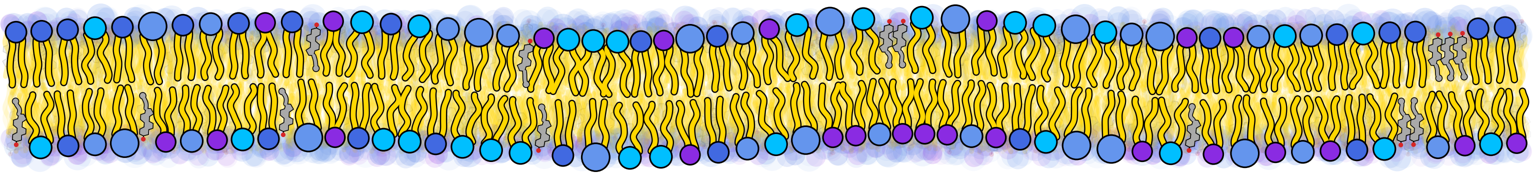

Lipid Membranes

Assembly, Geometry,
Elasticity, and Asymmetry

Problems

—— Version 1.1 ——

BY
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Problem sets for the 2024 Boulder Summer School on
“Self-Organizing Matter: From Inanimate to the Animate”

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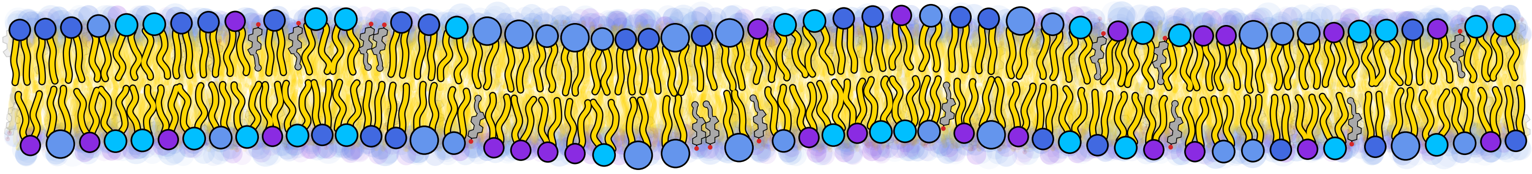

Organization

These problems are categorized as “Closed Problem” and “Open Problems”, where the former are the opposite of the latter. For the Closed Problems I know the solution, even though I have not actually written it up. For the Open Problems I *might* have some ideas for how to proceed, but I have no answer yet, and I can only venture guesses as to whether your particular approach might ultimately turn out to lead to a good solution, or at least a good way to rephrase the problem so that it might become accessible to other means. These are actual research questions. They are exciting (I think) and messy (I'm pretty sure). Proceed with caution, but dare dive into them. Who knows, maybe we can all write a paper about it one day?

The Closed Problems are a good way for you to practice the material I am trying to teach, and I urge you to try at least some of them. But you can dive into the Open Problems even if you haven't done the Closed ones. You might lack some training; true. But you might also lack some preconceptions, which might help. Do not shy away from Open Problems simply because the Closed ones look difficult.

Contents

1 Closed problems

1.1 Setting the stage

1. I claimed that chemical reactions happen at the eV scale and are thus not bothered by temperature. Does that strike you as plausible? If so, why do you think chemistry labs have Bunsen burners? Let's think a bit harder.
   Consider a chemical reaction that requires crossing an energy bar-

rier E_{barrier}. To be specific, let's say $E_{\text{barrier}} = 1\,\text{eV} = 40\,k_\text{B}T_{\text{room}}$. Thermal energy is too small to cross it (in a sense, we're $39\,k_\text{B}T_{\text{room}}$ "short"), but there's a small chance that a pretty *enormous* thermal fluctuation kicks you over it, and we expect that chance to be proportional to the Boltzmann factor $\text{e}^{-E_{\text{barrier}}/k_\text{B}T}$. That's teeny-tiny, but if we have a mole of material, we get lots of opportunities to try. Now: what happens if we change the temperature from 300 K to 600 K? Instead of $39\,k_\text{B}T_{\text{room}}$ we're now "only" $38\,k_\text{B}T_{\text{room}}$ short—it doesn't seem we made much headway. But that's of course the entirely wrong way to think about it. Doing it properly, how much more likely is it that the same chemical reaction is now triggered by a thermal fluctuation?

(You also just learned something deep about cooking.)

2. The interaction energy between two ions in solution is given by $U(r) = q_1q_2/4\pi\varepsilon_0\varepsilon_\text{r}r$, where q_1 and q_2 are the ionic charges, r is their separation, and ε_r is the dielectric constant.

   a) The so-called *Bjerrum length* ℓ_B is defined as the distance at which two monovalent ions in solution have an interaction energy equal to $k_\text{B}T$. Find a formula for it and calculate its value for water at room temperature!

   b) The dielectric constant of water actually depends a bit on temperature. A good approximation between 0 °C and 100 °C, due to Malmberg and Maryott (1956), is

   $$\varepsilon_\text{r}(T) \approx 10^{2.48151-0.001972(T/\text{K})} \approx \text{e}^{5.71388-0.004541(T/\text{K})} . \qquad (1)$$

   Show that this implies that the strength of electrostatic interactions in water *increases* with temperature. Does that strike you as weird?

   c) That the dielectric constant ε_r depends on temperature is a stark reminder that the electrostatic interaction in water is really a *free* energy. As such, it has an *energetic* and an *entropic* "component:" $\psi(r) = \psi_E(r) - T\psi_S(r)$, if you will. Leaning on some standard thermodynamic lore, such as $F = E - TS$, or $S = -\partial F/\partial T$, calculate $\psi_E(r)/\psi(r)$ and show that for the specific temperature dependence from Eqn. (1) the energetic part of the interaction *has the "wrong" sign* (meaning, two like charges in water would attract, if interaction *energy* were the only thing that matters. (The fact that they still repel means that the entropic part, $\psi_S(r)$, must overcompensate this blunder. This puts a new spin on the discovery that electrostatic interactions in water become stronger as the temperature increases.)

3. In Sec. 1.3, Problem 6c, specifically: Eqn. (9)) you will be asked to derive an approximate formula that relates the bending rigidity of *one leaflet* of a lipid membrane, κ_m, with its thickness d and the elastic modulus Y of the material from which it is made:

$$\kappa_\mathrm{m} = \frac{Yd^3}{12} \ . \tag{2}$$

   (In case you're wondering: the whole lipid *bilayer* has a rigidity that's just twice that: $\kappa = 2\kappa_\mathrm{m}$.) If you haven't derived it (yet), just believe it for now and proceed.

   a) If I tell you that $\kappa_\mathrm{m} \approx 15\,k_\mathrm{B}T$ and $d \approx 2\,\mathrm{nm}$, what value for the elastic modulus would you predict? Google! What type of matter does this suggest?

   b) A membrane's bending rigidity κ is *not* of order $k_\mathrm{B}T$. And it's not of order of hundreds of $k_\mathrm{B}T$ either. Ponder why the few-tens-of-$k_\mathrm{B}T$ value might be biophysically attractive! *(Hint: throwing another energy scale into the mix: the energy released by the hydrolysis of a single ATP molecule under physiological conditions is around* $20\,k_\mathrm{B}T$*, sometimes also a bit more.)*

   c) What if modern nanotechnology buffs decided to build such membranes out of steel? We'd like to keep the bending rigidity at the value I told you, for the reasons you hopefully unearthed in the last part, but we replace it with a modern-era material that has a modulus of 200 GPa. What thickness would it have to have? Does this strike you as realizable? What does it say, maybe in retrospect, about the fact that all of us are squishy beings?

4. I said that soft matter systems are particularly easy to drive out of thermal equilibrium. Can you imagine arguments that would show how they would also be great examples where the concept of active matter arises?

1.2 Self assembly of amphiphiles

1. While deriving the size distributions for cylindrical micelles, we encountered some nontrivial sums, which I claimed have closed

forms. Let's derive them. Our goal is to evaluate expressions of the type

$$S_n(x) = \sum_{n=1}^{\infty} k^n x^k \qquad \text{with } |x| < 1 \text{ and } n \in \mathbb{N}_0 \ . \tag{3}$$

The way to solve this is, curiously, by exploiting a tiny bit of calculus and some slick notation.

a) Remind yourself that you (hopefully) already know $S_0(x)$.

b) Define the differential operator $\hat{D} := x\frac{\mathrm{d}}{\mathrm{d}x}$. Now observe that x^k is an *eigenfunction* of that operator with *eigenvalue* k. Use this to rewrite the sum $S_1(x)$ using $S_0(x)$ and the differential operator $\hat{D}$.

c) Realize that this trick can be generalized into a formal expression for $S_n(x)$ that involves S_0 and powers of $\hat{D}$.

d) Explicitly evaluate $S_n(x)$ for $n \in \{1, 2, 3\}$, which we need in the notes.

2. Chemists might describe linear aggregation of monomers into chains as follows. We have monomers, which we denote as "X_1" and n-mers, which we denote by "X_n". And we assume that there are chemical equilibria that describe the addition of a monomer to an n-mer to create an $(n+1)$-mer, which look like this:

$$\mathrm{X}_n + \mathrm{X}_1 \xrightleftharpoons{K} \mathrm{X}_{n+1} \ . \tag{4}$$

The key to a simple analytical answer is assuming that the chemical equilibrium constant K is *the same* for all these reactions. Using the law of mass action, show that this leads *exactly* to the same exponential size distribution function for cylindrical micelles, which we derived in the notes using different means.

3. The mystery around the paradoxical result in connection with planar bilayer statistics, and its resolution, bear some striking resemblance to a similar conundrum (and its solution) in quantum mechanics, that typically arises when one first encounters Bose-Einstein condensation. Those of us who have learned about that material: ponder these two cases and narrate the similarity and differences!

1.3 Geometry and Elasticity of lipid membranes

1. Consider a smooth function $f(x)$. Given two close points x and $x + \mathrm{d}x$, as well as their two associated function values $f(x)$ and $f(x+\mathrm{d}x)$, find a simple heuristic argument for calculating the arc length $\mathrm{d}s$ which the function covers between these two points, and from there write down an expression that mirrors $\mathrm{d}A = \mathrm{d}x\,\mathrm{d}y\,\sqrt{1 + (\boldsymbol{\nabla} f)^2}$ in this simpler one-dimensional case!

2. Consider again a smooth function $f(x)$, and construct the *local normal* at some point $(x_0, f(x_0))$. Now also construct the normal at the slightly displaced point $(x_0 + \delta x, f(x_0 + \delta x))$. These two lines (generally) intersect at some point $(x_\mathrm{m}, y_\mathrm{m})$. In the limit $\delta x \to 0$ this point becomes the center of a circle that, if it touches the curve at the original point $(x_0, f(x_0))$, does not merely match the *slope* but also the *curvature*, and indeed the inverse radius of that circle is called the curvature of the curve at that point. Use this reasoning to calculate the curvature of $f(x)$ at a given point. Compare against the exact 2d result we derived for Monge gauge.

3. The principal curvatures for the two surfaces described by the functions $f_1(x, y) = \frac{1}{2}(x^2 - y^2)$ and $f_2(x, y) = xy$ in the notes (evaluated at $x = y = 0$) turned out to be identical, and the two principal directions were rotated by 45°. I claimed this happend because the *entire surfaces* are identical, just rotated with respect to one another by 45°. Show that this is true.

4. The upstairs-downstairs business with coordinates seems a bit odd. This problem strives to demystify this situation by explaining that two types of single-index objects exist which are fundamentally different in the way they transform under coordinate changes. And while this sounds like a big nuisance, this is really awesome, because it means we can construct *scalars* that are completely independent of the choice of coordinates.

    a) Say we have chosen a set of local coordinates $\{u^i\}$. Now we get second thoughts and rather take a different set, $\{\overline{u}^j\}$. To be able to translate back and forth between these choices, we must require there to be a relation of the form $\overline{u}^j = \overline{u}^j(u^i)$ (*i. e.*, the new coordinates are functions of the old ones), and also an inverse, $u^i = u^i(\overline{u}^j)$ (*i. e.*, the old coordinates can also be written as functions of the new ones). Moreover, these functions have to be sufficiently smooth, so that higher derivatives, if needed, also translate properly. (Technically, they are *diffeomorphisms.*) Now, these functions can be scarily nonlinear, but at any given point on our surface, we can expand these coordinates and recognize that the *local* relation is linear. To see this, simply look at the differential:

$$\mathrm{d}\overline{u}^j = \frac{\partial \overline{u}^j}{\partial u^i}\mathrm{d}u^i = M_i^j \mathrm{d}u^i \ . \tag{5}$$

Now here comes the fun bit: the differential $\mathrm{d}u^i$ is an object with an upstairs index, and it happens to transform from old to new coordinates with a matrix that describes the derivative of the new coordinates with respect to the old ones. But check out the tangent vectors $\boldsymbol{e}_i$. Show that when we transform those, *we get the inverse transformation behavior*, *i. e.*, a transformation with the *inverse* matrix: the derivative of the old coordinates with respect to the new ones!

b) Let's now say we have an object that arises as the contraction of one upstairs index with one downstairs index. Something like $U^i V_i$. Show that if we change our coordinates, such that $U^i \to \overline{U}^i$ and $V_i \to \overline{V}_i$, then the contracted object $\overline{U}^i \overline{V}_i$ has numerically the same value as the old one, *i. e.*, *it remains invariant under a change of coordinates.* In other words, it describes a piece of geometry that is finally free of the idiosyncrasies of a specific parametrization.
Hint: use what you have just discovered about the transformation matrix for objects with an upstairs vs. *a downstairs index. Observe in particular that the entire magic depends on the existence of the two transformation laws, and that it is hence* essential *that when we create these index contractions via the Einstein summation, one index is upstairs and the other one is downstairs. For instance, an object such as* $\sum_i U^i V^i$ *would* not *be invariant under coordinate transformations.*

5. Let's get some practice with the more general $\boldsymbol{X}(u^1, u^2)$ parametrization!

a) Recall how we wrote Monge gauge in a more fancy way:

$$(u^1, u^2) \mapsto \boldsymbol{X}(u^1, u^2) = \begin{pmatrix} u^1 \\ u^2 \\ f(u^2, u^2) \end{pmatrix} \in \mathbb{R}^3 \qquad \text{(Monge)} \ . \tag{6}$$

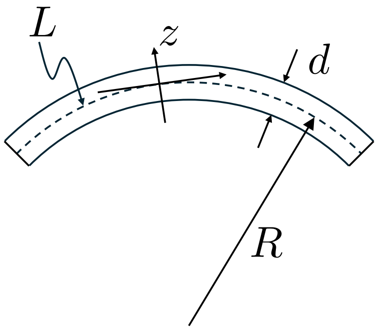

Figure 1 | Illustration of a thin plate bent along the "L-direction" into a circular arc of radius R. Set up a local coordinate system that measures the radial displacement from the mid-surface of the plate, but its origin lies at that mid-surface.

Use the definitions you find in Sec. 3.2.2 of the notes to work out the tangent vectors $\boldsymbol{e}_i$, metric g_{ij}, inverse metric g^{ij}, metric determinant g, normal vector $\boldsymbol{n}$, curvature tensor K_{ij} (which will look nice) and the shape operator K_i^j (which will unfortunately look less nice). By patiently working out the derivatives in Eqn. (3.12a), show that this boils down to the same expression as the trace of the matrix K_i^j you just evaluated.

b) Same game, but now for the description of a sphere using general coordinates

$$(u^1, u^2) \mapsto \boldsymbol{X}(u^1, u^2) = r \begin{pmatrix} \sin(u^1)\cos(u^2) \\ \sin(u^1)\sin(u^2) \\ \cos(u^1) \end{pmatrix} \in \mathbb{R}^3 \ . \tag{7}$$

Find $\boldsymbol{e}_i$, and g_{ij}. Observe that g_{ij} is diagonal and hence spherical coordinates are orthogonal. Calculate g and recognize $\sqrt{g}$ as the spherical Jacobian. Explicitly calculate $\boldsymbol{n}$ and see that, as hopefully expected, it is equal to $\boldsymbol{X}/|\boldsymbol{X}|$. Finally, calculate g^{ij}, K_{ij}, and K_i^j. When the dust has settled, you should find that $K_i^j = \delta_i^j/r$, showing that the two principal curvatures are identical and always equal to $1/r$, which we would of course expect for a sphere of radius r!

6. Consider a thin elastic solid plate of length L, width w, and thickness d. Imagine bending it, as illustrated in Fig. 1, such that its mid-plane now assumes a constant curvature of radius R. This will cost bending energy. In this problem our goal is to work out how this *bending* energy is related to the *stretching* energy of little volumes inside the plate because, clearly, pieces of the plate

on the "outer side" of the mid-plane are stretched, while pieces on the "inner side" are compressed.

a) Let's set up a radial coordinate system such that $z = 0$ sits at the plate's mid-plane. Slice up the plate into lots of thin concentric shells of thickness $\mathrm{d}z$ and let us make two critical assumptions: first, the mid-plane does *not* change its length along the "L-direction"; second, stretching any one of these thin slices will not change its thickness or width.[1] Given that, what is the length change of a thin slice that is a distance z away from the mid-plane?

b) If the resulting volume change of any such infinitesimal slice costs an infinitesimal elastic energy of the form

$$\mathrm{d}E = \frac{1}{2} Y \frac{(\mathrm{d}V - \mathrm{d}V_0)^2}{\mathrm{d}V_0} \ , \tag{8}$$

where Y is the so-called "Young modulus" (this is quite analogous to the area stretching equation (3.40) in the main notes), calculate the total elastic deformation energy of the bent plate by integrating up the infinitesimal costs of all slices.

c) Show that your result from (b) can be written as a bending energy density, $e = \frac{1}{2}\kappa(1/R)^2$, where the bending constant is given by

$$\kappa(\text{thin plate}) = \frac{Yd^3}{12} = \frac{K_A d^2}{12} \ , \tag{9}$$

where $K_A = Yd$ simply defines a 2d stretching modulus from the 3d Young modulus Y.

d) If you picture a lipid bilayer of thickness d as two elastic plates of thickness $d/2$ and use the bending energy results you've derived so far for these individual leaflets, show that the bending constant κ of a bilayer relates to the bilayer stretching constant K_A in the following way:

$$\kappa(\text{bilayer}) = \frac{K_A\, d^2}{48} \ . \tag{10}$$

The number "48" in the denominator is not quantitatively correct in real life. People have derived alternatives based on

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[1] Technically, this means we assume that the *Poisson ratio* of the material is zero.

better models (including Poisson ratio corrections, accounting for the polymeric nature of lipid tails, *etc.*). Overall, my feeling is that this argument is too crude to be trusted quantitatively, and efforts to make it more precise are maybe misguided; but it gives you a good order-of-magnitude idea.

7. Let's think about the surface of water. It has a surface tension σ, and we already know how to account for this. But water also has a density ρ, and raising or lowering some small packet of water above or below the resting level $f = 0$ under a gravitational acceleration g will cost energy (at sufficiently large scales). Convince me that within linear Monge gauge this can be accounted for by the energy

$$E = \int \mathrm{d}^2 r \left\{ \frac{1}{2}\sigma\big(\boldsymbol{\nabla} f\big)^2 + \frac{1}{2}\rho g f^2 \right\} = \frac{\sigma}{2}\int \mathrm{d}^2 r \Big\{ \big(\boldsymbol{\nabla} f\big)^2 + \big(f/\ell_\mathrm{c}\big)^2 \Big\} , \tag{11}$$

where $\ell_\mathrm{c} = \sqrt{\sigma/\rho g}$ is the so-called *capillary length.*

   a) What is the Euler-Lagrange equation satisfied by $f(\boldsymbol{r})$? What are its solutions in one dimension (*i. e.*, for functions $f(x)$)?

   b) Calculate the numerical value of ℓ_c for water and convince yourself that this, combined with your finding from part (a), makes sense in light of everyday experience.

   c) In analogy to our calculation for the power spectrum of a Helfrich membrane, Eqn. (3.70), calculate the power spectrum $\langle |f_{\boldsymbol{q}}|^2 \rangle$ for the thermal fluctuations of a water surface.

   d) Transform your result into real space and calculate the root-mean-square roughness $\langle f^2 \rangle^{1/2}$ of such a water surface. You may use a continuum approximation for the sum over $\boldsymbol{q}$-modes, but you will need to make some reasonable assumptions about the largest and smallest q-value.

8. Consider a rectangular paper strip of width w lying on a table such that a piece of length L reaches over the edge and sags under its own weight—see Fig. 2. Our goal in this problem is to calculate the shape of that strip in the limit of small sag, predict how much it sags, and from there determine the paper's bending rigidity.

   a) If κ is the bending rigidity of the paper, ρ its mass per unit area, and g is the gravitational acceleration, argue that the energy of a paper described by the shape $f(x)$ is given by

$$E = w \int_0^L \mathrm{d}x \, \left\{ \frac{1}{2}\kappa\big[f''(x)\big]^2 + \rho g f(x) \right\} . \tag{12}$$

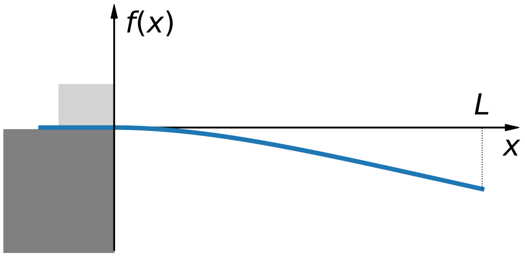

Figure 2 | Illustration of a piece of paper extending over the edge of a table, "clamped down" such that it initially extends horizontally over the edge but then begins sagging under its own weight.

There are a few approximations going on here—list them carefully and argue why they are permitted in the limit of small sag. (What in fact *is* "small sag" in the first place?

b) Perform the functional variation and show that the differential equation from which we will get $f(x)$ is

$$f''''(x) + \ell^{-3} = 0 \tag{13}$$

with some characteristic length ℓ. What is ℓ in terms of our given parameters?

c) What are the boundary conditions we need to apply at the two ends? The ones at $x = 0$ are fairly obvious, but the ones at $x = L$ are not, and you need to revisit our more careful thinking from Sec. 3.3.4 in the notes.

d) Now solve the differential equation—finding, as usual, a general solution and a particular solution. Show that in terms of the scaled variables $\tilde{x} = x/L$, $\tilde{\ell} = \ell/L$, and $\tilde{f} = f/L$, the solution can be written as the *universal shape*

$$\tilde{f}(\tilde{x}) = -\frac{\tilde{x}^4 - 4\tilde{x}^3 + 6\tilde{x}^2}{24\,\tilde{\ell}^3} \,. \tag{14}$$

e) Calculate the sag $s = |f(L)|$ as a function of all parameters, and solve this equation to determine κ in terms of parameters in the problem, as well as s. Now do a literal "table top" experiment: measure s and determine κ for some paper.

f) Making some reasonable estimate about the thickness of your piece of paper, and using Eqn. (9), estimate its stretching modulus K_A. Does its magnitude strike you as "large"? (Well, it has dimensions, so what would be a good point of comparison?)

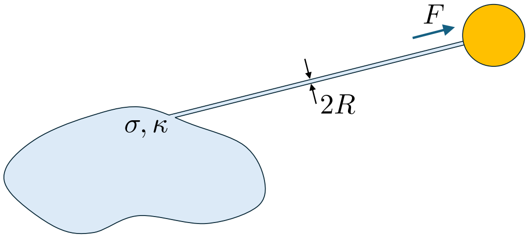

Figure 3 | An experimentalist pulls a thin long tether of radius R out of a cellular membrane; holding it requires the force F. Suitably set up, such an experiment can be used to measure the tension σ in the membrane, but a lot more is possible when you pull ingenious extra tricks.

9. A famous biophysical measurement on cells is to determine their surface tension. This works by attaching a microbead to them (somehow…) and then pull that bead away (many microns) in such a way that we can measure the force—see Fig. 3. Let us assume we can describe the cell's biomembrane with the Helfrich model, meaning we ascribe a tension σ and a bending rigidity κ to it. We now wish to learn how these parameters relate to one another, and how we can use that to measure interesting things.

   a) How much energy E is needed to pull a tether of radius R and length L out of a cell?
   (Hint: the calculation will show, and experimentalists know very well, that $R \ll L$, and so you do not need to worry about the ends of the tether.)

   b) The experimentalist controls L, but not R. The tether radius will adjust such that the energy of the whole tether is minimal. From this, find an equation that gives R in terms of σ and κ. Using typical values for those that I have hidden in these notes, calculate typical R values. Could you optically resolve them?

   c) The force to hold the tether is the derivative of the energy with respect to the length (at constant value of R). Show that it is given by

   $$F = 2\pi\sqrt{2\kappa\sigma} \; . \tag{15}$$

   That means: if we know F and κ, we can determine σ without having to know R. What are typical values for F? Google and convince yourself that this is exactly the right range for optical tweezers.

10. Consider a binary mixed lipid membrane *above* its demixing point (*i. e.*, no phase separation!) where the two types of lipids possess different spontaneous curvatures $J_{0,1}$ and $J_{0,2}$. Could the fact that their energy depends on the local curvature—*i. e.*, the composition and curvature are now coupled degrees of freedom—affect the power spectrum of membrane fluctuations? Would we maybe even get a different value for the bending rigidity if we naïvely fitted the "usual" Helfrich spectrum? Let's find out!

    Here's a minimal model for this. A Helfrich Hamiltonian with a simple mixing free energy for one leaflet:

    $$H_{\rm m} = \frac{1}{2}\kappa_{\rm m}\big[J - J_0(\phi)\big]^2 + g(\phi) \ , \tag{16}$$

    where J is the curvature and ϕ is the mole fraction of one of the two phases. For simplicity we assume that the leaflet's bending rigidity $\kappa_{\rm m}$ is not ϕ-dependent. For the mixing free energy g we make a simple mean field ansatz

    $$g(\phi) = \frac{k_{\rm B}T}{a_\ell}\big[\phi\log\phi + (1-\phi)\log(1-\phi) + \chi\phi(1-\phi)\big] \ , \tag{17}$$

    where χ is the Flory-Huggins mixing parameter and a_ℓ is the area per lipid.
    To couple composition and curvature, we make the (simplified) assumption that the spontaneous curvature of a mixture is just the linear combination of the curvature of its lipid components—proportionally weighted by their mole fractions:

    $$J_0(\phi) = J_{0,1}\phi + J_{0,2}(1-\phi) \ . \tag{18}$$

    a) If the average composition is $\bar{\phi}$, we can write

    $$\phi = \bar{\phi} + \psi \ , \tag{19}$$

    where ψ describes the (hopefully small?) composition fluctuations around the mean. By inserting this into Eqn. (17), show that it can be written as

    $$g(\psi) = \text{const.} + \text{const.} \times \psi + \frac{1}{2}B\psi^2 + \mathcal{O}(\psi^3) \ . \tag{20}$$

    Since we will subsequently only be interested in quadratic fluctuations, it turns out we really only need the quadratic term. You may drop the others. Find an expression for B!

b) Let us define the average curvature $\bar{J}_0$ and the curvature difference ΔJ_0 as

$$\bar{J}_0 = \bar{\phi} J_{0,1} + (1-\bar{\phi}) J_{0,2} \ , \tag{21a}$$

$$\Delta J_0 = J_{0,1} - J_{0,2} \ . \tag{21b}$$

Using this, show that the Hamiltonian of one leaflet can be written as

$$H_{\mathrm{m}} = \frac{1}{2}\kappa_{\mathrm{m}}\big(J - \bar{J}_0 - \Delta J_0\,\psi\big)^2 + \frac{1}{2}B\psi^2 \ . \tag{22}$$

c) Let's define the *bilayer* Hamiltonian as the sum of two monolayer Hamiltonians. Assume both sides contain the same mixture, and hence the same *averages*, but the local composition *fluctuations* will depend on the leaflet, so we need to define *two* fields $\psi_+(\boldsymbol{r})$ and $\psi_-(\boldsymbol{r})$. Write down the full Hamiltonian $H(J,\psi_+,\psi_-)$, where J is the bilayer curvature. *Hint: (1) Assume that J also describes the curvatures of each individual leaflet—so no parallel surface corrections. (2) Even if the lower leaflet also has curvature J, the lipids in it point the other way! Be careful about what this implies for the sign of the spontaneous curvature in that leaflet.*

d) In linear Monge gauge, we have $J = -\Delta f(\boldsymbol{r})$. At that level of approximation, we will now be interested in quadratic fluctuations of the three fields $f(\boldsymbol{r})$ and $\psi_\pm(\boldsymbol{r})$. Explain why this means that we can without harm drop $\bar{J}_0$ from our expression (and indeed all the other composition field terms that were constant or linear in ψ)!

e) Let's Fourier transform our fields! Here's the ansatz:

$$f(\boldsymbol{r}) = \sum_{\boldsymbol{q}} \tilde{f}_{\boldsymbol{q}}\, \mathrm{e}^{\mathrm{i}\boldsymbol{q}\cdot\boldsymbol{r}} \quad \text{and} \quad \psi_\pm(\boldsymbol{r}) = \sum_{\boldsymbol{q}} \tilde{\psi}_{\pm,\boldsymbol{q}}\, \mathrm{e}^{\mathrm{i}\boldsymbol{q}\cdot\boldsymbol{r}} \ , \tag{23}$$

with the usual conditions on the wave vectors and the Fourier coefficients to make the expansion real. Show that in Fourier space the Hamiltonian can be written as

$$H_{\mathrm{m}} = L^2 \sum_{\boldsymbol{q}} \frac{1}{2}\boldsymbol{v}_{\boldsymbol{q}}^\dagger \mathsf{M}_{\boldsymbol{q}} \boldsymbol{v}_{\boldsymbol{q}} \ , \tag{24}$$

where

$$\boldsymbol{v}_{\boldsymbol{q}} = \begin{pmatrix} \tilde{f}_{\boldsymbol{q}} \\ \tilde{\psi}_{+,\boldsymbol{q}} \\ \tilde{\psi}_{-,\boldsymbol{q}} \end{pmatrix} \tag{25}$$

and where $\mathbf{M}_{\boldsymbol{q}}$ is a hermitian (but not diagonal!) 3×3 matrix which you need to find!

f) Now off to fluctuations! In the lecture I used the simplest version of the *equipartition theorem*, which states that if we have a quadratic degree of freedom $\frac{1}{2}Kx^2$, then its expectation value is $\frac{1}{2}k_{\mathrm{B}}T$, and hence $\langle x^2\rangle = k_{\mathrm{B}}TK^{-1}$. There is a beautiful higher-dimensional generalization of this, proved in any StatMech book that probes even minimally into field theory, and it looks like this: if we have degrees of freedom $\{x_1, x_2, \ldots, x_N\}$, collected into a vector $\boldsymbol{x} = (x_1, \ldots, x_N)^\top$, and a quadratic energy of the form $\frac{1}{2}\boldsymbol{x}^\dagger\mathbf{K}\boldsymbol{x}$, with a symmetric ("kernel") matrix $\mathbf{K}$, then the expectation value of the product of any two degrees of freedom is

$$\langle x_i^* x_j\rangle = k_{\mathrm{B}}T\left(\mathbf{K}^{-1}\right)_{ij} . \tag{26}$$

Use this to calculate $\langle|\tilde{f}_{\boldsymbol{q}}|^2\rangle$.
Hint: Yes, you'll need to invert $\mathbf{M}$. *Feel free to use* MATHEMATICA® *or anything similar to do so.*

g) Unless you made a mistake, this spectrum looks like a tensionless Helfrich curvature fluctuation spectrum—in the sense that it is proportional to $1/q^4$. We will now insist that we can write it as $k_{\mathrm{B}}T/(L^2\kappa_{\mathrm{eff}}q^4)$ with some *effective* bending rigidity κ_{eff}. Show that this satisfies

$$\frac{\kappa_{\mathrm{eff}}}{\kappa} = 1 - \frac{\kappa\,\Delta J_0^2 a_\ell\,\bar{\phi}(1-\bar{\phi})}{2k_{\mathrm{B}}T\big(1-2\chi\bar{\phi}(1-\bar{\phi})\big)} \qquad \Big(\text{if } a_\ell\,\Delta J_0^2 \ll 1\Big) , \tag{27}$$

where $\kappa = 2\kappa_{\mathrm{m}}$ is the "bare" bilayer bending rigidity (*i. e.*, the one you would measure if there weren't any composition curvature shenanigans).

h) The case $\chi = 0$ was recently discussed in (Lessen et al., 2022) as a possible mechanism for why under certain circumstances people measuring the fluctuations of mixed membranes might measure "softened" values for the rigidity, *if their mechanism actually measures the true thermal equilibrium.* To see how big this effect might be, take $\kappa = 30\,k_{\mathrm{B}}T$, $\Delta J_0 = 0.3\,\mathrm{nm}^{-1}$ (that's pretty sizable, but not yet as big as it could be), $a_\ell = 0.65\,\mathrm{nm}^2$, $\phi = 0.3$, and $\chi = 0$ (ideal mixing). Can the inclusion of non-ideal mixing ($\chi \neq 0$) make the effect bigger?

1.4 Membrane asymmetry

1. A bending energy of the form $\frac{1}{2}\kappa(J - J_{0\mathrm{b}})^2$ can also be defined on the *leaflet* level, with individual monolayer bending rigidities $\kappa_{\mathrm{m}\pm}$ and spontaneous monolayer curvatures $J_{0\mathrm{m}\pm}$. Unlike $J_{0\mathrm{b}}$, the monolayer values $J_{0\mathrm{m}\pm}$ are generally nonzero, because there's no up-down symmetry in a single leaflet that would enforce this. Assuming that the two leaflets can slide past each other when the bilayer is bent, and ignoring stretching issues for this pure bending question, show that the bending-associated spontaneous curvature $J_{0\mathrm{b}}$ can be written as a function of the monolayer elastic parameters!
   (Hints: (1) The subtle difference in curvature a distance z_0 out or in from the bilayer midplane will turn into a higher order effect, so you can ignore it for this question. (Or you can check that this is in fact so.) (2) There is a subtle minus sign hiding in this question. As a control: do you get the expected answer when $J_{0m+} = J_{0m-}$?)

2. One of the hallmarks of membrane asymmetry are the two measures of spontaneous curvature, $J_{0\mathrm{b}}$ and $J_{0\mathrm{s}}$, appearing in the combined bending and stretching equation
   $$e^{\star}_{\mathrm{bend}} = \frac{1}{2}\kappa(J - J_{0\mathrm{b}})^2 + \frac{1}{2}\kappa_{\mathrm{nl}}(J - J_{0\mathrm{s}})^2 \ , \tag{28}$$
   especially the fact that they can be different from one another. Show that, sadly, both of them drop out of the physics in linear Monge gauge under periodic boundary conditions. This for instance means that a standard fluctuation analysis would not be able to learn anything about either of these values.

3. Consider an asymmetric membrane which has the lipid POPC on one side and the lipid POPE on the other. From the computer simulations of Venable et al. (2015) we take that they have virtually identical monolayer bending moduli $\kappa_{\mathrm{m}} \approx 15\,k_{\mathrm{B}}T$ but very different leaflet spontaneous curvatures: $J_{0\mathrm{m}}(\mathrm{POPC}) = -0.032\,\mathrm{nm}^{-1}$ and $J_{0\mathrm{m}}(\mathrm{POPE}) = -0.213\,\mathrm{nm}^{-1}$. Using the formula you might have derived in problem 1, this gives a bilayer curvature of $J_{0\mathrm{b}} = 0.09\,\mathrm{nm}^{-1}$ using the convention that POPC is "up" or "outside". Let's think a bit about vesicles we could make from such strongly asymmetric membranes.
   a) What's the radius of a vesicle in which both the overall torque and the differential stress are relaxed? On which side is POPE?

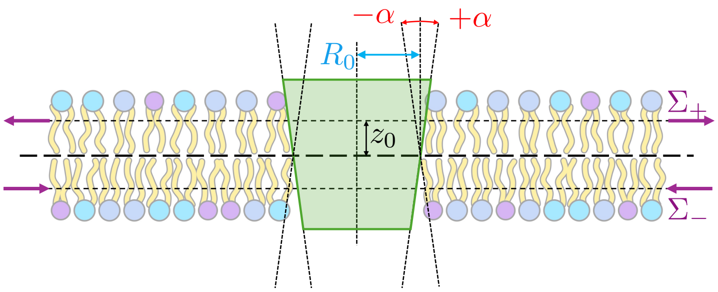

Figure 4 | Transmembrane protein embedded in a differentially stressed membrane. The indicated conformational change, which flips the truncated cone angle from $+\alpha$ to $-\alpha$, requires work to be done against the differential stress.

b) Eicher et al. (2018) succeeded in making vesicles with this type of lipidomic asymmetry, but with radii of $R \approx 60\,\mathrm{nm}$. In fact, they made them in both variants: $\mathrm{POPC}^{\mathrm{out}}/\mathrm{POPE}^{\mathrm{in}}$ as well as $\mathrm{POPE}^{\mathrm{out}}/\mathrm{POPC}^{\mathrm{in}}$. If we assume that the formation process selects the number of lipids on both side such that the overall bending rigidity from Eqn. (28) is minimal, what would be the differential stress for these two types of vesicles? Which side is under tension?

4. Some transmembrane proteins (such as ABC transporters) undergo conformational transitions that change their cross-sectional area in a way that differs between the two leaflets. As a toy example, consider the situation illustrated in Fig. 4: a protein shaped like a truncated cone "pivots" its orientation at the bilayer midplane in such a way that the cone angle changes from $+\alpha$ to $-\alpha$. If the membrane is under a differential stress $\Delta\Sigma$, what work needs to be done (or is done) due to this motion? Pick numbers for all the relevant parameters of the problem that strike you as biophysically plausible and express this free energy change in units of $k_{\mathrm{B}}T$. Do you think this could be relevant?

2 Open problems

1. Think about developing an experimental system that can measure *differential stress.* Not the overall leaflet tension $\Sigma = \Sigma_+ + \Sigma_-$; no, their *difference*, $\Delta\Sigma = \Sigma_+ - \Sigma_-$. Some obvious general approaches would be

    - designing particular small molecules with tension sensitive properties;
    - designing larger molecules (*e. g.* using DNA origami) to probe leaflet tension;
    - using molecules existing in nature (say, proteins) and using them in a new way;
    - exploiting certain physical properties of membranes (*e. g.* phase transitions) to arrive at proxies for $\Delta\Sigma$.
    - ....???

    Try to be as specific as possible in your description of the measurement process and the physical phenomenon you're hoping to exploit.
    Now ask yourself:

    - What are the major challenges that might cost the largest headaches and the most tweaking and tinkering?
    - What confounding factors exist? Phenomena that give a "signal" but are not related to differential stress, or at least not related in an easy way?
    - What could go wrong? Where are the fatal trip lines that might invalidate this approach?
    - How long would such an experiment take, from start to finish? A week? A month? A year? Longer than a PhD thesis? How much would it cost?

2. The model by Varma and Deserno (2022) discussed in the notes and the lecture, which describes the distribution of cholesterol in asymmetric membranes, accounts for leaflet stretching (or compressing), entropy, and the free energy of partitioning in a Flory-Huggins type of way. What it *does not* account for is

    - The fact that all these lipids have specific lipid spontaneous curvatures, including cholesterol, and these enter the bending energy density even if the membrane is flat.

   - The area of a membrane is not the sum of the individual areas of each lipid. This "additivity assumption" is particularly badly violated with cholesterol, whose addition to membranes can actually *shrink* them because cholesterol can increase the order of the lipids, which then pack better and use up less space. The resulting "negative differential area" is quite vividly demonstrated in the work by Leeb and Maibaum (2018), see especially Fig. 4.

   How could one include one or both of these new effects in the Varma model?

3. The Gaussian curvature modulus $\bar{\kappa}$ of lipid membranes is often irrelevant, because for one-component membranes without edges, for which also the topology never changes, this only contributes a constant to the free energy. But if we are for instance interested in fission or fusion, we would need to know its value for making progress. There are very few methods that have been proposed for doing so—either in simulation or experiment. Take a fresh look at the problem and ask yourself: If we really needed to know $\bar{\kappa}$, how could we determine it? Go through the same set of questions that I also listed above for the problem how to measure $\Delta\Sigma$.

4. People generally believe that Cholesterol flips vastly more rapidly than phospholipids, but some contrarian view exist. If you wanted to measure the flip-flop rate, how would you do it? (The goal here is to find something that's not already done!)

5. People also disagree about the question how cholesterol partitions between the two leaflets of the plasma membrane—let's just say, the human red blood cell. The discrepancy in opinions is huge: claims that the cholesterol content in the outer leaflet exceeds that of the inner one by more than an order of magnitude (Liu et al., 2017) coexist with claims that 60%–70% (Mondal et al., 2009) or even 80% (Courtney et al., 2018) of the total cholesterol content resides in the inner leaflet. Try to think about experiments that would be able to tell. Again, consider all the caveats and concerns I listed in the "measuring $\Delta\Sigma$ question" above.

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