and logistic growth** and the simple **Gaussian competition model.**

Models and Teaching: Models are commonly used in teaching, especially to avoid dissection, vivisection, and lengthy set-ups. They can be very useful, both in saving animals and time, especially where a system is well understood. They are not complete substitutes for real experience, but for many students and situations, they are an efficient and useful way to learn.

Support for the Davson-Danielli model came from electron microscopy. Electron micrographs of tissue sections fixed in osmium tetroxide (a fixative that imparted an electron dense coat on surfaces) revealed membranes as solid dark lines about 75 Å (angstroms -- 10^{-10} m) thick. In 1959 **Robertson** used a different fixative and stained his specimens with potassium permanganate. This gave much better resolution. He found that membranes consisted of a light area about 35 Å thick surrounded by dark lines each 20 Å in thickness. The membranes in Robertson's electron micrograph were interpreted as consisting of a protein-lipid-protein sandwich, consistent with the Davson-Danielli model. Furthermore, all cellular membranes had the same structure that led Robertson to conclude that they were identical. This led him to his "unit membrane hypothesis" which stated simply that all membranes had essentially the same sandwich like structure.

Conflict – Problems with the Davson-Danielli Sandwich and Unit Membrane

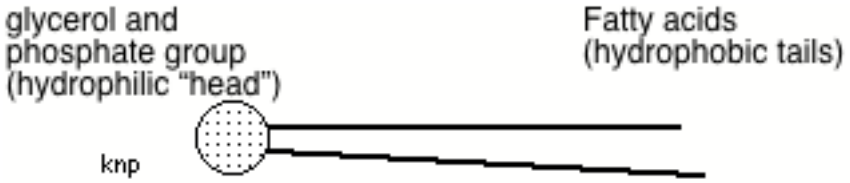
Despite the unifying effect of Robertson's unit membrane hypothesis, problems developed when it was found that each membrane-bound organelle had its own particular function. ***How could membranes vary in function if they all had the same structure?*** (Remember the notion of form and function). Variations in membrane thickness were also observed; this cast further doubt on Robertson's generalization. Furthermore, the Davson-Danielli model seemed inadequate to explain some membrane properties, e.g., how could lipid soluble material pass through membranes regardless of size if a protein cap protected the lipid?

Revolution in the Membrane World – The Fluid Mosaic Model
Singer and Nicholson

As evidence mounted which undermined the accepted model of membrane structure, Singer & Nicholson proposed a new model in 1972 that was more consistent with experimental data. This model, called the **fluid mosaic model**, emphasized the **dynamic** nature of membranes in sharp contrast to the static Davson-Danielli model. According to Singer & Nicholson:

- the molecular structure of the membrane is not rigid and fixed, but rather flows - especially the bimolecular layer of lipids.
- the lipids are not capped with a solid protein coating. Instead, proteins are dispersed throughout the membrane, leaving many portions of the lipid bare and exposed to the extra- and intracellular environments. It is through these bare areas that lipid-soluble molecules pass.

A crucial part of the model was the realization that the membrane lipids were typically di-glycerides with any of several types of phospholipid attached to the remaining carbon atom of the fat's glycerol (see macromolecule notes). The result was a fat molecule with two long hydrophobic "tails" and a more compact hydrophilic "head". Remember that these are often drawn like this:



- In addition to being attached to both lipid surfaces (peripheral proteins), proteins are also embedded in the lipid matrix itself (integral proteins).
- Transmembrane proteins extend throughout the entire membrane from the outer to the inner edges.

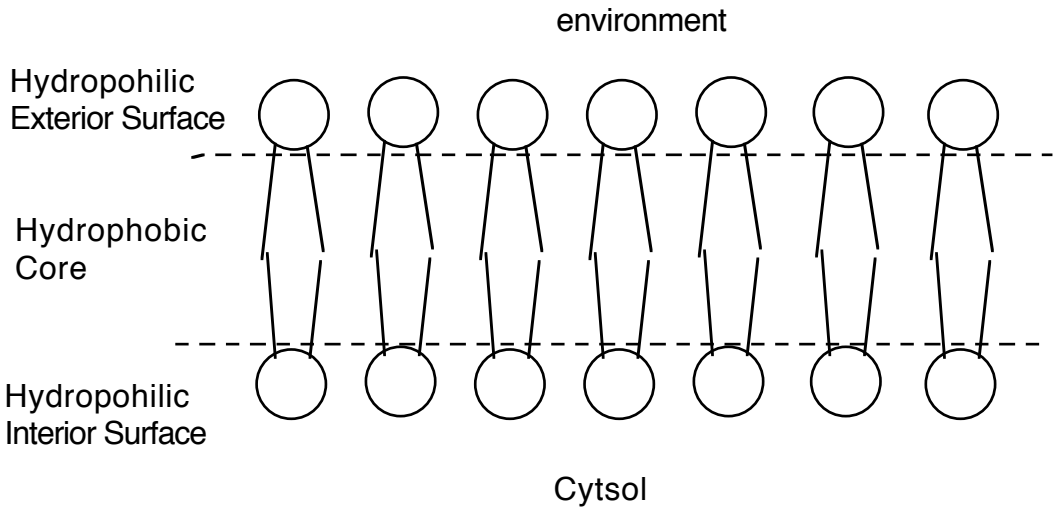
Some evidence for this model:
Behavior of lipids in water:
Phosphoglycerides with two fatty acids tend to self assemble into globular structures where the polar "heads" move to the interface with water and form polar bonds with it. These are called micelles (see macromolecule notes). The arrangement is stable because the lipid tails are excluded by the water – to the extent that if they try to leave the structure, the water excludes them and pushes them back towards the center of the micelle. Moreover, the non-polar tails interact with each other (weakly, albeit) via van der Waals interaction.

Micelles have many uses. One is that they can be made by detergents. The centers tend to hold onto non-polar (greasy materials applied to clothes from the

skin and food) while the entire structure is suspended in water. Thus, greases can be removed from clothes etc much better than with water only.

What would happen to a micelle if it were transferred from an aqueous solution to a non-polar solution?

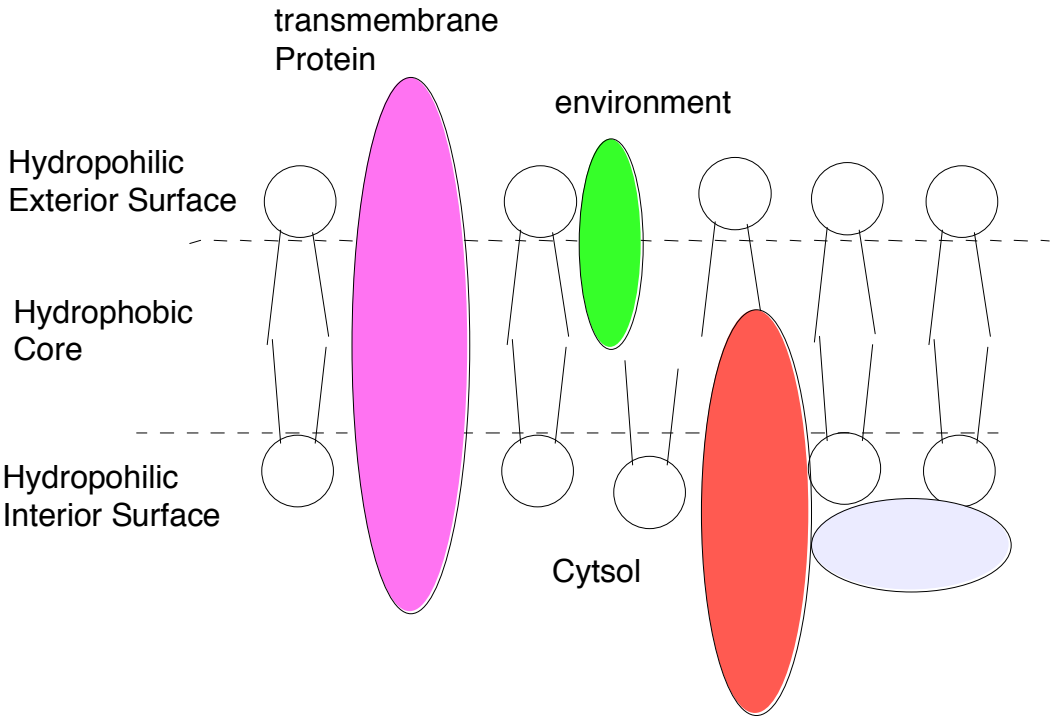
The same principles apply to the **lipid bilayer** and make it stable. On the outer and inner surfaces of the membrane are the polar heads of the diglycerides and the fatty acids make up the core:



What would be the effect of putting a lipid bilayer in a non-polar environment or adding a detergent?

Notice that in the model above, the individual diglycerides are constrained to remain in their layer – *they can neither flip to the other side nor can they leave the membrane*. Notice also that the structure is stable due to hydrophobic (van der Waals) and hydrophilic interactions. On the other hand, notice that an individual diglyceride is perfectly free to move within the plane where it is found. In fact, individual lipid molecules are not anchored down and they do move rapidly about – this is termed **membrane fluidity**.

Finally, let's add the proteins. The ones you see here can either be transmembrane, associated with the hydrophobic core, or located exclusively one side or the other. Notice also that these proteins do not need to be anchored – they are potentially free to move about in the membrane and many do. On the other hand, many are associated with the cytoskeleton and are more "locked in place".



What sort of features would the surface of a transmembrane protein need to remain in the membrane with two sides sticking into the aqueous cytosol and environment? (Check out Figure 6.3 in Sadava text)

What is the cytoskeleton, what is it made of it, where is it found? (See Chapter 3 in Sadava)

Characteristics of the membrane:

To a large extent, membrane specificity can be explained by the specific proteins contained in different membranes. Some proteins act as enzymes, others as signal receptors (we will discuss each of these later in the semester), and others as transporters to move ions or polar molecules across the membrane. Additionally, other proteins primarily have structural functions and serve as anchor points for materials in and outside of the cell; moreover, some also help to give the membrane a specific shape and rigidity which cannot be obtained from the lipid bilayer. **The exact characteristics of the membrane will therefore be determined by**:

1. the characteristics of the lipid bilayer, principally its fluidity
2. the types of membrane proteins that are present (primarily a question of gene regulation -- we will learn more about this later)
3. the membrane proteins' states of regulation (have the genes encoding these proteins been turned temporarily off or on? Again, more on this later in the semester)
4. the number of each type of membrane protein