Membranes #1
Sheetz, Core 2004

Paper for Friday (those who have not done a paper yet should do this one)
Yun Liang, Wei Yu, Yan Li, Zhenye Yang, Xiumin Yan, Qiongping Huang, and Xueliang Zhu. (2004) Nudel functions in membrane traffic mainly through association with Lis1 and cytoplasmic dynein. J. Cell Biol. 164:557-566. http://www.jcb.org/cgi/doi/10.1083/jcb.200308058

Suggested Readings:
David Boal, Mechanics of the Cell Cambridge Press, Chapters 5 and 7.
M. Edidin, (2003) The State of Lipid Rafts: From membranes to cells. Ann Rev Biophys Biomol Struct
Vas, V. and K. Simons (2004) Lipid Rafts. Ann Rev Biophys Biomol Struct (on line as prepub)
M.P. Sheetz, (2001) Cell control by membrane-cytoskeleton adhesion. Nature Rev.: Molec. Cell Bio. 2:392-395.

Lipid Bilayer Membranes

One of the critical features of cells is that they are separated from the surrounding environment by a barrier that allows them to preserve their identity, take up food and give off waste. For eukaryotic cells the barrier is typically a plasma membrane composed primarily of glycoproteins and a lipid bilayer that is 5 nm thick. The lipid bilayer forms the permeability barrier and glycoproteins are responsible for regulating the traffic of material to and from the cytoplasmic space. We will talk about the important physical features of the plasma membrane.

Hydrophobic Effect and the Bilayer Phase

The physical basis of the lipid bilayer membrane is the hydrophobic effect, i.e. the inability of hydrocarbons to hydrogen bond with water. At the interface between hydrocarbon and water, there is a higher energy state for the water because hydrogen bonds are lost. It is energetically favorable for the hydrocarbon to associate with hydrocarbon and to minimize the surface area of contact with water. Amphiphilic compounds such as fatty acids have hydrocarbon regions and hydrophilic regions. Fatty acids can stabilize hydrocarbon in water by covering the hydrocarbon surface with their hydrophilic regions while their hydrocarbon regions associate with hydrocarbons, i.e. like associates with like. When fatty acids (soaps) are suspended in water, they form micelles, spherical complexes of tens to hundreds of fatty acid molecules with hydrocarbon on the inside and carboxylic acid groups on the outside. The size of the micelle and its shape are determined in part by the relative surface area occupied by the hydrocarbon and hydrophilic portions of the molecules. When the hydrophobic portion is much smaller in area than the hydrophilic, micelles tend to be small with a high

curvature. When the two portions are approximately equal, micelles are much larger and have reduced curvature. In terms of free energy, the mixing of hydrocarbon and water causes a decrease in entropy (another way of saying this is that the entropy of water is decreased at the interface between hydrocarbon and water). A practical consequence is that hydrophobic interactions are stronger at high temperatures than low (this is why microtubule assembly occurs preferentially at high temperatures).

To create a hydrophobic barrier around cells, nature has developed phospholipids, which have nearly equal areas of their hydrophobic and hydrophilic portions. Phospholipids typically have two fatty acid chains esterified to a glycerol backbone and on the third glycerol hydroxyl there is a phosphate group which is derivatized with a hydrophilic moiety such as ethanolamine, choline, inositol or serine. Bilayers are two dimensional complexes of lipids with their phosphate groups at the water-bilayer interface and fatty acids internal.

As a rule of thumb, molecules with a fatty acid chain of 4 carbons or less can have reasonable solubility in water. Above 8 carbons, molecules bind strongly to a membrane or proteins with hydrophobic pockets (transport proteins that carry either phospholipids or fatty acids through cytoplasm have been identified). Energy of binding increases (~1.2 kcal/mol for each carbon) and the force needed to pull a phospholipid from the membrane is only about 10 pN.

Physical Properties of Biological Membranes

Thickness	5 nm
Area per Lipid molecule	0.5 nm^2
Lipid Fluidity	Fluid but high viscosity (100 X water, about 1 Poise)
Diffusion coefficient	10^{-8} lipid to 10^{-14} cm^2/sec for some proteins
Stretch (elastic modulus)	very little (4% at lysis, tension of 10 mN/m)
Asymmetry in vivo	Plasma membrane asymmetric (Outside PC and Sphingomyelin; Inside PE, PS and PI, PIP, PIP2)
Bending Stiffness	2×10^{-19} Nm
Shear modulus	very low
Membrane Dynamics	Endocytosis of an area = plasma membrane/hr.

Bending Stiffness of Membranes

A major factor in the shaping of membranes is the bending stiffness of the bilayer. Erythrocytes assume a biconcave disc shape because it is the lowest bending energy shape for the surface area to volume ratio of the erythrocyte. If we have a flat membrane and we form a tether by pulling vertically on the membrane, then the change in energy of the membrane (dE) is

$$dE = (B/2)(1/R_t)^2 dA \qquad (14)$$

where B is membrane bending stiffness (measured values range from 1.6 to 2.7×10^{-19} Nm and we will assume a value of 2×10^{-19} Nm), R_t is the radius of the curved membrane, and dA is the area of the membrane that is moved from the flat region to the curved region.

Fluid Shear Resistance

The lipid bilayer components of biological membranes are fluid; therefore, there is no shear elasticity of the bilayer, only viscous resistance to shear. Membrane proteins associated with the bilayer often form an extensive network that has elastic deformation properties on the time scale of seconds. However, when deformations are held for minutes, the cytoskeleton dynamics results in an accommodation to the new shape. In the deformation of cells the lipid is normally a passive component, which flows around moving proteins.

Membrane Diffusion

The combination of the relatively slow diffusion rates of membrane proteins and rapid exchange of membranes, means that regional differences in protein concentration can be easily created in membranes.

Problems:

1. If the bilayer has an elastic modulus of 10 mN/m for dA/A = 0.04 and we assume that half of the bilayer has half of that elastic modulus, then the addition of an amphiphilic compound that expands one half of the bilayer by 0.5% will cause an expansive tension in the other half of the bilayer of ___?

2. In the video, we observed that a tube of membrane could go to several beads on a string. If we consider only bending stiffness of the membrane (2×10^{-19} Nm), then which is the lower energy configuration, a tube of constant diameter (0.2 microns) or a combination of a sphere and a tube of smaller diameter (overall length is 10 microns, i.e. 6.28 microns squared of membrane). (a partial proof is fine using the formula (14) above to calculate the energy needed to form a couple different spheres and tubes from a flat bilayer)

3. A membrane protein has a diffusion coefficient of 10^{-10} cm^2/sec and yet it travels 1.5 microns in 25 sec toward the leading edge. What is the probability that the protein would move in this way from a simple consideration of one-dimensional diffusion (remember the Gaussian (Normal) distribution curve)?

Membrane Asymmetry and Surface Potential

There are many consequences of membrane asymmetry. It is a critical aspect of membranes that is tied to many different cell functions.

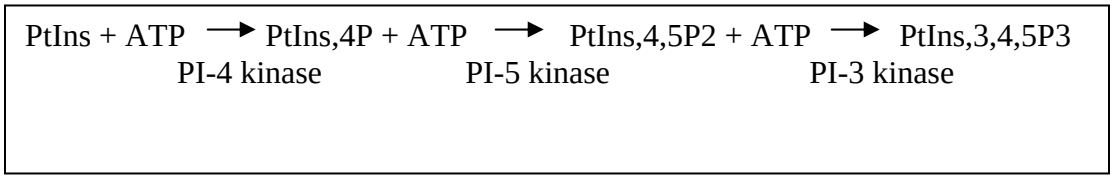
Lipid Asymmetry and Flippases

If we consider the normal lipid composition of a plasma membrane such as the erythrocyte, the outer surface lipids are neutral except for the glycolipids and in that case the charges are separated from the membrane surface by the length of the carbohydrate molecules. At the cytoplasmic surface, we find over 90% of the phosphatidyl serine and inositol which constitute 12-20% of the total phospholipids and 24-40% of the

cytoplasmic half of the bilayer. Since those lipids are negatively charged, there is a very high density of negative charges on the cytoplasmic surface (0.5-0.8 charge/nm^2). There are many cationic proteins and/or cationic domains of proteins that are localized to the plasma membrane cytoplasmic surface and may neutralize a significant fraction of the lipid charges. Although binding of the lipids to proteins will decrease the effective anionic lipid concentration, increased flipping of phosphatidyl serine to the external half of the bilayer is typically found after the cytoplasmic ATP concentration drops or cells are damaged. Appearance of phosphatidyl serine on the external surface is diagnostic of cells entering apoptotic (cell death) pathways and commercial apoptosis kits utilize phosphatidyl serine binding proteins as a reliable assay for apoptosis. An ATP-dependent flippase is present that can restore the normal asymmetry of phosphatidyl serine. Thus, it appears that the asymmetry is important to the cell. Further, the asymmetry could not simply be the result of asymmetric synthesis of the lipids, which would irreversibly equalize over time without a flippase. Energy-dependent concentration of phosphatidyl serine has been observed and is a critical component in the preservation of cell viability, i.e. apoptosis occurs when lipid asymmetry is lost.

Inositol Lipids and Changes in Level of Charge

The most highly charged lipids are the most dynamic in cells. Phosphatidyl inositol mono-, di-, and tri-phosphates rapidly turn over in cells and have been linked to many signaling pathways. Hormonal signaling (calcium release), cell chemotaxis, actin assembly and secretion are all linked to the hydrolysis or synthesis of phosphorylated inositols. The basic metabolic pathways are noted below



Major roles have been proposed for PI-4,5P2 in many cell functions. It is the most abundant phosphorylated inositol and, when hydrolyzed by phospholipase C, is the source of IP3 (1,4,5 triphosphoinositol) and diacylglycerol. IP3 causes calcium release from internal (ER) stores and diacylglycerol activates protein kinase C (PKC) to phosphorylate serine groups on membrane associated proteins.

Recent studies have linked the production and perhaps the dynamics of PtIns-3,4P2 and PtIns-3,4,5P3 with chemotaxis and cell asymmetry generation. The kinase, PI-3 kinase, is a major enzyme in chemotactic pathways but how kinase activity is linked to the asymmetric assembly of actin and migration is not clear.

Charge Pairs in Solution

The physical properties of charges in a water environment have important effects on the behavior of charged nucleic acids, proteins and lipids. Take the case of a charge pair such as a sodium cation and a chloride anion dissolved in water. If we go back to our consideration of diffusion, then it is clear that they will diffuse independently except that charge-charge attraction will draw them together. A balance will be struck on average between the tendency to diffuse away from each other and the attractive electrostatic force. The mathematical description of this balance is provided by the Debye-Huckel model. We can assume that the distribution of counterions around a point charge will follow the Boltzmann relation

$$n_i = n_0 e^{-U/kT} \quad (15)$$

where n_i is the number density (concentration) at the ith point which is different in energy from the 0^{th} point by U, T is the temperature, and k is the Boltzmann constant ($k = 1.38 \times 10^{-23}$ J°K^{-1}). U is only electrostatic energy that is dependent upon the potential (Ψ_i) at the ith point

$$U = ze_o\Psi_i \quad (16)$$

where z is the charge on the counterion and e_o is a unit charge ($e_o = 1.6 \times 10^{-19}$ C). The potential will decrease rapidly with distance from the first charge (or a protein or a lipid surface) particularly in a concentrated salt solution.

To understand the relationship between the salt concentration and the change in potential with distance, a useful parameter is the Debye length, which corresponds to the effective distance between the two counterions. Debye length is calculated from the equations above using a number of approximations and derivations for Ψ_i.

$$\kappa^{-1} = L_D = (\varepsilon_o\varepsilon kT/2e_0^2N_AI)^{1/2} \quad (17)$$

where ε_o is the dielectric constant, ε_o is the permittivity of free space ($\varepsilon_o = 8.854 \times 10^{-12}$ C^{2}N^{-1}m^{-2}), N_A is Avagadro's number ($N_A = 6.023\ 10^{23}$ molecules/mole) and I is the ionic strength ($I = 1/2\Sigma c_i z_i^2$, where c_i is the concentration of the ith ion and z_i is the charge).

Debye length for 0.1 M NaCl, $L_D = 0.96$ nm,

0.01 M NaCl $L_D = 3.04$ nm

0.01 M MgCl2$L_D = 1.75$ nm

Surface Potentials (Guoy Chapman Double layer)

Because density of anionic charges is very high on the cytoplasmic surface of the plasma membrane, we should consider the consequences. When the overall concentration of ions is low, the counterions can diffuse very far from the surface, giving rise to a surface potential. The Debye length is the distance from the surface at which the surface potential drops to 1/e of the original potential.

$$\Psi_x = \Psi_o e^{-\kappa x} \quad (19)$$

Debye length increases with increasing temperature and with decreasing ion concentration.

Concentration at Charged Surfaces

We have talked about the concentration of proteins at membrane surfaces as a means of increasing protein-protein interactions. The surface potential at the cytoplasmic face of the plasma membrane can affect the concentration of ions in the region as well as the pH. If we assume that the concentration of anionic lipids is 33% of the total lipids on that surface, then the surface potential in 0.1 M NaCl will be about 50 mVolts negative. According to the Boltzmann distribution, the concentration of a cation at the surface (c_s) will be greater than the concentration in cytoplasm (c_c) because of a lower energy due to interaction with the surface charge.

$$c_s = c_c e^{+ze\ 0.05/kT} = c_c e^{+8 \times 10^{-21}/(1.38 \times 10^{-23} \times 300°)} = c_c e^{1.9} \quad (20)$$

This applies for all singly charged ions and corresponds to roughly a 4.5-fold increase in the concentration. For a divalent cation, the concentration would be about twenty-fold

greater ($e^{3.8}$). These are theoretical values and the large size of hydrated ions in solution will change the observed values dramatically.

Because at 0.1 M salt the potential drops to 1/e in about 1 nm, the surface potential is only 50 mV at the surface and at 5 nm from the surface the potential will be only $1/e^5$ of what it is at the surface. Regional changes in the distribution of anionic lipids or the binding of proteins to the surface through cationic domains would result in major inhomogeneities in the surface potential (Peitzsch et al., 1995; Murray et al., 1999).

Problems:

1. A membrane channel has a large cytoplasmic domain that covers a circular area 4 nm in diameter (pore is in the center) at the membrane surface and neutralizes the negative lipid charges beneath it. If the potential at the uncovered membrane surface is –50 millivolts, and the solution contains 0.1 M of monovalent salt. What is the pH at the mouth of the pore and edge of the protein?

2. We want to understand the importance of the cytoplasmic surface potential to the transmembrane potential. Transmembrane potentials are typically –100 millivolts (negative inside the cell). If the cytoplasmic surface has a charge of –50 millivolts, what is potential gradient across the 5 nm of the bilayer? Dielectric breakdown of biological membrane occurs at about 1 volt across the membrane. What is the fraction of the potential gradient across the membrane at dielectric breakdown that is contributed by the cytoplasmic surface potential given above?

References:

Murray, D., A. Arbuzova, G. Hangyas-Mihalyne, A. Gambhir, N. Ben-Tal, B. Honig, and S. McLaughlin. 1999. Electrostatic properties of membranes containing acidic lipids and adsorbed basic peptides: theory and experiment. *Biophys J.* 77:3176-3188.

Peitzsch, R. M., M. Eisenberg, K. A. Sharp, and S. McLaughlin. 1995. Calculations of the electrostatic potential adjacent to model phospholipid bilayers. *Biophys J.* 68:729-738.

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