Homework & Journal Club assignment (J. L. Robertson)

1. Read *Singer, S., Nicolson, G.* (1972). The Fluid Mosaic Model of the Structure of Cell Membranes Science 175(4023), 720-731. https://dx.doi.org/10.1126/science.175.4023.720

2. Identify 2 new hypotheses presented in the fluid mosaic model (note, there are many)

- Hypothesis 1 - Cell membranes are comprised of a "*mosaic structure of alternating globular proteins and phospholipid bilayer was the only membrane model among those analyzed that was simultaneously consistent with thermodynamic restrictions and with all of the experimental data available.*"
- Hypothesis 2 -The "*mosaic appears to be a fluid or dynamic one and, for many purposes, is best thought of as a two-dimensional oriented viscous solution.*"
- Hypothesis 3 - The Fluid Mosaic Model is "*applicable to most biological membranes, such as plasmalemmal an intracellular membranes, including the membranes of different cell organelles, such as mitochondria and chloroplasts.*" … BUT, only applies to "functional membranes" not myelin, or lipoprotein membranes of small animal viruses.
- Hypothesis 4 – "*phospholipids and proteins of membranes do not interact strongly; in fact, they appear to be largely independent*".
- Hypothesis 5 – "*while the largest portion of the phospholipid is in bilayer form and not strongly coupled to proteins in the membrane, a small fraction of the lipid is more tightly coupled to protein*", i.e. there are annular lipids that are different from bulk lipids.
- Hypothesis 6 – "*The globular protein molecules are postulated to be amphipathic as are the phospholipids"* and *"The amphipathic structure adopted by a particular … molecule, and therefore the extent to which it is embedded in the membrane, are under thermodynamic control; that is, they are determined by the amino acid sequence and covalent structure of the protein, and by its interactions with its molecular environment, so that the free energy of the system as a whole is at a minimum."*
- Hypothesis 7 – *"An integral protein molecule with the appropriate size and structure, or a suitable aggregate of integral proteins … may traverse the entire membrane".*
- Hypothesis 8 – "*functional cell membranes have a long-range mosaic structure with the lipids constituting the matrix.*". Should generally be no long-range order in a mosaic membrane with a lipid matrix, i.e. over the order of a few tenths of a micrometer or greater. Long range random arrangements of protein are the norm, wherever non-random distributions are found, there must be a protein-dependent mechanism arising from them.
- Hypothesis 9 – *"The physical or chemical perturbation of a membrane may affect or alter a particular membrane component or set of components".* This can lead to a redistribution of membrane components allowing for new thermodynamic interactions.
- Hypothesis 10 – Membrane protein aggregation or clustering as a signal for malignant transformation in cancer (as opposed to differential exposure of cryptic sites of agglutinin receptors).
- Hypothesis 11 – Cooperative phenomenon. An effect that is initiated at one site and transmitted to another remote site by some structural coupling between the two sites.

3. Describe the rationale and experimental evidence supporting the fluid mosaic model in contrast to previous hypothesized models

- **Hypothesis 1:**
  - A thermodynamic rationale is provided to make the connection that in order for hydrophobic and hydrophilic interactions to be maximized, that the most energetically favorable structure would be one where the hydrophobic portions of the membrane were buried and hydrophilic layers face the water. This suggests that the proteins, which contain hydrophobic regions, should also be embedded in the hydrophobic core in contradiction to the Davson & Danielli trilaminar model proposing proteins monolayers on the lipid surfaces, which is not thermodynamically stable.
  - There are two types of proteins that are observed to associate with cell membranes. The first class are easy to remove, will dissociate free of lipids and remain soluble/folded. The second class, which is the majority with over 70%, require extreme measures to isolate (detergents, bile acids, denaturants, organic solvents), remain associated with lipids and tend to aggregate when lipids are removed. This indicates that there are two different physical behaviors of membrane proteins, in line with ones that would be peripheral and ones that are integral, or membrane embedded. In contrast, the Davson & Danielli model would not necessarily predict these two distinct classes of behaviors.
  - Integral membrane proteins are heterogeneous, i.e. there is not one predominant type. This is not supportive of a specialized layer of protein that is critical to the membrane structure.
  - Integral membrane proteins have higher alpha helical content, as measured by circular dichroism, compared with known soluble proteins.
  - Differential scanning calorimetry of intact mycoplasma membranes shows similar phase transition profiles to the membranes formed from lipid extraction of the same cell types. This is also supported by x-ray diffraction and spin label dynamics studies. This results is not in line with the previous Benson model (did not discuss, but presents lipoprotein particles that are repeated along the barrier), which would predict distinctly different properties of the lipids in the membrane compared to the lipid bilayers formed from the lipid extracts.
  - Dynamics of lipids from one side of the membrane to the other is slow, in line with the thermodynamic presentation of a lipid bilayer structure.
- **Hypothesis 2:**
  - Under physiological conditions, and except for myelin, functional cell membranes are fluid rather than crystalline. Here they mean that crystalline is ordered. This is supported by EPR spin-labeling experiments, x-ray diffraction and DSC.
  - Also see **Hypothesis 8**.
  - Membrane lipids of poikilothermic organisms contain a larger fraction of unsaturated fatty acids the lower their temperature of growth.
- **Hypothesis 4:**
  - Little change in DSC for membranes with protein or membrane extracts that contain mostly lipids.

    - Enzymatic release of phosphorylated amines from intact erythrocyte membranes perturbs the physical state of the fatty acids, but not the secondary structure of phospholipase C.
- **Hypothesis 7:**
    - Freeze-etching experiments showing protrusions and divots in partner leaflets.
    - Exposure of integral proteins at both intracellular and extracellular membrane surfaces, by proteolysis or chemical labeling.
- **Hypothesis 8:**
    - Distribution of protein molecules would be expected to be ordered if the protein was the scaffold or matrix. Electron microscopy of flattened human erythrocyte membranes examining antigen membrane proteins indicate that they are randomly distributed. While ferritin showed clustering, the clusters remained random. Freeze-etching also shows the same random distributions.
    - If the membrane consisted of integral proteins dispersed by the lipid bilayer matrix, the membrane would be a two-dimensional liquid like solution where the protein was dissolved in the lipid bilayer. Thus, the mosaic structure would be dynamic. Integral proteins would be expected to diffuse at rates determined in part by the effective viscosity of the lipids. On the other hand, if the protein was the matrix, then they predict that the long-range structure would be static. There would be large activation energies for proteins to diffuse, and so no diffusion is expected from a protein matrix.
    - Temperature dependence of x-ray diffraction also support rhodopsin particles being in a planar liquid-like state in the membrane, despite the high density of protein and the appearance of periodicity in the diffraction (i.e. long-range ordering). Adsorption of BSA alters the rhodopsin distribution, showing perturbation or rearrangement of molecules, which contradicts a model in which the rhodopsin molecules form a tight lattice structure.
    - Frye and Edidin studies. The proteins distributes across the entire membrane, and yet the membrane barrier still persists. This argues that it is not the protein that serves as the barrier matrix. Lowering the temperature decreased the mixing rate. This is in line with a fluid membrane, and that it is not the proteins comprising the matrix, unless the mechanism involved the proteins would being removed and rebuilt.
    - Mindich studies demonstrated that protein and lipid ratios can be varied widely while preserving membrane properties. This contradicts a fixed protein matrix.

- **Hypothesis 11**: Trans cooperativity refers to allostery, i.e. changes in shape. Here they refer to a change in the membrane (i.e. membrane + protein) at one location that is then transmitted to another location in the membrane + protein. This may refer to a membrane protein aggregate/oligomer binding a ligand on one side of the membrane, inducing a conformational rearrangement changing the function.

- **Hypothesis 11:** Cis cooperativity, refers to changes produced over the entire membrane. For example killing effects of bacterial lysins, growth hormone, fertilization (?). Monod-Wyman-Changeeux allosteric model of protein cooperativity has been extended to the membrane in a similar way. Proteins exist in two states, one with a higher binding affinity

than the other. The binding of a single ligand to one protein couples to the change in conformation of the other proteins. In the fluid mosaic model, this can be coupled to the aggregation or self-assembly of the proteins/receptors to provide the cooperativity. This would predict that the cooperativity would have kinetics corresponding to the diffusion of the molecules in the viscous membrane. However, in the lipoprotein model, it is expected that the cooperativity would happen much faster. Colicin cooperativity happens on minute time scales, much slower than would be expected for the other model by ms.

4. Identify a limitation in their logic and propose an experiment that could test their hypothesis further.

- Hypothesis 1: One of the rationales is that the membrane thickness is 75-90 Å. Not sure what thickness this is referring to? => It would be important to examine the membrane thickness broadly across many organisms and organelles to make this generalization, as well as image reconstituted membranes.
- "*None of the evidence so far obtained for the bilayer form permits us to say whether the bilayer is continuous or interrupted*". The broad phase transition profiles allows for the possibility of different membrane phases, such that the cooperative unit is small, on the order of 100 lipids => prediction of lipid rafts! Microscopy studies of smaller phases separated regions by super-resolution microscopy.
- "*None of the experiments mentioned above is sufficiently sensitive and quantitative to prove whether 100 percent of the phospholipid is in the bilayer form*". They speculate that as much as 30 percent of the lipid could be in a different state. Prediction of lipid droplets and lipoparticles.
- Hypothesis 4: Many membrane proteins require certain lipids for expression of their activity, suggesting lipids do interact with proteins. => Examine whether these are truly specific. Do these lipids act as ligands following specific association isotherms?
- Hypothesis 5: They state that there is no satisfactory evidence for such as distinctive lipid fraction at that time. => EPR studies to examine changes in lipid dynamics around proteins vs bulk.
- Hypothesis 1: They indicate that certain protein interactions are not considered in their model, such as aggregation, a.k.a oligomerization, or interaction of integral membrane proteins with a peripheral protein that could be attached to the cell exterior (or interior). => EM to visualize connections of proteins to cytoskeleton or external matrix, studies of oligomerization/aggregation in membranes by fluorescence microscopy (e.g. single-molecule FRET or photobleaching).
- Hypothesis 1: Their model indicates a rather uniform mosaic membrane, but they state that they are not considering specific lipid interactions with the integral membrane proteins that may lead to altered thickness across local regions in the membrane. They therefore present their cartoon of their model as an average of the cell membrane, that does not provide local resolution. => SANS or other membrane structural methods with and without protein. Dependency of protein conformation on global membrane thickness.
- The Fluid Mosaic Model does not elaborate on which component is the mortar and which are the bricks in the membrane. I.e. what is the matrix or the solvent?

=> they outlined many experiments to test whether properties of the membrane depend on lipid viscosity.

- Hypothesis 8: There is no evidence that short-range order does not occur, and may arise via protein-protein interactions. => Again, study protein-protein interactions in membranes by microscopy or other approaches.
- Hypothesis 8: Order is observed in certain cellular environments. For instance, synapses, and the 2D crystalline lattices of bacteriorhodopsin. => But do larger components, i.e. clusters diffuse as if they are in a fluid membrane?
- Some experiments suggest that lipids are not readily interchangeable between membranes, arguing against free diffusion. Wilson and Fox studied induction of beta-galactoside and beta-glucoside transport systems in E. coli that cannot synthesize unsaturated fatty acids. Feeding fatty acids in the cell medium allowed for control of oleic (C18:1) vs. linoleic (C18:2). The cells have a temperature dependent response of the transport rate that depended on oleic vs. linoleic incorporation into the membrane. If linoleic acid was introduced for a short pulse after growth on oleic media, then the transport would appear like linoleic, indicating that linoleic acid was incorporated locally and not mixed. But, this does not seem like a controlled experiment…. The transport proteins may interact strongly or specifically with the new lipid. What are the expected kinetics of lipid exchange in a fluid membrane? What about other changes that could occur in the cell that might confound the interpretation of these results?
- Membrane asymmetry, e.g. oligosaccharides. Organization of different lipids in the leaflets indicates some special non-random organization of lipids. => Still, if fluidity is demonstrated in each leaflet it would support 3D organization but fluid behavior in 2D.
- Intracellular membranes. Frye and Edidin's results present the fluid nature of the outer plasma membrane, but there is not much evidence of inner membranes. => do all of these studies in membranes extracted from intracellular organelles. Can use sucrose gradients to separate out membrane fractions.
- Differential hypotheses for Hypothesis 10 – Membrane protein aggregation or clustering as a signal for malignant transformation in cancer (as opposed to differential exposure of cryptic sites of agglutinin receptors). => Testable by ferritin conjugated agglutinins. Mild proteolysis with malignant transformed cells would be observe to form clusters by EM.
- Hypothesis 11 – cis type cooperativity in signaling and colicin effects. => Would be affected by changes in the fluid properties of the membrane, either temperature of lipid composition.
- Cell-cell and sell substrate interactions may be an alternate hypothesis. Involves apposition of intense local electric fields => would this be dependent on electric shielding and the salt concentration?
- Multivalent antibody binding alters the distribution of the antigen in the membrane in an non-native way => test other labeling methods.

5. Come prepared to discuss your reading and rationale on Thursday.