2.4: Membrane Compressibility

Compressibility is a measure of the relative volume change of a substance in response to stress. **Lipid membrane** compressibility has been extensively studied and multiple models developed. Researchers have employed a combination of classic biochemical methods and computational analyses for the purpose of characterizing the elastic nature of mono- and bilayers. Membrane compressibility can also be understood in the context of **biological systems**. It is theorized that cell membranes evolved billions of years ago to provide a barrier between the cell and its surrounding environment. This barrier is elastic by nature, giving it the ability to compress and spread to protect the structural integrity of the cell even in times of mechanical deformation.

For example, under conditions of osmotic stress or during inclusion of proteins, phospholipid bilayers respond by expanding and shrinking. These processes have evolved to effectively preserve vital intercellular, intracellular, and intramembranous biological processes. **Red blood cells (RBC)** were the first cell used as a model system by which to study biological membrane compressibility. Much of the early insights into membrane deformations were gleamed by these studies conducted in the 1970s and 1980s. RBCs continue to be a great model in which to investigate membrane compressibility, due in part to the fact that they contain a variety of biologically important lipid types including Phosphatidic acid (PA), Phosphatidylethanolamine (PE), Phosphatidylcholine (PC), Phosphatidylserine (PS), Phosphatidylinositol (PI), Lysophosphatidylcholine (LPC), and Sphingomycin (SPH).

Membrane Elasticity

Elasticity refers to the ability of a system to reverse a deformation when the deformation is independent of elongation and contraction. In other words, a membrane essentially acts like a rubber band and so to understand membrane compressibility, we study membrane elasticity. The elasticity of a membrane directly determines the possible area per lipid molecule ration that a membrane can have.

Hooke's Law states that the force needed to extend or compress a spring by some distance, X, is proportional to that distance. To some extent, all materials exhibit Hookean behavior when under a small strain or stress. – including biological systems. This is again a simplified way in which to study a biological lipid membrane, but it does prove useful for some applications.

Young's modulus is used to predict how much a material sample will extend under a specific tension or shorten under a specific compression. Therefore, Young's modulus is also known as the tensile modulus or elastic modulus and is the main modulus studied for biological applications (more below). The Young's modulus of small inhomogeneous samples - such as cells and tissues - an be studied using atomic force microscopy (AFM). For example, AFM has been used to determine the Young's modulus of cells, which range from approximately 500 Pa – 100 kPa depending on the cell type. Actin filaments and microtubules have a Young's modulus magnitudes higher – measured at approximately 1 GPa (109 Pa), indicating that actin filaments are exponentially more "stiff" than cell membranes.

Elasticity refers to the ability of a system to reverse a deformation when the deformation is independent of elongation and contraction. Hooke's Law states that the force needed to extend or compress a spring by some distance, X, is proportional to that distance. To some extent, all systems can be thought of in terms of Hooke's Law – including biological systems. This is of course a simplified way in which to study a biological lipid membrane, but it does prove useful for some applications.

Stretching and Shearing of Biological Membranes

Classical elasticity theory is used to investigate energy changes that result from stretching or shearing. When studying fluid phospholipid bilayers, one does not need to account for shear since the lipids are in a "fluid" liquid crystalline state at physiological conditions (with the exception of myelin sheaths that surround neuronal axons). Thus the shear modulus is by definition zero. Instead, we use viscosity as a similar measure.

Stretching, however, is important to understand in relation to biological membranes as it does cost energy and therefore directly affects the structure and potential function of the membrane itself. In the most simplistic form, we can model the energy change (E_{stretch}) for a membrane at zero external stress with an area A_0 that is stretched to a size $A > A_0$ using the following equation:

$$E_{\text{stretch}} = \frac{1}{2} K_{\text{stretch}} \frac{(A - A_0)^2}{A_0}, \tag{2.4.1}$$

where the modulus $K_{stretch}$ is a proportionality constant between a quadratic deviation of the area from its unstressed state and the respective energy.

The lateral stress (Σ) represents the tension in the membrane when it is under such a strain:

$$\Sigma := \frac{\partial E_{stretch}}{\partial A} = K_{stretch}\,\frac{A - A_0}{A_0} =: K_{stretch}\,u \tag{2.4.2}$$

where the **dimensionless strain** is defined as

$$u = \frac{A}{A_0} - 1. \tag{2.4.3}$$

Recall Hooke's law mentioned above: stress is proportional to strain. For this application, the constant of proportionality is the stretching modulus $K_{stretch}$. Unlike the elastic area **compressibility modulus** (K_a) (also just called "compressibility modulus"), $K_{stretch}$ can be measured experimentally with relative ease using micropipette experiments to determine area change. Typical $K_{stretch}$ values are around 250 mN/m.

Researchers have used this equation to mathematically determine the rupture strain of biological membranes – and have determined it is only a few percent. This means that, compared to more elastic materials, biological membranes have a low stretch ability – or in other words, they can only stretch a small amount before they will "break" and the cell will burst.

However, some specialized membranes – such as membranes involved in endocytosis and exocytosis - can undergo more extreme deformation. This is to be expected, as these membranes must move large molecular "packages" in and out of cell membrane compartments without resulting in membrane collapse and subsequent cell death.

Mechanical Elastic Moduli of Homogeneous Lipid Layers

As opposed to heterogeneous phospholipid layers, homogeneous bilayers are often characterized using only three mechanical elastic moduli:

1. The area expansion (compression) modulus (K_a)
2. The bending modulus (K_b)
3. Edge energy (Λ) – not described here. See Evans *et al.* 2003 for further reading

Elastic area compressibility modulus

Homogeneous lipid mono- and bilayers can be modeled as two-dimensional structures, and therefore the membrane compression modulus K_a is defined within one plane. The bulk compressibility of a lipid bilayer is reported to be approximately 5.6 x 10^{-7} kPa^{-1}. For comparison, the value for water is 4.5 x 10^{-7} kPa^{-1}.

Studies show that monolayer compressibility is largely dependent on membrane thickness. Bilayer compressibility, on the other hand, is less dependent on thickness. This idea seems counterintuitive and therefore has been studied in depth. The reason for this: bilayers resist expansion mostly to decrease the exposure of their hydrophobic tails to water – which due to the hydrophobic effect is more thermodynamically favorable. Because of this, bilayer compressibility is less dependent on thickness and more dependent on the surrounding solution.

Studies have attempted to quantify the elastic area compressibility (K_a) modulus of one leaflet of a homogeneous lipid bilayer, and the most simple approximation is that it is equal to 2γ, where gamma is the surface tension of the water-lipid interface. With typical gamma values for one leaflet ranging from 20-50mN/m (or J/m2) per leaflet, K_a is approximated to be 80-200mN/m for a lipid bilayer.

Bending modulus

The bending modulus (K_b) is defined as the amount of energy that is required to deform a membrane from its "normal" curvature to a different curvature. We know that a lipid bilayer is composed of two monolayer leaflets, so you can imagine that for one leaflet to be bent, the other must be stretched. Because of this relationship, researchers have observed that as membrane thickness increases, the more each leaflet must deform to accommodate the curvature. The area compression modulus, bending modulus and bilayer thickness (t) have been related using the following equation (Boal *et al.* 2002):

$$K_b = K_a\,t \tag{2.4.4}$$

This relationship, although profoundly simplified, can be extremely useful. If one experimentally determines K_b and t, then K_a can be estimated with ease – acting as a starting point for more accurate measurements. Again, you must keep in mind that this is an

approximation and one that is generally limited to small deformations. However, since most biological membranes can only support a few percent strain before rupturing, this equation has served as a good general model.

To determine the energy of bending for a biological membrane, one can essentially consider a membrane as a two-dimensional surface that bends into a third dimension. Weak bending is a type of deformation that costs significantly less energy than stretching. The bending modulus of a general single elastic sheet (shown here as '*K*') is defined according to:

$$\kappa = \frac{1}{12} K_{stretch}\, h^2 \tag{2.4.5}$$

where h is membrane thickness; with the bending energy per area written as:

$$e_{bend} = \frac{1}{2}\kappa \frac{1}{R^2} \tag{2.4.6}$$

where R is the radius. Since lipid bilayers are composed of two sheets, one change must be made to the equation for K shown above. The modified relation between stretching and bending modulus is often found in the literature as follows (for two uncoupled sheets):

$$\kappa = \frac{1}{48} K_{stretch}\, h^2 \tag{2.4.7}$$

This equation allows for the bending modulus to be calculated by directly measuring $K_{stretch}$.

Last, the **Helfrich model** of membrane bending elasticity is used to describe the energy associated with membrane curvature. This model is far more advanced than earlier, more simplistic ones – modeling a lipid monolayer as a three-dimensional elastic layer. Because of this, Helfrich theory has been extensively used in studying the biology of cells, and has proven an excellent tool for quantifying biologically relevant membrane phenomena. For further reading and quantification of this theory, see further readinga.

Studying Unilamellar Vesicles: Adiabatic Volume Compressibility

Adiabatic compressibility is often used to study unilamellar lipid vesicles. The adiabatic (or isentropic) compressibility is the compressibility of a system in which no heat is exchanged with the environment. Adiabatic compressibility is typically used to understand the compressibility in a homogenous system, and becomes simply a function of isothermal compressibility, heat capacity coefficient and volume expansion coefficient – all of which can be determined by the heat capacity of a lipid system.

Because of this, one must ask to what extent the water surrounding a membrane contributes to heat buffering during transient membrane compression. There is some controversy over whether or not heat is exchanged between a lipid membrane and the surrounding water. However, this approach has been successful and therefore many researchers have concluded that heat exchange between membranes and the aqueous environment must be negligible in the megahertz regime.

Studying Lateral Compressibility of Phospholipid Bilayers

Many models have attempted to accurately describe cell membranes and cell membrane deformation. The most widely accepted is the fluid mosaic model, proposed by Singer and Nicolson in 1972. This model looks at biological membranes as two-dimensional fluid-like bilayers in which the lipids can move freely about. However, this is only a starting point.

A more accurate, yet also simplified, model is to look at a cell membrane as a lipid bilayer plus a membrane skeleton composed of two main categories of proteins: those that are partially embedded in the bilayer (**peripheral membrane proteins**) and those that are fully embedded in the bilayer (**integral membrane proteins**). This model incorporates more heterogeneity when performing calculations – which is essential to studying highly heterogeneous phospholipid bilayers.

One of the earlier attempts to characterize phospholipid bilayer deformation was by a study in 1982 (Lis *et al.* 1982). The researchers claim that the deformation of a phospholipid bilayer is highly non-linear, and therefore cannot be described in terms of a simple modulus.

In performing these experiments, researchers have found distinct differences between lipid monolayers, homogeneous lipid bilayers, and phospholipid bilayers. For example, upon compression, monolayers do not undergo a phase transition whereas some bilayers do. For example, the lateral compression of certain lipids (e.g. dilauryl PC and dimyristoyl PC) forces their acyl chains through a "melted" to "frozen" transition at temperatures higher than their T_M.

Current state of this research: many have argued that the lateral compressibility of phospholipid bilayers must be studied experimentally instead of modeled. This is largely because of the heterogeneous nature of phospholipid bilayers found in living systems. These studies continue today, and probably will for a while to come – as phospholipid systems are not only complex, but can also be unpredictable and even technically difficult to study in their natural (not reconstituted) environment.

Therefore, additional studies must be conducted to better understand lateral compressibility on critical cellular processes such as:

- membrane assembly
- mobility and segregation of membrane components
- enzyme-mediated membrane permeability
- incorporation of proteins or drugs into the membrane

Factors Affecting Compressibility in Biological Systems

Temperature

Most phospholipid bilayers exist in a liquid-crystalline state near physiological temperature, but will undergo a phase transition from a liquid-crystalline state to the gel phase as it approaches its melting temperature (T_m). This transition takes place when temperatures are lowered. Studies have shown that during the phase transition, lateral area of the membrane decreases by approximately 25%.

Pressure

Interestingly, compressing a biological membrane under otherwise physiological conditions can induce phase transition. Thus, an increase in pressure mimics the effects of a decrease in temperature. This relationship is modeled using the Clausius-Clapeyron equation:

$$\frac{dT_m}{dp} = \frac{T_m \Delta V}{\Delta H} \tag{2.4.8}$$

Thus the pressure raises the T_M without affecting ΔV or ΔH. However, due to their heterogeneous nature biological membranes do not often undergo phase transitions. Instead the effects of pressure are confined to ordering the bilayer. Because of this increase, when pressure is applied to a phospholipid bilayer, it can result in the membrane releasing some of their peripheral and/or integral membrane proteins.

Lipid Tail Length and Saturation

Studies have shown that the membrane compression modulus (K_a) varies with tail length and unsaturation.

Solution Type

Because membrane stretching and compression exposes hydrophilic phospholipid tales to the aqueous environment, K_a of lipid bilayers varies strongly with solution conditions.

Membrane Composition

Since biological membranes consist of lipids, proteins, cholesterols, and other molecules, they cannot be characterized in the same manner in which unilamellar vesicles or even homogeneous monolayers can be understood. The compressibility of bilayers is directly dependent on their composition – such as presence of cholesterol or presence of double bonds. For example, the area compressibility modulus (K_A) of a membrane increases as the percent cholesterol increases. Additionally, proteins can induce curvature in membranes by transporting lipids or by embedding themselves into the bilayer.

Molecular packing

As membranes cool, they lose fluidity. This results in the acyl groups fully extending and the head groups packing tightly and dehydrating. In respect to hydration, another study in 1985 determined the effects of hydration on egg and dioleoyl lecithin and found that at low hydrations, the head groups are much less tightly packed compared to those more hydrated (White *et al.* 1985). This is assumed to be due to osmotic pressure driving the head groups apart.

Tools are used to study membrane compressibility?

It is difficult to experimentally determine the compression modulus of a phospholipid bilayer. Therefore, one method often employed is to measure how vesicles swell in response to osmotic stress. However, this is an indirect and sometimes inaccurate

method due to polydispersity in vesicle size.

A more direct method of measuring K_a is the pipette aspiration method. This too can pose issues, as biological membranes are thin and fragile by nature and are difficult to study in this capacity without the cell dying. However, much important information has been gleamed using this method. Put in simple terms, this assay is essentially one where the researchers sucks part of a membrane up into a pipette, measures the length that is sucked into the pipette, then calculates the change in area to determine the elasticity of that particular membrane.

Atomic force microscopy (AFM) – also mentioned briefly above - has also been used to directly measure mechanical properties of bilayers, but this method is still under development. Because of these difficulties, the compression modulus is often calculated using the values of its determining characteristics.

Additional methods and techniques used to study lipid mechanics include (but are not limited to):

1. Single molecule tracking (fluorescent or radiolabeled probes): to asses the movement of lipids under certain conditions
2. ^{2}H-NMR: to determine bilayer dimensions
3. Langmuir Trough: to measure area per lipid
4. NEMD method: membrane area change
5. Phase contrast microscopy
6. Differential scanning calorimetry: to measure exotherms during a liquid crystalline to gel phase transition and endotherms during the reverse.
7. Fourier transform infrared spectroscopy (FTIR): lipid phase transitions
8. X-ray diffraction: to determine area per lipid as a function of water activity for different lipids (molecular packing)
9. Optical microscopy: to measure the volume of a bilayer during pressurization
10. Neutron diffraction: to determine molecular packing

Applications of Membrane Compressibility

Ion movement

When an ion penetrates a membrane, a very slight shape change takes place and thus has a corresponding energy cost – known as the **membrane deformation energy**

$$\Delta G_{\text{mem}} = \frac{1}{2}\int_{\Omega}\left[\frac{K_a}{L_0^2}\left(u^- - u^+\right)^2 + \frac{K_c}{2}\left(\left(\nabla^2 u^-\right)^2 + \left(\nabla^2 u^+\right)^2\right) + \frac{\alpha}{2}\left(\left(\nabla u^-\right)^2 + \left(\nabla u^+\right)^2\right)\right]\mathrm{d}x\mathrm{d}y$$

where u^+ is the shape of the upper leaflet and u^- the lower leaflet; L_0 the equilibrium thickness of the membrane; K_a the compression modulus; K_c the membrane bending modulus; and α the surface tension. Furthermore, this equation in combination with a domain of interest, X, ranging from position x-y, and with respect to variations in u+ and u^-, can be additionally used as a tool to determine the shapes of each leaflet.

Protein and small molecule movement

The values of K_a and K_b affect the ability of proteins and small molecules to insert into the phospholipid bilayer. The reverse is also true: bilayer mechanical properties have been demonstrated to affect the function of mechanically activated ion channels.

Ordering effects of pressure on cell structures

The ordering effects of pressure were discussed earlier. Examples of this include the rat liver mitochondria, human erythrocytes, and fish synaptic and brain myelin vesicles in which the ordering effect of pressure can be determined by measuring the increase in temperature required to offset the pressure. These values have been determined to be approximately 0.13 – 0.21 °C mPa^{-1}.

Effects of high pressure on cells

Cells are often exposed to hydrostatic pressure. This affects numerous cellular processes and reactions – albeit in a reversible manner. For example, the cytoplasm of eukaryotic cells can be reversibly “liquefied” under conditions of high pressure. Additionally, studies on amphibian epithelia have shown that changes in pressure can affect the passive permeability of sodium through the cell membrane.

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