BE/APh 161: Physical Biology of the Cell, Winter 2023
Homework #6

Due at the start of lecture, 2:30PM, February 22, 2023.

Problem 6.1 (Diffusion along a polymer, 10 pts)**.**
Some proteins, such as polymerases, diffuse along DNA prior to finding their binding sites. If a protein diffuses along DNA, its root mean square displacement along the filament scales as $\sqrt{t}$. How does the root mean square displacement *in space* scale with time?

Problem 6.2 (The persistence length , 10 pts)**.**
In lecture, we defined the persistence length to be the length ξ_p such that

$$\langle \mathbf{u}(s) \cdot \mathbf{u}(s') \rangle = \mathrm{e}^{-|s'-s|/\xi_p}. \tag{6.1}$$

Show that it follows from this definition that the persistence length is

$$\xi_p = \lim_{L\to\infty} \langle \mathbf{R} \cdot \mathbf{u}_0 \rangle, \tag{6.2}$$

where $\mathbf{R}$ is the end-to-end distance of a polymer and $\mathbf{u}_0$ is the unit tangent at $s = 0$. Explain intuitively why this expression is the persistence length.

Wormlike chains and endosome capture

In the next several problems, we will work with wormlike chains with an application of capture of endocytic vesicles into endosomes in mind. These problems were inspired by conversations with Marcus Jahnel and by Murray, Jahnel, et al., Nature, ***537***, 107–111, 2016. You should do the problems in order. It is up to you, but you may wish to do all of these problems in a single Jupyter notebook.

Endosomes are organelles that are involved in intracellular transport. When vesicles are brought in via endocytosis, they fuse with endosomes. As depicted in Fig. 1, endosomes are decorated with long proteins called endosome-tethering factor, namely EEA1. These tethers have a long, stiff **coiled coil** domain consisting of long alpha helices that are twisted around each other (hence the name, "coiled coil"). Murray, Jahnel, and coworkers exposed an interesting mechanism for how these long tethers serve to fuse vesicles with the endosome. Upon binding to the surface of the vesicle, the protein Rab5 binds EEA1. This binding event results in disruption of the coiled coil structure, thereby making the tether much more flexible. The more flexible chain then serve as an entropic spring to bring the vesicle and the endosome together.

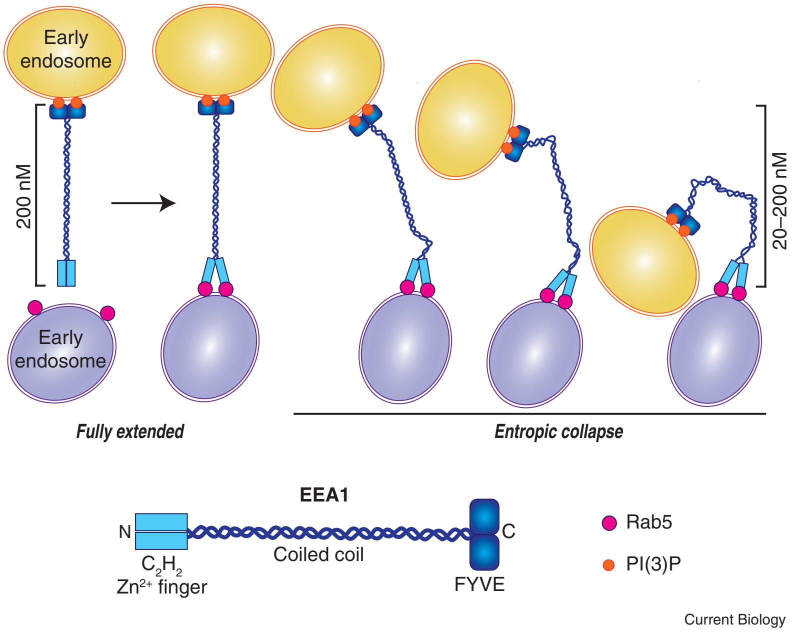

Figure 1: Schematic of vesicle fusion with endosomes mediated by EAA1. Image taken from Das and Lambright, *Curr. Biol.*, **26**, R927–R929, 2016.

Because the EEA1 coiled coil is stiff when not bound to Rab5 and semi-stiff (it's not *completely* flexible) when bound to Rab5, we need to treat its stiffness when studying it. We therefore need to employ the wormlike chain.

Problem 6.3 (Freely rotating chains and the wormlike chain, 35 pts).
We saw in class that a random walk on a lattice can be an effective model for a flexible polymer. We discussed that the statistics of a freely-jointed chain and those of a Gaussian coil are the same, to reasonable approximation, as those of a random walk on a lattice.

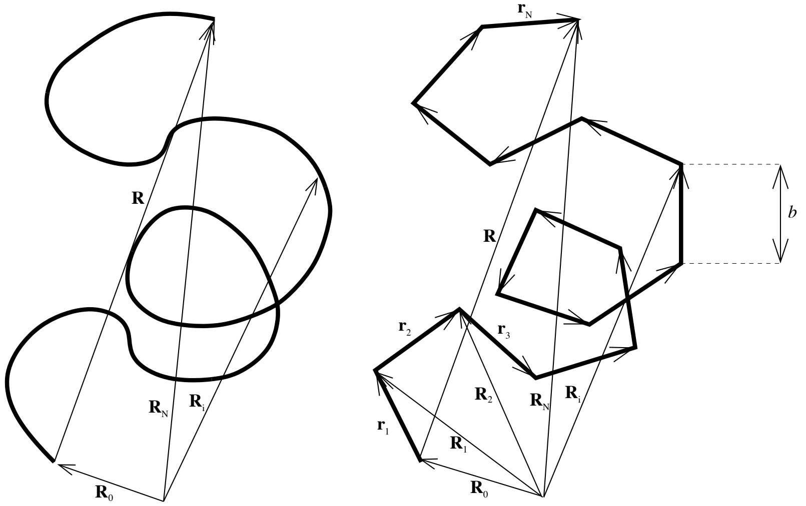

Figure 2: Schematic of a polymer modeled as a freely-jointed chain.

In a freely-jointed chain, shown in the schematic in Fig. 2, we model a polymer as rigid segments, each of which is connected to its neighbors by completely flexible joints. The angle between successive segments may take on any value with equal probability. Let $\mathbf{u}_i$ be the unit vector point along segment i such that

$$\mathbf{u}_i = \frac{\mathbf{R}_{i+1} - \mathbf{R}_i}{|\mathbf{R}_{i+1} - \mathbf{R}_i|}. \tag{6.3}$$

The next segment has a unit vector $\mathbf{u}_{i+1}$, such that

$$\mathbf{u}_i \cdot \mathbf{u}_{i+1} = \cos\theta, \tag{6.4}$$

where θ is the angle between the two segments. The segment $i+1$ can also swivel azimuthally to $\mathbf{u}_i$ with angle ϕ. For a freely jointed chain,

$$\theta \sim \text{Uniform}(0, \pi), \tag{6.5}$$

$$\phi \sim \text{Uniform}(0, 2\pi), \tag{6.6}$$

which is to say that any angle of the polar and azimuthal angle off of the $\mathbf{u}_i$ axis is equally likely.

For the FJC is useful for describing flexible chains, but missed the effects of stiffness. We discussed a wormlike chain in class; it is modeled as a space curve where the energy of a configuration of the chain is

$$E_{\text{chain}} = -\frac{EI}{2}\int_0^L \mathrm{d}s \left|\frac{\mathrm{d}\mathbf{u}}{\mathrm{d}s}\right|^2 = -\frac{\xi_p k_B T}{2}\int_0^L \mathrm{d}s \left|\frac{\mathrm{d}\mathbf{u}}{\mathrm{d}s}\right|^2, \tag{6.7}$$

where the key parameters are the length L of the chain and its persistence length ξ_p. Working with this continuum description has serious analytical challenges. It is also difficult to work with numerically; approximations are almost always necessary.

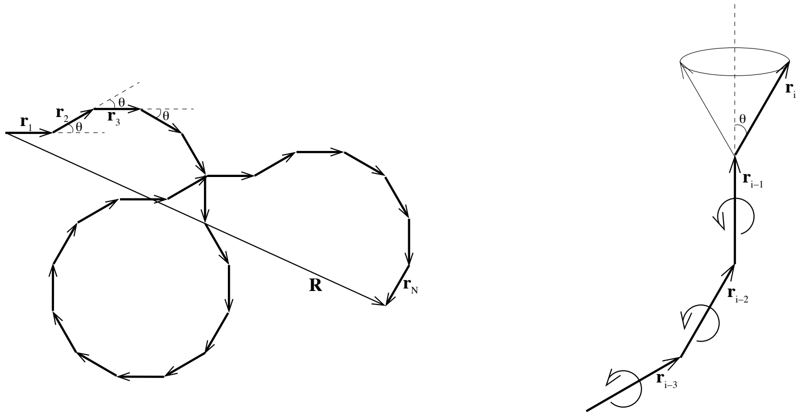

Figure 3: Schematic of a polymer modeled as a freely-rotating chain. To the left, a representation of a two-dimensional freely-rotating chain. To the right, a section of a freely-rotating chain in three dimensions. The angle between any to adjacent segments is θ, but the azimuthal angle with respect to the axis formed by an adjacent segment may take on any value, hence the name "freely-rotating chain."

The first approximation we will consider is the **freely-rotating chain** (FRC). This is a discrete version of a wormlike chain. Like the freely-jointed chain, the chain consists of N segments; we will call the length of each segment l. The angle between each segment is set to be θ, such that

$$\mathbf{u}_i \cdot \mathbf{u}_{i+1} = \cos\theta \;\; \forall i. \tag{6.8}$$

This is in contrast to the freely-jointed chain, where θ may take any value between zero and π. However, like the freely-jointed chain, the azimuthal angle between two segments in three dimensions can take on any value between zero and 2π with equal probability.

It can be shown (outside the scope of this homework) that this freely-rotating chain is a wormlike chain in the limit where the number of segments N goes to infinity, the segment length l goes to zero, and the angle θ goes to zero such that Nl remains constant, as does the quantity $l/(1 - \cos\theta)$. These two constants have physical meaning. The first is the total length of the polymer, $Nl = L$. The second is the persistence length,

$$\xi_p = \frac{l}{1 - \cos\theta}. \tag{6.9}$$

a) To approximate configurations of a wormlike chain, we can generate freely-rotating chains. In their paper, Murray, Jahnel, and coworkers measured profiles of EAA1 coiled coils and plotted them as bouquet plots, as in Fig. 2e and 2f of their paper. Write computer code to generate FRCs and make bouquet plots similar to those in the paper. You should work in dimensionless units, considering various values of ξ_p/L and plotting the position of the chains with length scaled by the total length L of the polymer. Make bouquet plots for $\xi_p/L = 0.01, 0.1, 1,$ and 10. Comment on what you see.

   It may be challenging for you to implement a FRC in three dimensions. You can start by doing the much simpler 2D chain, and then attempt to do a three-dimensional chain. When you plot the three-dimensional chain, you will have to plot a projection of the chain onto a plane. If you are having too much trouble, you can forego the three-dimensional calculation and only report the two-dimensional results for a total loss of two points.

b) For the same persistence length-to-total length ratios in part (a), generate many chains and compute the mean square end-to-end distance, $\langle \mathbf{R} \cdot \mathbf{R} \rangle$. How do your results compare to the result we derived in lecture,

$$\frac{\langle \mathbf{R} \cdot \mathbf{R} \rangle}{L^2} = 2\,\frac{\xi_p}{L}\left(1 - \frac{\xi_p}{L}\left(1 - \mathrm{e}^{-L/\xi_p}\right)\right)? \tag{6.10}$$

   Also use your results to compute $\langle \mathbf{R} \cdot \mathbf{u}_0 \rangle$. Do your results agree with the result of Problem 6.2?

Problem 6.4 (The Blundell-Terentjev approximation of a Wormlike chain, 25 pts). If we define $\mathbf{r}(s)$ to be the position of a wormlike chain a distance s along its path, then the unit tangent to the curve is

$$\mathbf{u}(s) = \frac{\mathrm{d}\mathbf{r}}{\mathrm{d}s}. \tag{6.11}$$

When modeling wormlike chains, it is therefore necessary to enforce that these are indeed unit vectors;

$$\mathbf{u}(s) \cdot \mathbf{u}(s) = \frac{\mathrm{d}\mathbf{r}}{\mathrm{d}s} \cdot \frac{\mathrm{d}\mathbf{r}}{\mathrm{d}s} = 1. \tag{6.12}$$

Both in terms of analytics and numerics, this constraint is difficult to enforce. In a series of papers, Blundell and Terentjev worked out approximate results for wormlike chains where this constraint is relaxed. *Locally*, they allow

$$\frac{\mathrm{d}\mathbf{r}}{\mathrm{d}s} \cdot \frac{\mathrm{d}\mathbf{r}}{\mathrm{d}s} \neq 1, \tag{6.13}$$

but *on average*, they have

$$\left\langle \frac{\mathrm{d}\mathbf{r}}{\mathrm{d}s} \cdot \frac{\mathrm{d}\mathbf{r}}{\mathrm{d}s} \right\rangle = 1. \tag{6.14}$$

Their key result was that they could write a probability density function for the end-to-end distance of a chain. Its unnormalized expression is

$$P(\gamma) \propto \int_{-\infty}^{\infty} \mathrm{d}\zeta\, \mathrm{e}^{i\gamma\zeta} \left(\frac{\sqrt{i\zeta}}{\sin\sqrt{i\zeta}} \right)^{d/2}, \tag{6.15}$$

where d is the dimension of the problem (either 2 or 3) and

$$\gamma = \frac{EI}{2k_BTL}\left(1 - x^2\right) = \frac{\xi_p}{2L}\left(1 - x^2\right), \tag{6.16}$$

where $\mathbf{x} = \mathbf{R}/L$, where $\mathbf{R}$ is the end-to-end vector of the chain, and $x^2 = \mathbf{x} \cdot \mathbf{x}$. In two dimensions this integral can be evaluated exactly (try it if you love contour integration!), and the result gives an unnormalized probability density function

$$P(\gamma) \propto \sum_{n=1}^{\infty} (-1)^{n+1}\, n^2\, \mathrm{e}^{-n^2\pi^2\gamma}. \tag{6.17}$$

In three dimensions, the integral is not tractable, but Blundell and Terentjev derived a very effective approximate formula,

$$P(\gamma) \propto \exp\left[-\pi^2\gamma - \frac{1}{\pi\gamma} \right]. \tag{6.18}$$

Written in terms of the dimensionless end-to-end vector $\mathbf{x}$, this is

$$P(\mathbf{x}) \propto \exp\left[-\frac{\pi^2\xi_p}{2L}\left(1 - \mathbf{x}\cdot\mathbf{x}\right) - \frac{2L}{\pi\,\xi_p\left(1 - \mathbf{x}\cdot\mathbf{x}\right)} \right]. \tag{6.19}$$

Unless indicated, we will use this as the PDF for a WLC.

a) Verify that the above PDF has the appropriate limiting behavior for small ξ_p/L.

b) Plot the approximate PDF $P(x)$ for a WLC in three dimensions. Do this for various values of ξ_p/L.

c) In this part of the problem, we will compare the Blundell-Terentjev PDF/CDF with the histogram/ECDF generated from samples of a FRJ. For comparisons against the FRJ, it is easier to compare the **radial distribution** instead of the distribution exactly. Instead writing the end-to-end vector $\mathbf{x}$ in spherical coordinates, where x is its magnitude, we have

$$P(x,\theta,\phi) = A\exp\left[-\frac{\pi^2\xi_p}{2L}\left(1-x^2\right) - \frac{2L}{\pi\,\xi_p\,(1-x^2)}\right], \tag{6.20}$$

where θ and ϕ are the polar and azimuthal angles of a spherical polar coordinate axis system, not to be confused with the similarly named angles in our FRC model, and A is a constant of proportionality (a normalization constant). We can marginalize over θ and ϕ to give

$$\begin{aligned} P(x) &= \int_0^{2\pi}\mathrm{d}\phi\int_0^{\pi}\mathrm{d}\theta\;\sin\theta P(x,\theta,\phi) \\ &= 4\pi A\exp\left[-\frac{\pi^2\xi_p}{2L}\left(1-x^2\right) - \frac{2L}{\pi\,\xi_p\,(1-x^2)}\right], \end{aligned} \tag{6.21}$$

Because the normalization condition is

$$\int_0^1 \mathrm{d}x\,x^2\,P(x) = 1, \tag{6.22}$$

we can solve for A by integrating over the radial direction x, since.

$$A = \left(4\pi\int_0^1 \mathrm{d}x\,x^2\exp\left[-\frac{\pi^2\xi_p}{2L}\left(1-x^2\right) - \frac{2L}{\pi\,\xi_p\,(1-x^2)}\right]\right)^{-1}, \tag{6.23}$$

The function

$$P_{\mathrm{rad}}(x) = x^2 P(x) \tag{6.24}$$

is referred to as the radial probability density function, which integrates to unity by integrating over x (without the radial component of the volume element of spherical polar coordinates).

Compute a histogram or ECDF of x^2 based on your simulations in Problem 6.3 (3D if you could complete it; otherwise use the 2D result) and overlay that with the theoretical radial PDF above (or CDF computed from it). If you only completed the 2D simulation in Problem 6.3, use the theoretical 2D result. Do the results match? If they do not, why do you think they deviate?

d) Write down the an expression for the free energy, $F(x)$, of a WLC in three dimensions under the Blundell and Terentjev approximation. Find the critical persistence length, ξ_p^{crit}/L above which an extended state has the minimal free energy and below which a crumpled state has the minimal free energy. Make a plot of the free energy as a function of x for various values of ξ_p/L, making sure to also include and highlight the curve for the critical persistence length. Are there parameter values for which there a multiple minima of the free energy?

e) Defining f to be the applied force at the ends of a polymer, with $f > 0$ being compressive and $f < 0$ being extensional, write down the force-extension curve for the Blundell-Terentjev model. Plot the dimensionless force-extension curve for various values of ξ_p/L and x. *Hint:* Think about how you can use the free energy to write an expression for the force-extension curve. Think also about how you need to modify the free energy expression in part (d) to include force and extension.

f) The Marko-Siggia model presented in Chapter 10 of *PBoC2* is only valid for extensional (nor compressive) applied forces. Plot the force-extension curve of the Blundell-Terentjev model along with the Marko-Siggia model of equation 10.26 of *PBoC2*:

$$\frac{f\xi_p}{k_BT} = \frac{1}{4}\left(1 - \frac{1}{(1-x)^2}\right) - x. \tag{6.25}$$

Note that this equation differs from equation 10.26 of *PBoC2* because we have defined positive force to be compressive. I have also adopted the notation of this problem.

Problem 6.5 (Coiled-coils and forces, 20 pts).
We will now use the results from the Blundell and Terentjev approximations for a WLC to model forces on vesicle by coiled coils and collapsed coiled coils.

a) For a polymer of length L, find the critical persistence length where the equilibrium end-to-end length of the polymer is greater than zero.

b) Murray, Jahnel, and coworkers measured the contour length of the EEA1 coiled coil to be 222 nm. In the absence of Rab5, they measured its persistence length to be 246 nm. (Their method of measuring this was very interesting; I encourage you to read the paper.) In the presence of Rab5, they measured the persistence length to be 74 nm. Updated measurements (Jahnel, personal communication, to be published) give values of

contour length	210 nm
persistence length without Rab5	250 nm
persistence length with Rab5	50 nm

How do these measured values relate to the critical persistence length you determined in part (a)? What does that mean EEA1's putative function for merging vesicles?

c) Calculate the force that the EEA1 polymers exert on a vesicle when it becomes flexible upon docking. How fast can it move a vesicle? (You might want to refer to BioNumbers to find typical sizes of vesicles and endosomes.)