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Primer

Membrane biophysics

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In physics, we usually want to reduce the behavior of the system to fit it to a formula. In biology, however, all the details of the system are important; their organization makes for life. Thus the organization of proteins in the plasma membrane is critical for various biological processes that occur on membranes. My purpose here is to introduce readers first to the opportunities for learning that abound in membrane biophysics, then to describe physical forces that determine membrane structure and function (especially those that impact on membrane fusion), and finally to discuss how recognizing these forces lets us think about the lateral organization of lipids and proteins in biological membranes — one of the outstanding problems in the field.

What is membrane biophysics?

Consistent with the fact that about a third of the dry weight of a cell is membrane, almost half of all proteins encoded by a eukaryotic genome are membrane proteins. Thus roughly half of biological processes occur on membranes, and each of these processes will have aspects of its function that fall into the realm of physics. Just as water is the solvent for soluble proteins, the phospholipid bilayer membrane is the solvent for membrane proteins and forms the basis of the biological membrane. A semi-crystalline array that is ordered in some aspects and disordered in other aspects, a membrane has both a fluid and a solid character (Figure 1). It is only two molecules thick but can have an area of a millimeter squared (e.g. eggs) or a length of many meters (e.g. axons in giraffes). The phospholipid bilayer itself is stable for a range of different lipid compositions, and bilayers self-assemble upon sufficient hydration of these lipids. Membrane properties and self-interactions can therefore be extensively studied without proteins *in vitro*. Our understanding of the physical nature of the membrane backbone comes mostly from studies of the spectroscopic, microscopic, and electrophysiological properties of phospholipid bilayers in the absence of proteins.

Historically, physicists were attracted to aspects of biology that were intrinsically physical, such as biological electricity (e.g. the historic controversy between Galvani and Volta [1]). Chemists and physicists differ in their approach to biology as exemplified by the issues of electron transport and the Mitchell hypothesis [2,3]. Chemical pathways invoking high-energy intermediates fit the developing notion of cellular energy stored as ATP. However, Mitchell's physical hypothesis of cells storing energy in a field and in a proton gradient across a membrane was alien to biochemists focusing on pathways of intermediary metabolism: acceptance of the hypothesis took time.

Scientists who identify themselves as membrane biophysicists examine mechanisms of channel behavior, cable properties of neuronal cellular processes, membrane fusion, membrane fission, membrane organization, membrane lipid phase behavior, protein clustering, and many other topics. While cell biologists aim to identify and characterize the molecules that comprise the structures that allow the membrane to carry out these various processes, membrane biophysicists (and physical chemists) wonder how these processes work — what are the forces, energies, and pathways that explain the activity? There is a biophysical question associated with every protein and lipid in a membrane, with every one of their activities, and with all of their interactions. In voltage-dependent channel gating, it is now proposed by some that a peptide migrates across the lipid part of the membrane in response to the membrane potential. In membrane structure, it is proposed that the lipid environment controls protein clustering for activity. In apoptosis, we think that lipids are part of an apoptotic pore. In viral fusion, we think that lipids are physically stressed by proteins inducing local membrane curvature. In membrane trafficking, we think that proteins induce switch-like changes in membrane tubule diameter, rapidly cycling from wide to narrow.

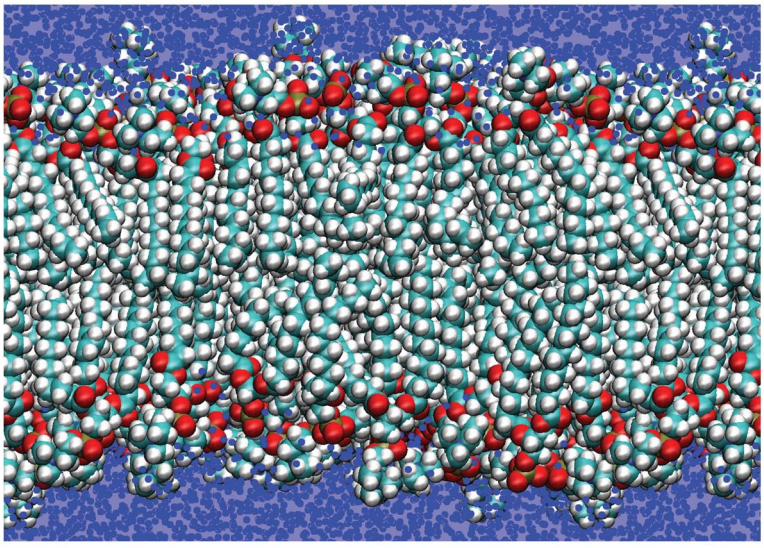

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Figure 1. A molecular dynamics simulation snapshot of a bilayer comprising the phospholipid DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) at 30°C. The simulation was performed on the NIH biowulf cluster using CHARMM software.

Through the work of Van't Hoff, Fick, Gibbs, Nernst, and Einstein in the 19th and 20th centuries came our current understanding of osmosis, diffusion, diffusion potentials, and Brownian motion on the physical level [4]. These principles were then applied to biological problems of semi-permeable membranes in the 50s and 60s [5,6] and provided the intellectual basis of the ultimate acceptance of the Mitchell hypothesis and the Hodgkin–Huxley analysis of excitable membranes [7–11]. A further advance came with electron microscopy, itself a *tour de force* of physics and electrical engineering that brought cell biologists the discovery that all of the intracellular organelles are bound by bilayer membranes. Electron microscopy revealed the ubiquity of membrane fusion and fission (the simultaneous coalescence or separation of two membranes) in the processes of exocytosis and endocytosis (processes by which materials are secreted and internalized, respectively) and protein synthesis through the secretory pathway. Then came the use of reconstituted membranes to examine channels and fusion [12,13]. Studies of physical principles of membranes also revealed membrane–membrane interactions, including the discovery of the hydration force, the phase behavior of lipids, and the co-existence of lipid domains (all-important elements of the modern field of lateral membrane heterogeneity — see below).

Forces that dominate membrane structure and function

The primary force of membrane assembly is water's self-love, the hydrophobic effect that results from lipids having both polar and non-polar molecular regions [14]. Briefly, water is so unhappy next to the acyl chains that make up the lipid tails of phospholipids and next to certain amino acid residues (hydrophobic amino acids) that acyl chains and transmembrane domains pack together to minimize contact with water. On the other hand, the head group of a lipid is very polar and happy to be near water. The bilayer structure with two planar monolayers of lipids joined together by their tails is the solution to the problem of excluding water from hydrophobic tails and transmembrane domains.

To give some idea of how much energy stabilizes this structure, consider that it takes about 55 kcal/mole to remove a single acyl chain from a bilayer and put it into water [14]. That means the energy of stabilization of the membrane is equivalent to about 16 high energy phosphate bonds (ATP -> ADP) per lipid molecule! For this reason, membranes hate edges [15] — even a 20 nm edge of a ruptured membrane would expose about a hundred lipids or cost 11000 kcal/mole. Since no other physical force comes close to generating this amount of energy and since more than 95% of the hydrophobic lateral area of a typical biological membrane is occupied by lipids, the hydrophobic effect dominates in biological membranes.

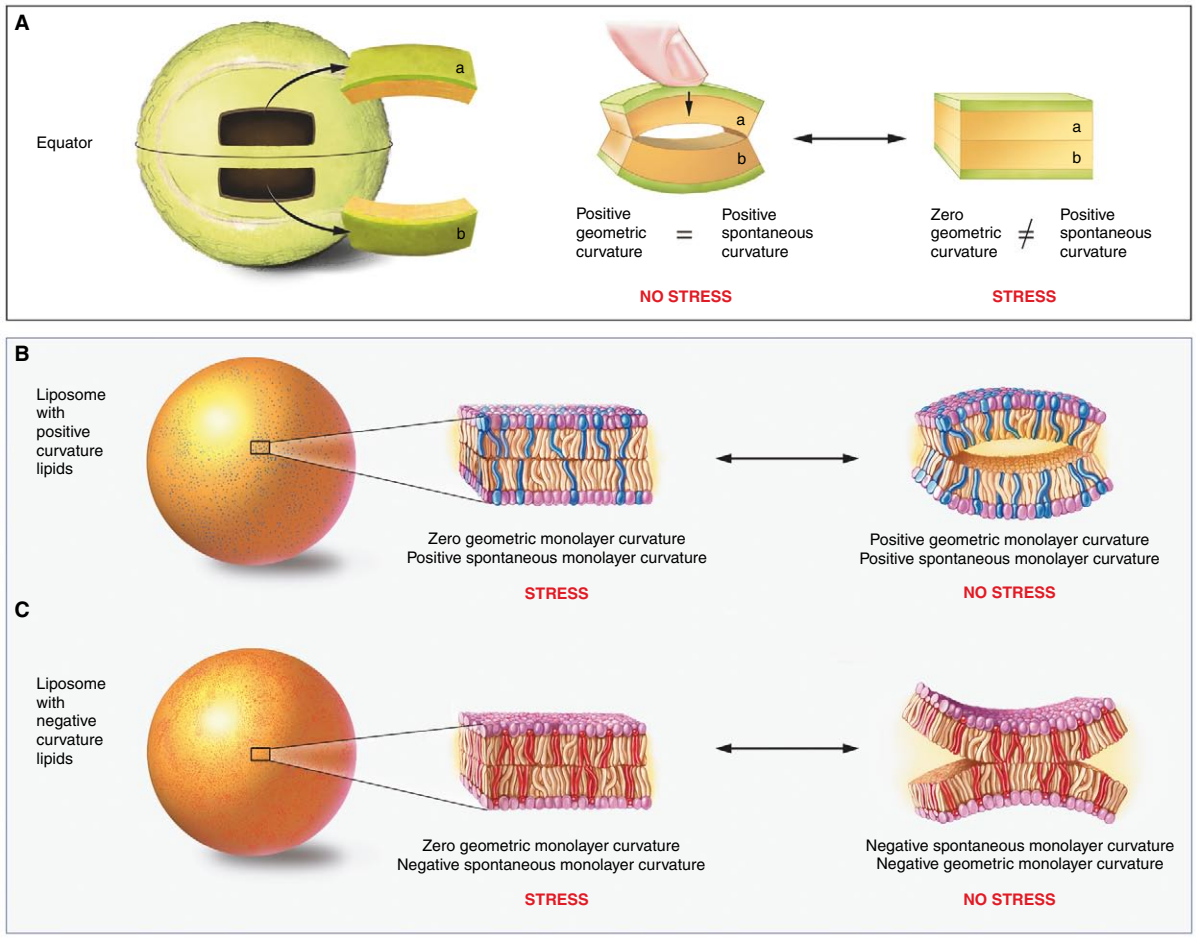
In addition to the hydrophobic effect, other intermolecular forces act to determine the net surface tension of a phospholipid membrane. The lateral distance between lipids in a bilayer membrane at equilibrium is a balance between several factors: compression caused by the Van der Waals attraction of the acyl chains [16,17]; separation caused by the entropic motions of the acyl chains to occupy all available configurations and thus push each other out of the way; close packing at the level of the carbonyl groups that form the junction between the head group and the hydrocarbon tail to avoid water incursion; and generally expansive interactions of the head groups with each other and solvent components due to their hydration, ion binding, and endogenous electrostatics [18,19]. Any outside force that attempts to change this equilibrium lateral distance (about 8 Å per lipid head group) meets stiff resistance. The elastic modulus of a membrane is about 20 kT/nm. Furthermore, membranes cannot withstand any appreciable stretching and break at about 3% stretch, regardless of composition [20].

Because membrane shape represents the intrinsic desire of lipids to occupy structure, membrane curvature is proving to be a very important feature of a number of biological processes. The mathematical basis for the analysis of the energetics of membrane curvature and the contributions of lipids and different proteins to various intracellular structures involved in membrane trafficking has recently been reviewed [21]. One lesson learnt was that local spontaneous curvature of the membrane promoted by protein inserting and binding to membranes is more likely to lead to the formation of intermediates of curved organelles (e.g. during budding or endocytosis). Protein polymerization into coats can then stabilize bent membranes into vesicles whose intrinsic bilayer curvature can be tuned by the presence or absence of local curvature agents, thereby allowing variable driving forces for subsequent changes in membrane curvature [22].

Intrinsic to the study of membrane biophysics is the study of the composition and structure of the phospholipid bilayer. Here the X-ray diffraction studies of membranes by Luzatti and his colleagues were critical [23]. To create a repeating structure for diffraction, they prepared multilayers of membranes. Hence, in addition to determining the electron density of material across the bilayer, they could measure the repeat distance between bilayers or the hydration volume between layers, and also determine the phase of the structure (e.g. lamellar, hexagonal, inverted hexagonal, liquid, gel, or liquid ordered). This study therefore had in its origin both the hydration forces and the phase behavior of lipids [18,19]. The fact that the phases could interconvert following changes in osmotic pressure led to the proposal that lipids are polymorphic and to the idea of spontaneous curvatures of lipid monolayers [23,24]. The central concept linking the spontaneous curvature of a lipid to a working concept of 'effective shape' is that, when

Box 1

A didactic tool for understanding spontaneous curvature.



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(A) An ordinary tennis ball is shown with cuts to produce two rectangular pieces of the tennis ball surface (a and b). Since the tennis ball is made of layers having different areas, there is a natural tendency of these pieces to curl. That is, in the absence of any outside forces, they spontaneously exhibit curvature, here denoted by the geometric curvature (a measure of the actual observed curvature [21]). In the middle of (A), this spontaneous curvature of each layer is seen when the two pieces are loosely assembled together. Since these pieces of ball are elastic, it is possible to flatten these layers by applying outside force, i.e. the curvature can go to zero (neutral) by applying pressure with fingers. However, the pieces are now stressed, and by removing fingers they will spring back to their intrinsic, spontaneous curved state. This demonstrates three features of the bending elasticity of all sheets: 1) there is a spontaneous curvature, and when geometric curvature is the same as spontaneous curvature, there is no curvature stress, 2) curvature stress is created when the sheet is deformed to any other curvature, and 3) in the absence of external forces, the curvature stress is relieved by bending back to the spontaneous curvature. (B) A large phospholipid vesicle is shown in water (light blue background), composed of mostly neutral lipids (yellow) but with a significant fraction of lipids having a positive spontaneous curvature (blue, e.g. lysolipids that have only one acyl tail). In this thought experiment we cut out such a small piece of membrane that it is essentially flat and we can see the individual lipids. Since membranes hate edges in water, in this cartoon we place air (yellow) around all the exposed hydrocarbon tails. The bilayer is flat, despite the fact that it is composed of two monolayers that would tend to bend if separated. Thus these flat monolayers are stressed, experiencing 'elastic frustration'. If we could allow yellow air to split the bilayer into two monolayers, we would then see them relax and exhibit their spontaneous curvature, as shown in the last figure of this panel (note that this disjoint bilayer can never exist, this is only a cartoon!). Since this lipid mixture has overall positive curvature, each monolayer would bend to bulge the head groups out. Essentially, the requirements of monolayer contiguity enforced by the hydrophobic effect are the 'fingers' pushing the monolayers together in aqueous solution. Similarly to the tennis ball pieces, this pushing takes work, and thus stores energy that can be used for work, e.g. for forming bent intermediates. (C) The same as (B) except the composition is mostly neutral lipids with a significant fraction of lipids having negative spontaneous curvature (red, e.g. phosphatidyl ethanolamine, which has a small poorly hydrated head). Now the monolayers wish to bend in the opposite direction than those in (B).

Many thanks to Lydia Kibiuc for the artwork, Sol Gruner for the tennis ball idea, and Leonid Chernomordik and Michael Kozlov for their critique of the figure.

an experimentally determined structure of a monolayer is equally partitioned into its different lipids, one can equally divide the lipidic volume into a number of self-similar lipids, whose shape would represent an average volumetric shape that each lipid occupies. Since lipids are fluid, each lipid rapidly interconverts between many isomeric configurations. Head group charge, methylation, motion, solvent component interactions, and other factors can shrink or expand head group hydration, changing the 'effective volume' of the phospholipids. 'Effective shape' is an idealization since no one lipid ever holds one shape for very long, but, as a practical matter, the idea of lipid shape makes curvature-driven hypotheses easier to imagine.

The bending energy, then, is the work required to bend the monolayer away from its intrinsic shape towards a new shape, representing a new kind of cellular energy in addition to high-energy bonds and gradients across membranes, as it is energy that can be stored in the 'bent spring' of a membrane (Box 1) [21]. Since, in general, the biological membrane is constrained to a lamellar phase, and its shape can be formed by proteins acting as a scaffold, the incorporation of lipids with differing intrinsic curvatures does not change the geometric curvature of the membrane, but it does alter the work required to promote further changes, such as bending a monolayer towards the center of the bilayer membrane sufficiently to create a pore lined by headgroups, or bending a monolayer away from the center of the bilayer to create a stalk between two proximal membranes [25]. Since a small vesicle (such as a sonicated liposome or an internal vesicle of a multivesicular body) has an outer membrane with a positive intrinsic curvature, if its lipids have slightly negative monolayer curvature (and almost all naturally occurring phospholipids are slightly negative in intrinsic monolayer curvature), then the lipids would find it very easy to bend away from the membrane and form a stalk.

Electrostatics also have a major role in membrane structure and function [26], but to a lesser extent than the hydrophobic effect, bending, tension, and elasticity. A significant fraction of lipids bear negative charge at physiological pH, and many proteins and glycoproteins have a multitude of charges. Surface charge in turn attracts a cloud of counterions, most easily modeled simply as a layer of negative charge at the membrane surface and a layer of positive charge a short distance (the Debye length) into the aqueous phase. Of course, actual binding of ions, such as calcium, neutralize charge (the Stern layer) and there are many instructive complexities; phospholipid membranes are a textbook example of the physics of charged surfaces in ionic solution [27]. Specific positive charge on protein surfaces explains the binding of extrinsic proteins to membranes. Also, surface concentrations of polyphosphate inositide lipids interact with specific domains of signaling and structural proteins (complementary binding domains), and some of these lipids may in turn regulate curvature by regulating the binding of curvature-active protein domains to membranes. Studies of cells expressing mutant versions of some of these proteins show that the curvature mechanisms first detected with lipid bilayers also apply to membranes containing these proteins *in vivo* [21].

One example: membrane microdomains

The challenge in the current field of membrane microheterogeneity is to discover its inner logic and categorize the types of membrane microdomains that allow for optimal organization and efficiency of the various processes that go on at the membrane surface. It may be that there are aspects of the membrane's composition that hinder or help proteins to do the work for which they are selected, and there must be mechanisms by which the right lipids get to these proteins. Evolutionary pressures have left us with membranes having hundreds of different lipid species, representing a selective pressure at some point for each of them. These hundreds of lipid species and thousands of membrane protein species make for a large universe of potential subsets of specific lipids and proteins; each subset may cooperate to form a type of functional unit of the biological membrane. How could such hypothetical units be organized in fluid membranes?

Surprisingly, the gel phase of a membrane offers clues to good organizational principles for proteins that need to form macromolecular complexes in the plane of the membrane. These complexes are real lattices with real lattice energies and result in the packing of crystalline hydrocarbons at the right temperature. For example, hexane has a discrete intermolecular spacing of 4.1 Å when frozen, compared to an average spacing of 4.5 Å when melted [28]. By controlling the number of lipids between them, proteins quantize the distance between themselves, perhaps nucleating a small volume where the intercalating lipids are ordered at temperatures higher than their transition temperatures.

Just as a biological membrane is a mosaic of lipids with very different curvatures that all stay flat despite their individual predilections, a biological membrane is also a mosaic of lipids with very different melting points, even those whose preferred state at physiological temperature is the gel state (solid) [29]. Thus we may imagine that proteins have a critical role in acting as well-defined solid objects in the membrane that impose constraints on lipid configurations and thus favor certain lipids for their nearest neighbor. With a huge variety of protein transmembrane domain sequences and a large variety of lipids, it is reasonable to imagine that proteins and lipids evolved together to build up biological structure in the membrane, and perhaps each membrane complex has its own specific protein and lipid compositions [30]. Thus the growing evidence for biological membranes having significant heterogeneity may be more a reflection of this large degree

of organization than the global duality of liquid-ordered *vs*. liquid-disordered microdomains that is seen in three-component phospholipid- and cholesterol-containing bilayers.

Summary

Membrane biophysics is a vast field, in which life uses all of the physical forces and laws to organize physiological processes. The simple physics of the phospholipid bilayer often dominates the structure of the membrane to provide compartmentalization of cellular space — proteins work within the constraints of the bilayer to catalyze lipid metabolism, bend membranes, transport impermeant substances, organize microdomains, and many other essential processes of life.

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The path of DNA in the kinetochore

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The kinetochore is the protein–DNA complex at eukaryotic centromeres that functions as the attachment site for spindle microtubules. In budding yeast, the centromere spans 120 bp, there is a single microtubule per kinetochore, and the entire spindle is composed of 16 kinetochore microtubules plus four interpolar microtubules from each pole. There are >65 different proteins at the kinetochore, organized in at least six core multimeric complexes [1]. A spindle checkpoint network monitors the state of attachment and tension between the microtubule and chromosome. We present a model for the path of DNA in the kinetochore.

Replicated sister centromeres become maximally separated by 600–800 nm in metaphase [2]. Separation progressively decreases along chromosome arms such that sister chromatids are tightly juxtaposed at ~10 kb from the centromere [2]. The molecular glue linking sister chromatids, cohesin, is recruited to a 20–50 kb region surrounding the centromere at 3- to 5-fold higher levels than centromere-distal locations [3]. A major paradox is the accumulation of cohesin at regions of separated sister DNA strands. A second problem is the nature of the mechanical linkage coupling DNA to a dynamic microtubule plus-end. This linkage must resist detachment by mitotic forces while sliding along the polymerizing and depolymerizing microtubule lattice.

We propose that pericentric chromatin is held together via intramolecular cohesion (Figure 1), similar to a foldback structure proposed for the fission yeast centromere [4]. In contrast to fission yeast, the budding yeast core centromere (120 bp DNA wrapped around a specialized nucleosome