Lecture 4 - Membrane Dynamics

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November 27, 2023

1 Reference reading

Phillips, R. (2018). Physics of Biological Membranes

2 Lipid & membrane dynamics

Lipids in the membrane plane are capable of different motions or dynamics including:

- trans/gauche isomerisations (picoseconds)
- rotation around chemical bonds (picoseconds)
- rotation about the lipid axis (nanoseconds)
- lateral diffusion (microseconds)
- undulatory motions of the bilayer (milliseconds to seconds)
- flip-flop between leaflets (milliseconds to seconds, hours)

3 The main phase transition

The major determinant of lipid dynamics is the membrane phase. Previously, we discussed the several bilayer phases that are adopted by amphipathic lipids.

- gel (L_β) including sub-gel, gel, interdigitated gel and rippled gel
- liquid crystalline/disordered (L_α), including liquid disordered (L_d) and liquid ordered (L_o)

As the temperature increases, the main phase transition occurs from L_β to L_α represents a cooperative rotameric disordering of the hydrocarbon chains, from trans (extended) to gauche (kinked) configurations. In the gel phase, the acyl tails are fully extended maximizing the trans configurations of carbon-carbon bonds. As a result of the lipid extension, the membranes become thicker and while diffusion still occurs (consistency is like solidified bacon grease) the diffusion is about 1000x slower than in the liquid disordered phase. In the liquid crystalline phase, acyl chains are flexible, fluid and exhibit kinks due to gauche orientations. The membrane thickness is decreased and diffusion increases.

3.1 Differential scanning calorimetry

A direct way of measuring the main phase transition temperature from gel to liquid disordered, and some of the other gel states. This approach measures the heat capacity change as a function of system temperature, and reports the temperature at which the phases change, e.g. T_m for the main phase transition. In a pure lipid bilayer system, you can often measure the gel to ripple pre-transition and then the melting transition to the liquid crystalline phase.

Another method of measuring changes in membrane phase is by measuring the bilayer thickness by atomic force microscopy, but this requires created a supported bilayer on a silica slide. Gel phase leads to an increase in membrane thickness due to extended acyl chains.

3.2 Trends in main phase transition temperatures

For pure glycerophospholipid membranes with commonly used lipids in laboratory experiments, the phase transition temperatures can range from negative -60 to 80 °C. Remember that the higher the T_m, the more the temperature has to be increased in order to observe the gel to liquid crystalline phase transition. The phase transition temperature of pure lipids describes the intermolecular interactions with the other lipids in the leaflet and bilayer. In general, higher transition temperatures mean more stable lipid-lipid interactions.

- Independent of head-group, as you increase the number of carbons in the acyl chains, the intermolecular chain interaction increases and the transition temperature increases. For example, T_m of DLPC (C12:0) = -2 °C vs. Tm of DSPC (C18:0) is 55 °C. This is because of the increase in VDW interactions between neighboring acyl chains leading to ordering of the lipids, stabilizing the gel state. Introducing any amount of unsaturated bonds reduces the transition temperature, for example T_m of DSPC (C18:0) is 55 °C vs. T_m of DOPC (C18:1) is -17 °C.

- Unsaturation (double bond) in at least one of the chains drops the phase transition temperature dramatically. All are well below 37 °C and most are below 0 °C.

- Headgroup types introduce an offset to the T_m vs. chain length curves, generally depending on size, which reflects the nature of the headgroup to interact with water and ions in the bulk solution. For example, PE is a lipid that has considerably higher phase transition temperatures compared to PC lipids. For example, the T_m of DSPC (C18:0) is 55 °C vs. the T_m of DSPE (C18:0) is 70 °C. The main reason why this occurs is that PE has a much smaller headgroup size, and so it will tend to pack closer together and not interact with the surrounding water. The relationship is similar with PA lipids.

- Other intermolecular interactions PE also participates in hydrogen bonding with neighboring PE lipids, leading to a stabilization of packing in the gel state.

Note, many of the pure lipids examined have phase transition temperatures that are below the biological growth temperature. For instance, *E. coli* mainly contains POPE type lipids, and yet its T_m = 25 °C. How do these cells have fluid bilayers at 37 °C? This raises the important consideration that biological membranes are never just one type, but are mixtures, comprised of many types of lipid molecules. Lipids can be extracted by cells using an extraction procedure with different solvents (e.g. chloroform/methanol/water mixtures), and the different lipid types can be quantified by thin layer chromatography or mass spectrometry procedures.

Examining the polar lipid composition of *E. coli* membranes, there is 67% PE, 23% PG and 10% cardiolipin. While the chain type is not analyzed, we know from other studies that it is mainly of the PO type. As a guess, we could make a crude estimate by taking a a weighted average of the different T_ms to hypothesize a phase transition temperature for the *E. coli* membrane. Let us ignore the cardiolipin for now and just estimate this for a two component system: (67*(25 °C) + 33*(-2 °C))/100 = 16.1 °C, somewhere in between the two phase transition temperatures. If the lipids mixed perfectly, and maintained their phase transition properties as when they were in their respective pure lipid environments, then we might expect a sharp transition at this average temperature. However, this mixed membrane now contains at least three types of lipid-lipid interactions: PE-PE, PG-PG, PE-PG. Furthermore there is not perfect mixing, and in fact, can be clusters of different lipid patches. This all depends on the interlipid interactions, and the resulting phase properties can be modeled well using a 2D, grid-based Ising like model. The takeaway from this, is that lipid mixtures can exhibit both shifts in the phase transition temperature (away from the weighted average) and broadening due to loss of cooperativity, and sub-domain formation in these complex mixtures.

3.3 Cholesterol and the phase transition

The addition of cholesterol to the phospholipid bilayer can act as a buffer on the fluidity of the membrane. At the lower temperatures, where the gel phase transition would typically

occur, cholesterol disrupts chain cooperativity and disrupts the neighboring interactions, it therefore decreases the phase transition temperature. However, at higher temperatures, the cholesterol stabilizes some extended chain distributions so that fluidity is restricted. Altogether, it has a buffering effect.

4 Liquid-liquid phase separation in membranes

4.1 Diffusion in different membrane phases

Diffusion in the gel phase is slow, with a diffusion coefficient measured around D = 10^{-11} cm^2/s. Addition of cholesterol introduces a new phase, the liquid ordered (L_o) phase that spontaneously assembles and is distinguishable from the other liquid crystalline phase, called liquid disordered (L_d). The two phases can coexist in the liquid membrane.

- liquid ordered, L_o - which is sometimes referred to as rafts enriched with saturated lipids, sphingolipids and cholesterol. D = 2.5 x 10^{-8} cm^2/s.
- liquid disordered, L_d - mono and poly unsaturated lipids. D = 5 x 10^{-8} cm^2/s, just 2-fold faster than liquid ordered.

4.2 Membrane rafts

The liquid ordered phase is often referred to as membrane "rafts", and is associated with cholesterol, sphingolipids and sometimes membrane proteins. The observation of different liquid phases within lipid bilayers raises the question of whether this exists in biological membranes. In 1997, the concept of lipid rafts was proposed by Simons & Ikonen from experiments demonstrating detergent resistant membrane fractions that were heavily comprised of cholesterol and sphingolipids. The broad hypothesis is that the cholesterol in our plasma membranes serves a role as providing localization in the membrane via these spontaneously assembled liquid ordered domains that favor the partitioning or binding of key biological membrane proteins. These domains may be stabilized by the cytoskeleton, and they might be dynamic. Note, this hypothesis is still an active question in the field and there are many questions to be answered.

4.3 Measuring liquid-liquid membrane phase separation in vitro

Since the liquid ordered and liquid disordered phases are both liquids, a higher resolution approach to measuring these phase changes is by deuterium NMR or ESR spectroscopy. NMR offers technical advantages because ESR requires conjugation with spin labels. This yields information about the dynamics of the acyl chain configurations. With both approaches, the average orientation of the nth CH_2 segment along the chain and is expressed as a classical liquid crystal order parameter

$$S_n = \frac{3 < cos^2(\theta) > -1}{2} \quad (1)$$

In these experiments, protons can be replaced by deuterium in a site specific manner. θ is the angle of the C-D bond relative to the membrane normal. With NMR, the order parameter can be measured from deuterium quadrupole splitting measurements as a function of different conditions. The phases can be mapped out under many conditions such as temperature, salt, and varying lipid compositions to yield phase diagrams. For example, DOPC/DPPC/cholesterol exhibits changes in the L_o/L_d proportions as a function of temperature.

4.4 Measuring liquid-liquid membrane phase separation in vivo

By doping in fluorescently labelled lipids and making GUVs, these dynamic phases can be visualized within the membrane, revealing the remarkable co-existence of these different membrane environments within the same lipid bilayer. Some of the limitations of the ongoing studies in the study of 'rafts' comes from challenges in visualizing the domains in a biological setting without perturbing them. The major way of observing these domains is by microscopy and using either fluorescent lipids that differentially partition into the different phases, or fluorescent proteins that will localize to the different phases. For instance, RAS and GPI anchored proteins will localize to liquid ordered domains, as well as cholera toxin. Superresolution microscopy has provided a tool to visualize these small domains, as they are much smaller in biological membranes than in synthetic mixed lipid bilayers.

5 Lateral diffusion of lipids in the lipid bilayer

The diffusion of species in the membrane can be measured by fluorescence microscopy in several ways.

- Fluorescence recovery after photobleaching (FRAP). This first method (developed by Axelrod, Webb, Elson and others) followed the experiment of Frye and Edidin and further quantified the fluid behavior of lipid bilayers. Here, an area of the membrane containing fluorescent lipids, is bleached, and the recovery of the fluorescence is quantified. The diffusion constant, D, is measured by fitting the exponential recovery of the bleaching while considering the beam width.
- Fluorescence correlation spectroscopy (FCS). In the second method (developed by Magle, Elson, Webb), a microscope, usually a confocal setup but can be others, measures the autocorrelation function from the fluorescent fluctuations of diffusing particles. This function is fit to a diffusion model to obtain the diffusion time (τD) to obtain the diffusion coefficient, D.

- Single-particle tracking (SPT). For membranes, total internal reflection fluorescence microscopy is an excellent approach, as it illuminates just the membrane layer on the slide allowing for single-molecule visualization. Furthermore, the movement of particles in membranes is slow enough to be acquired with electron multiplying/charged coupled device (EM/CCD) cameras, but recent advances in complementary metal-oxide-superconductor (CMOS) camera technologies allow for much faster time resolution. As a result single lipids and proteins can be watched in real time, provided you can label the molecules fluorescently. The diffusion of these molecules can be measured by analyzing the mean square displacement as a function of the observation time.

5.1 Biological diffusion

In general, the diffusion of lipids and membrane proteins is a couple of magnitudes slower than diffusion in water. For lipids, it is on the order of 10^{-8} cm^2/s. Note, diffusion coefficients are often reported as cm^2/s or $\mu m^2/s$, and it is important to know how to convert between these so that you can read the literature.

In pure membranes, membrane proteins are slightly slower than lipids but not by much. This is because of the differences of diffusion in 2D vs. 3D. In 3D, such as diffusion in water, the diffusion coefficient can be described by the Stokes-Einstein equation. We can see that there is a dependency on viscosity of the solution as well as the size of the diffusing species, exhibiting a 1/R dependency. However, in 2D, diffusion does not behave in the same way. This is described by the Stokes paradox, where there is no creeping flow around a disk or cylinder in 2D. Therefore, the diffusion coefficient is described by another model, the Saffran-Delbruck 2D hydrodynamic model. While we see that the diffusion still depends on viscosity, but now viscosity of the membrane and the bulk water, the dependency on the size of the diffusing particle is reduced, now ln(1/R). This means that the diffusion in two-dimensions does not depend strongly on the size of the diffusing species. This has been tested experimentally by reconstitution of purified proteins and measurements of the diffusion coefficient by FCS. The relationships are clear, the diffusion of membrane proteins in membranes contains little information about the particle size, supporting the Saffran-Delbruck model. However, things change once we move to biological membranes. First off, the diffusion is generally slower, and now there does appear to be a stronger dependency of the diffusion on the size of the species. The thing to consider here, is that there are often intra- and extra-cellular loops that penetrate outside of the 2D membrane. In a biological situation, these may encounter other anchoring proteins in the cell.

5.2 Different diffusional behaviors in cells

Single particle tracking experiments in real cell membranes was critical to demonstrate the anomalous diffusion in biology. When protein molecules are fluorescently labelled, they

behave according to four different categories

- Stationary ($<r^2> \sim 0$)
- Simple Brownian diffusion ($<r^2> = 4Dt$)
- Confined Brownian diffusion (plateau)
- Directed diffusion

These observations support a hypothesis that the cytoskeleton acts to localize protein domains in the membrane.

6 Lipid flip-flop (transverse dynamics)

Lipids can also undergo motions between the two leaflets but this is rare and far slower than the dynamics within the leaflet. The earliest studies (Kornberg & McConnell 1971) measure the rates at 2 x 10^{-5} per second (i.e., 2 x 10^{-8}/ms), yet these measurements may be slower due to the large spin labels. Cholesterol is predicted to have flip flop rates on the microsecond timescale. Addition of proteins can accelerate the transverse lipid exchange rate. Proteins such as flippases, floppases maintain lipid gradients to overcome this.

7 Molecular dynamics simulations of membranes

Lipid bilayers are complex solvent environments in that they exhibit 3-dimensional structure and chemical heterogeneity. While experimental methods can inform on some of these mechanical and dynamic information, we generally lack resolution to report on these behaviors in specific environments in the membrane such as around membrane proteins. Computer modeling provides access to developing physically consistent molecular models, and while they are still being validated, they can recapitulate many important membrane properties.

When carrying out a molecular dynamics simulation of a membrane, an important thing to consider is the timescale required to observe what is of interest. The membrane is viscous, and lipids move slowly, so there must be a strategy in place to assess convergence of the dynamics to be sampled. For this, there are different strategies, such as coarse-grained vs. all-atom force-fields, that allow for extension of the simulation into appropriate timescales. In some cases, a coarse-grained force-field, which has been parametrized to reproduce fundamental membrane behaviors such as lipid area and membrane thickness, makes a simulation possible even if it is a reduced physical model. Examining an average lipid system, about 30 nm x 30 nm, we can see that it requires at least 10 microseconds for full exploration of all lipids in the box. This is typically a prohibitive timescale for all-atom

molecular dynamics simulations. However, with coarse-graining and sufficient sampling, molecular dynamics simulations can model changes in local membrane structure, lipid configurations, presence of water, and preferential solvation in mixed lipid systems. New analysis programs, such as MOSAICS can be used to determine local changes in membrane structure, composition and dynamics, particularly around membrane proteins. And both coarse-grained and all-atom force-fields are under constant development to recapitulate membrane properties.