Membrane Proteins

- By number of molecules, there are more lipid molecules in the membrane, because a lipid molecule is smaller than a protein.
- In a typical plasma membrane, there are 50 lipid molecules to each protein molecule.
- Schwann cells or oligodendrocytes wrap layer upon layer of their plasma membrane around the axon – It is called Myelin sheath.

Audio:
Although the properties of lipids are important for the function of membranes, many of the properties of membranes are due to the presence of membrane-associated proteins. In the last lecture we saw that different membrane organelles and systems had differences in their abundance of specific lipids and similarly, different membrane types and amounts of proteins. Lipid molecules generally outnumber proteins in a typical membrane with ratios of about 50 or more to 1. However, since the lipids are very small, the total mass of protein in a membrane may be greater than that of the lipid molecules. Myelin is a membrane system formed by supporting cells of the nervous system called Schwann cells. This membrane system forms an electrically insulating membrane barrier around nerve cells and it’s more than 75% lipid in composition. At the other extreme, the inner membrane of mitochondria is about 75% protein and most membranes are somewhere in the middle of this spectrum with about a 50-50 ratio of lipids and proteins.

Types of Membrane Proteins

Proteins interacts with membrane in three ways
- Integral proteins: transmembrane proteins
- Anchored proteins: cytosolic and non-cytosolic
- Peripheral proteins: cytosolic and non-cytosolic

Audio:
This slide shows an overview of the various classes of proteins found associated with membranes, which are distinguished by how they attach to the membrane. Integral proteins are embedded within the bilayer by hydrophobic interactions. These proteins may be associated with only one face of the bilayer, or they may be transmembrane proteins which cross both lipid layers. Anchored proteins are attached to the membrane through a linkage of some sort, such as through covalent attachment to glycolipids. Finally, peripheral membrane proteins are loosely attached, either to the lipid membrane surface or to another more tightly associated protein. Anchored and peripheral membrane proteins do not necessarily need to have hydrophobic domains because they don’t interact directly with the membranes.
Integral proteins – Three transmembrane proteins

- Single-pass transmembrane protein (could also have a lipid anchor as shown in 1)
- Multi-pass transmembrane protein
- B-barrel protein on the outer membranes

Audio:
I’m going to go through the various types of proteins in some detail, discussing how they associate with the membrane and the implications of that type of association for the functional role of the protein. Integral proteins are proteins that have significant regions of hydrophobicity, which attach the protein to the membrane. Some of the most important are the transmembrane proteins, because these are proteins that allow the inside and outside of a cell to communicate and interact with each other. Transmembrane proteins can have a variety of arrangements as shown on this slide. The simplest have a single alpha-helical coil of hydrophobic amino acids, which traverses the lipid bilayer. These proteins can also be covalently linked to lipids within the membrane, as shown in part 1 of this figure and this tends to further stabilize the membrane protein within the membrane. More complex proteins may have several alpha-helical coils, each of which traverses the membrane, as shown in part 2. Transmembrane proteins can also take the form of a beta-barrel and you can think of this configuration as being a two dimensional sheet of protein which is then rolled up into a tube. Many channel forming proteins are beta barrels and they allow regulated movement of material through a pore that’s in the middle of the barrel.

Slide #4

Characteristics of Integral proteins / Transmembrane proteins

- Usually β-helix; contains 20-25 aa; hydrophobic aa (green)
- Amphipatic: the cytosolic part and the exoplasmic part are usually hydrophilic

Audio:
The alpha helical domain of transmembrane proteins share a number of common features. The membrane-spanning region is generally between 20 and 25 amino acids in length because shorter proteins don’t form helices long enough to pass through the entire membrane. The amino acid composition of helical regions that pass through a membrane primarily consist of non-polar amino acids. A chart from chapter three of your book is shown on the upper right hand side of the slide and you can see that most of the amino acids, shown in the membrane-spanning domain of the protein on the left of the slide, are in fact, non-polar amino acids. Computer programs can conduct statistical analyses of the amino acid sequence of proteins and predict the likelihood that a region of protein is hydrophobic or hydrophilic, based on the concentration of polar and non-polar amino acids in the region. This type of analysis generates what’s known as a hydrophobicity plot. The example on this slide is the hydrophobicity plot of bacteriorhodopsin, which has seven membrane-spanning regions and you can see that on the chart. This type of analysis can be done on the genomic level and the results of such an analysis suggest that at least 20% of all proteins in our bodies have membrane associations of this type. Parts of proteins that enter into the cytoplasm, or external environment on either side are generally hydrophobic and contain a higher concentration of polar amino acids. Therefore, just like lipids, most
transmembrane proteins are amphipathic and have both hydrophobic and hydrophilic regions

Slide #5

Multi-pass transmembrane (integral membrane) proteins

- Transmembrane α-helices often interact with one another.
- Most helices pass entirely through before turning around, but there are also cases, where helices turn around after going through one leaflet (aquaporin).

7 helices bacteriorhodopsin (light driven pump); retinal chromophore (purple)

2 helices Aquaporin water channel are only half way through the lipid bilayer

Audio:
Most of the time, alpha helical domains of transmembrane proteins go entirely through the membrane and bacteriorhodopsin is an example of this, having 7 transmembrane domains, all of which go entirely through the lipid bilayer. However, some transmembrane proteins have regions in their interior which are protected from the hydrophobic membrane by the layers of surrounding protein. This environment allows protein loops that can be hydrophilic to form inside the membrane itself and it allows the proteins to make turns within the bilayer. This greatly increases the flexibility these proteins have in generating functional domains. Because individual alpha helices are connected by flexible non-helical linkages, the transmembrane helices can also shift their position relative to each other fairly easily and consequently, many transmembrane proteins that need to change their shape in a large way in order to perform a function, are going to made up of multiple alpha helical coils connected to each other in this way.

Slide #6

B barrel proteins

- B barrels formed from different numbers of β strands.
- Makes relatively large pores called porins (lets molecules in size of 10 kD)
- Porins are on the OM of bacteria, mitochondria, chloroplasts

Audio:
So we just discussed proteins that have multiple alpha helical coils, which can shift their position relative to each other. We’re now going to talk about beta barrels which are relatively stiff and unchangeable in their form. Beta barrels are formed from two dimensional sheets, or beta sheets of protein. The diagram of the way beta sheets are formed, shown on the top right of this slide, is from page 135 of your book. The individual strands of a beta sheet are ten, or even fewer amino acids in length and these strands can be constructed so that nonpolar amino acids face out and polar amino acids face in, and therefore beta barrel transmembrane proteins cannot be identified by a hydrophobicity plot, so it’s not clear how many of these proteins exist in a genome. The hydrophilic interior allows these proteins to have complex structures on the inside that can interact with elements found in the cytoplasm or extracellular environment. In addition, beta barrel proteins can be very large. For example, a number of internal membranes contain large pores made out of beta barrel proteins, called porins that facilitate movements of metabolized and other large molecules across the membrane. However, since the beta barrel, again, is
relatively rigid, these proteins cannot change much in their shape and so proteins that have to undergo large changes

Slide #7

**Anchored Proteins**

Linkages:
- **Cytosolic:**
  - Amide linkage between myristic acid and an N terminal glycine
  - Thioester linkage between palmitoyl or prenyl group and terminal cisteine
- **Exoplasmic:** GPI linkage between protein and glycosylphosphotidylinositol

Audio:
Entirely water soluble or hydrophilic proteins can also associate with membranes in a number of ways. The first, shown on this slide, is the attachment to the membrane using a covalent linkage to a membrane-soluble hydrocarbon chain. As shown on this slide, several linkage types are possible. Some proteins are attached by an amide linkage, formed between an N-terminal glycine and a fatty acid, and other proteins use a thioester linkage, which forms between an terminal cysteine and fatty acids. Finally, proteins found on the outside of cells are frequently found attached to the lipid glycosylphosphotidylinosital, which fortunately is generally abbreviated “GPI”. GPI linked proteins are attached to membranes in the endoplasmic reticulum and we’ll see how this happens in our lecture on the endoplasmic reticulum. Because anchored proteins do not cross the membrane, their activity is restricted to one side of the membrane or the other. Often, however these proteins are involved in coupling signals generated by other transmembrane proteins to regulatory mechanisms within cells and we’ll discuss specific examples of this when we cover cell signaling in the third part of this course.

Slide #8

**Peripheral proteins interacting w integral protein by ionic interactions**

- 7. Cytosolic peripheral protein
- 8. Extracellular peripheral protein

How do one distinguish between peripheral proteins and integral proteins?
- Peripheral proteins are released by high concentration of salt, which disrupts ionic interactions
- Detergents are needed to release integral proteins and hydrocarbon-anchored proteins

Audio:
Finally, some proteins associate with membranes through attachment to other proteins, as shown on this slide. These proteins can be identified experimentally by their relatively weak ionic association with membranes, which is easily disrupted using aqueous solutions of varying ionic strength, such as a high salt concentration. In contrast, integral and anchored proteins need to be isolated from membranes using detergents, which have to disrupt the integrity of the membrane
Common Post translational modifications

- If membrane proteins are glycosylated, carbohydrates on the exoplasmic side
- If membrane proteins have disulfide bonds, they are on the exoplasmic side. Sulfhydryl groups in cytosol side do not form disulfide bonds because cytosol is a reducing environment

Audio:
Once proteins are associated with membranes, they can undergo several types of modifications, including the addition of further linkages to carbohydrate molecules, like oligo and polysaccharides, as well as the formation of disulfide bonds, which may stabilize the shape of the protein. These modifications are generally found only on the exoplasmic proteins and not on cytosolic proteins and this is because the addition of carbohydrate molecules to proteins happens within the lumen of the endoplasmic reticulum and the golgi apparatus, which as we’ll see when we discuss these organelles, always end up on the outside of the cells after secretion. In addition, disulfide bonds are rare within the cytoplasm because the internal cytoplasm is a reducing environment, which breaks disulfide bonds. The glycosylation of exoplasmic proteins, produces a layer of carbohydrates on the outside of the cell, that coats the outer surface of the cell and this is known as a glycocalyx. The dark, fuzzy line, shown in the image on the right of the slide, shows this coating on an actual cell. The coating is involved in several very important cellular processes including cell protection, cell attachment to things in the outside environment and cell to cell recognition. For example, proteins that bind to carbohydrates, known as lectins, are involved in the interaction of cells during blood clotting, inflammation responses and egg fertilization.

Methods to study protein lateral diffusion

Audio:
When we covered lipids, we spent some time discussing their movement within the membrane. Proteins also move within the membrane and there are mechanisms that modify this behavior. Of course, movement across the membrane is rare, but rotational and lateral movement of membrane proteins is common. The diagram on this page shows the results of one type of experiment that can be used to study the movement of proteins within a membrane. Two different types of cells, each with unique membrane associative proteins are fused to create a single, larger cell. Initially, proteins unique to the individual cells are found in localized areas of the fused cell, but they rapidly disperse over the entire cell surface. On the left of this slide is data from this type of experiment that was published in a paper in the journal of Cell Biology. The images show fluorescently labeled red blood cells, incubated with a larger, non-fluorescent cell. The images on the left show the cells under normal light conditions and the images on the right show the fluorescent probe. The pair of images on the top shows incubation in the absence
of the fusion of the cells and the pair at the bottom shows the same set up, in the presence of factors that promoted the fusion of the two different types of cells and you can see that in the bottom set of images, the fluorescent marker has begun to transfer from the red blood cells to the larger, initially non-fluorescent cell.

**Slide #11**

**Fluorescent recovery after photobleaching (FRAP)**

Movie: First part- complete recovery, protein is totally and freely mobile. Second part: no recovery. Protein is stationary and stable

**Audio:**
Another way to study the movement of membrane proteins is by labeling them with fluorescent probes and then bleaching the probe in a localized area using a laser. There are two strategies for doing this. The first is known as fluorescence recovery after photobleaching or FRAP. With this method, the area that is bleached is small and the larger surrounding cell contains large amounts of unbleached protein. Immediately after the bleaching event, the bleached area would have no proteins that were fluorescent, however over time, the bleached proteins begin to move out of the bleached zone and unbleached subunits move into it and you can monitor this recovery of fluorescence in the bleached area with a video camera. Analysis of this kind of data produces the graph at the top right of the screen. The video shows an example of this type of experiment. In this particular instance, the recovery curve eventually reaches its initial value, before bleaching, which means that the area of the bleach was relatively small and the movement of the proteins was not restricted in any way.

**Slide #12**

**Fluorescence loss in photobleaching (FLIP)**

FLIP diagrams

**Audio:**
FLIP is another bleaching method that can be used to study the movement of fluorescently labeled components in cells. FLIP stands for “fluorescence loss in photobleaching”. With this method, one area of the cell is bleached over and over, and the intensity of fluorescence in a neighboring area is monitored, instead of the fluorescence in the area that’s being bleached. If fluorescently labeled proteins can move between the two regions, there’s going to be a gradual loss of fluorescence in the area that’s not being bleached and this is because the subunits are moving into that bleached area and being bleached. If all of the fluorescently labeled proteins in a cell move freely, they will all eventually move into that area and be bleached and the entire cell will eventually become dark, as shown in this diagram. The method does have some advantages over FRAP. For example, the fluorescent proteins which are being looked at or measured are not the ones that are being exposed to the potentially damaging laser light.

**Slide #13**

**Limited recovery after FRAP**
Diagrams of membrane micro-domains, bleached zone, as well as a graph.

**Audio:**
The graph shown on the previous two slides show complete recovery or complete loss of fluorescence during the FRAP and FLIP experiments, and that’s actually not a very typical result. The graph on this slide shows results of a FRAP experiment in a more common situation. Here, the curve in the recovery phase does not reach its initial, pre-bleach value. Instead, only 60% recovery is seen and this indicates that 40% of the proteins in the area surrounding the bleach zone were not able to move into the bleached area or alternatively, that 40% of the bleached subunits were unable to move out of the bleached area. There are several possible explanations for this, one of which is shown on the right hand side of the slide. Here, the membrane is not continuous, but rather it is divided into discrete micro-domains. Membrane components can move freely within the domains, as shown by the thick, solid arrows, but crossing from one domain to another is infrequent, as shown by the thin, dashed arrows. We’ll see at the end of this lecture that such domains are actually common in real cells. The video shows an example that mimics this type of result. The movie is showing photobleaching of a fluorescent protein, expressed in the membrane of a bacterium. The protein is mobile, so eventually the whole bacteria becomes evenly labeled after the bleach event. However, the bleached area is large and the bacteria is small and so the bleaching makes a large fraction of the total proteins non-fluorescent. As a result, the entire cells ends up being uniformly labeled, but dim and you can sort of this bacteria as a model for a single micro-domain in a larger cell.

Slide #14

**Methods for restricting the movement of proteins in membranes**

A: aggregation
B: tethered from outside;
C: tethered from inside,
D: tethered by another cell

Distribution of proteins in a polarized epithelial cell
- Protein A on the apical side
- Protein B on the basolateral side

**Audio:**
Proteins may be very large structures and their lateral movement within a membrane can be restricted simply by their size to a value of over a thousand times slower than that of the surrounding lipid molecules. However, just slow movement of proteins doesn’t create specialized membrane domains and those specializations are important for cells to perform their functions. So, as a consequence, cells have several strategies of further restricting the movement of proteins and they’re shown here and on the next few slides. For example, proteins may self-aggregate as shown in picture A, or be linked together on either the outside or inside of cells by other proteins as shown in B and C. Proteins can also be localized at contact points between cells, like that shown in D, or between a cell and the surface that it’s attached to. Another strategy is shown by the diagram at the right of the slide. Here, a polarized epithelial cell has a specialized membrane at its top or apical surface. The boundaries of the apical membrane are
defined by structures called tight junctions, which are formed by contact between two cells and these restrict the movement of proteins and even lipids from the apical surface to the sides of the cell. A unique lipid composition of the apical membrane is probably needed to provide cues that promote the delivery of the green labeled proteins A to the surface and the tight junctions then also restrict the movement of the A proteins into the lateral, and ultimately bottom of the cell. In contrast, protein B, which is found in the basal and lateral membrane surfaces also cannot move into the apical membrane, and as a result, these two membrane domains are specialized and unique.

Slide #15

Lipid Raft: micro-membrane domain

- Thick (thicker than 5nm) ordered membrane domain
- Rich in sphingolipids, glycolipids and cholesterol
- Rich in GPI-anchored proteins and proteins with a longer transmembrane region
- Important for membrane trafficking (endocytosis) and receptor-mediated cell signaling.

Audio:
Membrane rafts, which we’ve already discussed, are another structure that can restrict the movement of proteins into or out of a unique area. As we saw in the last lecture, membrane rafts are thicker regions of the membrane that are relatively stable and are created by including special mixtures of lipids, such as sphingolipids and glycolipids in a restricted membrane area. The unique composition of a lipid raft may promote the recruitment and retention of unique membrane proteins. Localized concentrations of membrane proteins are, in turn, important for numerous cell processes, such as endocytosis, where cells engulf membranes along with extracellular fluid or even other cells, as well as in signaling events, and we’ll be looking at all of this in more detail, later on in this course.

Slide #16

Creation of membrane “corrals” by cytoskeletal filament proteins

Diagrams and movie clip

Audio:
Finally, one of the first and best studied examples of the restriction of membrane proteins is shown in this slide. Red blood cells have a network of cytosolic protein filaments, which is attached to their cell membranes and support the membrane, giving the cell its unique shape. The arrangement of supporting proteins is shown at the top right of this slide, and diagramed in red at the bottom left. The presence of these proteins creates membrane micro-domains which are called corrals. Transmembrane proteins move rapidly and freely within an individual corral, but crossing from one corral to the next happens infrequently. The image at the bottom right shows the path of a protein within this membrane system. The protein spends most of its time moving around within several highly restricted areas, each of which is indicated by a different color. The video shows raw data for the movement of a gold particle, attached to a single red blood cell transmembrane protein, called Band 3. What you should look for as you while you watch the video is the movement of the gold particle over very short distances in one spot and then a sort
of, longer hop as the gold particle, the protein attached to it moves from one corral to the next.