

Homework #1: Biopolymers (For Problem 1 and 2, do work on here).

Problem # 1 - Polymerization Kinetics (15 pts)

Recall, in a simple example of polymerization kinetics there is an on and off rate for the monomers and we can define the polymerization with a differential equation. In reality, polymerization is more complex.

Consider actin & tubulin,

- subunits are not symmetric
 - they add to the two ends of a filament with preferred orientation giving rise to an oriented filament
- subunits carry ATP (actin) and GTP (tubulin) which hydrolyzed to ADP and GDP after polymerization.

Based on the ideal case and the illustration above, write down the equations for microtubule and actin. (5 points for equation and chart)

In general, $\frac{dn}{dt} = k_{on}C$ and $\frac{dn}{dt} = -k_{off}$ so $k_{on}C_{crit} - k_{off} = 0$

$C_{crit}^+ = \frac{k_{off}^+}{k_{on}^+}$ and $C_{crit}^- = \frac{k_{off}^-}{k_{on}^-}$ for the DP and TP side, respectively.

These ratios differ depending on which side (+ or -) and if it is DP or TP.

From these equations and the on/off rates, calculate the critical concentrations and fill in the table. Note the units for on and off rate are different.

	k_{on}^+	k_{off}^+	k_{on}^-	k_{off}^-	C_{crit}^+	C_{crit}^-
ATP-actin	11.6	1.4	1.3	0.8	0.12	0.62
ADP-actin	3.8	7.2	0.16	0.27	1.9	1.7
GTP-tubulin	8.9	44	4.3	2.3	4.9	5.3
GDP-tubulin	0	733	0	915	n/a	n/a

For actin, there is a condition called steady state treadmilling. Treadmilling occurs when one end of a filament polymerizes while the other end depolymerizes. This means the polymer neither shrinks nor grows. Based on your knowledge of polymerization kinetics, and the condition statement, can you derive the steady state concentration in terms of on and off rates for actin? (Hint, when you plug in numbers, $C_{std} = 0.17 \mu\text{M}$.) 10 points for this part.

$$\frac{dn^+}{dt} + \frac{dn^-}{dt} = 0$$

$$k_{on}^+ C_{std} - k_{off}^+ + k_{on}^- C_{std} - k_{off}^- = 0$$

$$C_{std} = \frac{k_{off}^+ + k_{off}^-}{k_{on}^+ + k_{on}^-}$$

Note, your steady state concentration is always between the positive and negative end critical concentration.

Problem # 2 - Derivation of Persistence Length (25 pts)

In class, I simply stated the equation for persistence length. Here you will attempt to derive the equation. Consider a continuous polymer of contour length L undergoing thermal fluctuations. We define a quantity s that runs from 0 to L and gives a parametrization by which each point on the polymer can be identified. We define the orientation at each point $\theta(s)$ as the angle the polymer makes with an imaginary horizontal line. At $s = 0$, the polymer end is fixed such that $\theta(0) = 0$.

Let's start with the approximation for the derivatives of some function $f(s)$. Hint: It stems from the first two terms of the Taylor series approximation. (2 pts)

$$\frac{df}{ds} \approx \frac{f(s + \Delta s) - f(s)}{\Delta s}$$

The equation above is a good approximation for small Δs as long as $f(s)$ is smooth. In our case, $f(s) = \langle \cos \theta'(s) \rangle$ where $\theta'(s) = \theta(s) - \theta(0)$ and $\langle \rangle$ indicates the time average.

A short aside for those wondering why cosine.

Let's say we are monitoring $\theta(s)$ over time and draw the probability distribution for it. The distributions near the fixed end or small s , then we expect that the chances the polymer to have a different orientation from $\theta = 0$ are very small, and so the distribution would be very sharp with a peak at $\theta = 0$. If we were to find the average cosine of the angle, we would find that $\langle \cos \theta \rangle \approx \langle \cos 0 \rangle = 1$. For large s , we would expect a much higher chance for the polymer to have a different orientation for 0 and the orientation is effectively random (uncorrelated from the fixed end) and so $\langle \cos \theta \rangle \approx 0$. In conclusion, $\langle \cos \theta \rangle$ decreases from 1 to 0 as s gets larger. In fact, this decrease occurs exponentially.

Back to the problem at hand! You can plug in our $f(s)$ in the approximation for the derivative you stated earlier. (1 pt)

$$\frac{df}{ds} \approx \frac{\langle \cos (\theta'(s + \Delta s)) \rangle - \langle \cos (\theta'(s)) \rangle}{\Delta s}$$

Now, if we let $\Delta\theta'(s) = \theta'(s + \Delta s) - \theta'(s)$. Rearrange this and replace $\theta'(s + \Delta s)$ in your expression from above. (1 pt)

$$\frac{df}{ds} \approx \frac{\langle \cos (\theta'(s) + \Delta\theta'(s)) \rangle - \langle \cos (\theta'(s)) \rangle}{\Delta s}$$

Note: $\Delta\theta'(s)$ and $\theta'(s)$ are independent quantities. Use the $\cos(a+b)$ expansion for the above expression. (1 pt)

$$\frac{df}{ds} \approx \frac{\langle \cos (\theta'(s)) \cos(\Delta\theta'(s)) - \sin (\theta'(s)) \sin(\Delta\theta'(s)) \rangle - \langle \cos \theta'(s) \rangle}{\Delta s}$$

Note: $\Delta\theta'(s)$ and $\theta'(s)$ are independent quantities. Use the identity $\langle ab \rangle = \langle a \rangle \langle b \rangle$ to further expand the expression. I will not go into where this comes from. (1 pt)

$$\frac{df}{ds} \approx \frac{\langle \cos (\theta'(s)) \rangle \langle \cos(\Delta\theta'(s)) \rangle - \langle \sin (\theta'(s)) \rangle \langle \sin(\Delta\theta'(s)) \rangle - \langle \cos \theta'(s) \rangle}{\Delta s}$$

Note, $\langle \rangle$ is time average but for our purpose everything is a function of space not time.

Phew! That is a pretty long expression. Let's start simplifying! $\Delta\theta'(s)$ and $\theta'(s)$ are equally likely to be negative or positive (symmetric about zero, odd functions like sine average to zero). Knowing this, write a simplified expression for df/ds . (1 pt)

$$\frac{df}{ds} \approx \frac{\langle \cos(\theta'(s)) \rangle \langle \cos(\Delta\theta'(s)) \rangle - \langle \cos\theta'(s) \rangle}{\Delta s}$$

Finally, recall $f(s) = \langle \cos\theta'(s) \rangle$ so factor this out and rewrite df/ds . (1 pt)

$$\frac{df}{ds} \approx \frac{\langle \cos(\theta'(s)) \rangle (\langle \cos(\Delta\theta'(s)) \rangle - 1)}{\Delta s}$$

$$\frac{df}{ds} \approx \frac{(\langle \cos(\Delta\theta'(s)) \rangle - 1)}{\Delta s} f(s)$$

Notice $df/ds = -C f(s)$ where $C = \text{constant}$. Can you justify why I placed a negative?

Consider cosine from $-\pi$ to π . For $\cos(0) = 1$, the constant is 0 and we get a totally different solution. For any other cosine in this interval, the cosine is between 0 and 1 so the term in the () will always be negative. (2 pts)

What is the solution for this differential equation $df/ds = -C f(s)$? (1 pt)

Use separation of variable to get

$$f(s) = e^{-Cs}$$

Note $C = 1/l_p$ where l_p is the normalization factor aka persistence length.

Note, from now on $\Delta\theta(s) = \theta'(s)$. Your final expression:

$$\langle \cos \Delta\theta(s) \rangle = \exp\{-s/l_p\}$$

Consider a segment of actin relation to a segment of DNA. Calculate the length of each segment such that the change in angle between the two ends of each segments is, on average, 25 deg. Assume the persistence lengths for actin and DNA are 15 microns and 50 nm, respectively. (5 pts)

Using $\langle \cos(\Delta\theta(s)) \rangle = e^{-s/l_p} \rightarrow$ solve for s .

$$s = -l_p \ln(\langle \cos(\Delta\theta(s)) \rangle)$$

$$\langle \cos(25 \text{ deg}) \rangle = 0.9$$

$$s = 0.1l_p$$

for actin, $s = 1.5$ microns

for DNA, $s = 5$ nm.

What does this mean? Physically for actin, on average, 1.5 micron span of thermally fluctuating actin would have a difference in angle of 25 degrees.

Earlier we considered a thermally fluctuating polymer. Now let's related flexural rigidity for an elastic beam. Recall, we derived the bending of an elastic beam earlier and derived its flexural rigidity EI . We now want to model a thermally fluctuating polymer as a curvy elastic beam; its persistence length is proportional to the flexural rigidity of the beam.

Consider an elastic 3-D beam with flexural rigidity EI bent 180 deg with constant curvature R . The elastic energy for this beam is

$$Q = EI\pi/2R$$

This can be rewritten for a general bend angle as

$$Q = EI\theta/2R$$

Recall the definition of arc length and express the bend angle in terms of arc length and curvature. Rewrite the above equation. (1 pt)

$$\theta = \frac{s}{R}$$

$$Q(\theta) = EI\theta^2/2s$$

This equation describes the internal energy of a beam of arc length s subject to constant curves such that the bend angle is θ radians.

Now here is where things will get a bit complicated but I do not expect you to know this so I will simply state it. If we were to submerge this polymer within a constant temperature heat bath, we could use Boltzmann's distribution to find the probability of finding a polymer with bend angle θ as

$$p(\theta) = \frac{1}{Z} e^{-Q(\theta)/k_b T}$$

Z is known as the partition function.

$$Z = \int_0^{2\pi} \int_0^\pi \exp\left(-Q(\theta)/k_b T\right) d\phi \sin\theta d\theta$$

We are taking the intergral with respect to a differential element of solid angle. We now seek to quantify the average amount of polymer curvature. We can do this by computing

$$\langle \theta^2 \rangle = \frac{1}{Z} \int_0^{2\pi} \int_0^\pi \exp\left(-Q(\theta)/k_b T\right) \theta^2 d\phi \sin\theta d\theta$$

Now do not worry, there is no way I am going to make you solve that. We are engineers and we love our small angle approximations! For small angles,

$$\langle \theta^2 \rangle = \frac{2k_b T s}{EI}$$

Now let's go back to what we derived before with cosine. What is the Maclaurin series for $\cos x$ (or Taylor series about 0)? Expand $\langle \cos \Delta\theta(s) \rangle$ to the 2nd order term. Note $\langle 1-x \rangle = 1 - \langle x \rangle$. (1 pt)

$$\cos x \approx 1 - \frac{x^2}{2} + \frac{x^4}{24} \dots$$

$$\langle \cos \Delta\theta(s) \rangle = 1 - \left\langle \frac{(\Delta\theta)^2}{2} \right\rangle$$

If the polymer is assumed to be a constant curvature beam of arc length s , then we can substitute in our expression for $\langle \theta^2 \rangle$ found right before your expansion and substitute. (1 pt)

$$\langle \cos \Delta\theta(s) \rangle = 1 - \frac{k_b T s}{EI}$$

Now note our earlier expression

$$\langle \cos \Delta\theta(s) \rangle = \exp\{-s/l_p\}$$

We can use another Taylor series expansion for $\exp\{-x\}$ for small x . Use this expansion to 1st order to rewrite the expression. (1 pt)

$$e^{-x} \approx 1 - x \dots$$

$$\langle \cos \Delta\theta(s) \rangle = \exp\{-s/l_p\} = 1 - s/l_p$$

See what just happened! You have two expressions for $\langle \cos \Delta\theta(s) \rangle$ that you simplified and you can set them equal to each other to get persistence length equation! Do this here: (5 pt)

$$\begin{aligned} \langle \cos \Delta\theta(s) \rangle &= \exp\{-s/l_p\} = 1 - s/l_p \\ \langle \cos \Delta\theta(s) \rangle &= 1 - \frac{k_b T s}{EI} \\ 1 - \frac{k_b T s}{EI} &= 1 - s/l_p \\ l_p &= \frac{EI}{k_b T} \end{aligned}$$

Problem # 3 - The primary cilium (30 pts)

The primary cilium is a microtubule-based structure on the surface on most cells, responsible for receiving signals [mechanical and chemical] from other cells and transmitting these signals to the nucleus, which in turn decides upon a cellular response.

Compute the buckling length of a primary cilium. As shown by the figure below, the primary cilium is made up of nine microtubules arranged in a ring as shown. Assume that each microtubule is not linked to its neighbor. Thus, each microtubule buckles independently. State which values you use for any constants (Young's modulus, membrane, tension, radius, and moment of inertia of the microtubule).

(5 points)

Assume $EI = 364 \times 10^{-25} \text{ N/m}^2$ where $E = 1.9 \text{ GPa}$.

$r = 12.5 \text{ nm}$

$F_{\text{mem}} = 50 \text{ pN}$. This is distributed over all 9 microtubules.

$$L_B = \sqrt{\frac{\pi^2 EI}{4F_b}} = \sqrt{\frac{\pi^2 (364 \times 10^{-25})}{\frac{4(50 \text{ pN})}{9}}} = 4.03 \text{ microns}$$

Note, I know the wording of this is funny so if you found the buckling length of the full cilium like this, you should around 1.34 microns and that is acceptable as well.

Now use parallel axis theorem to show that the bending moment of inertia of a collection of n microtubules is

$$I = \frac{\pi r^4}{4} \left(n + \frac{2n^3}{\pi^2} \right)$$

Assuming each microtubule is a solid rod of radius r and they are all tightly cross-linked to one another. Assume that there are enough microtubules that the circumference of the primary cilia can be approximated by the sum of the individual microtubule diameters. (20 pts)

The primary cilium is virtually circularly symmetric. Thus, let's find the polar moment of inertia to simplify things.

Recall,

$$J = J_c + Ad^2 \text{ where } d \text{ is the distance between the two axes.}$$

For generic n microtubules, you can estimate the circumference of the cilium as the sum of the diameters for each microtubule (imagine an n -sided polygon).

$$C = 2\pi R = n(2r) \text{ so } R = \frac{nr}{\pi}$$

$$\text{For one microtubule, } J_{MT} = \frac{\pi r^4}{2} + \pi r^2 R^2 = \frac{\pi r^4}{2} + \pi r^2 \left(\frac{nr}{\pi}\right)^2$$

$$\text{For the cilium, } J = nJ_{MT}$$

$$\text{Simplification yields } J = \frac{\pi r^4}{2} \left(n + \frac{2n^3}{\pi^2} \right)$$

Note, $I = \frac{J}{2}$ based on the properties of polar moment of inertia.

$$\text{Thus, } I = \frac{\pi r^4}{4} \left(n + \frac{2n^3}{\pi^2} \right)$$

Afterwards, use the formula to calculate the buckling length of primary cilium.

Use the formula for inertia and the E for a microtubule. (5 pts)

Assume, $n = 9$ and $F = 50$ pN.

Plug into

$$L_B = \sqrt{\frac{\pi^2 EI}{4F_b}} = 16.8 \text{ microns}$$

Problem #4 – Freely Joined Chain and Worm Like Chain Model (30 pts – 10 pts/question)

1. Summarize the manuscript in 150 words or less.
 - a. Include: Motivation, Methodology, Main Findings
 2. From lecture and from this paper, what are the differences between FJC and WLC model? What does this paper use?
 3. How was tension in the DNA molecule determined? Draw a FBD if it helps!
1. I wanted you to treat this like it was your own project pitch and simply state the motivation, methodology, and main findings of the paper. An example of 150 words is and less, is the abstract but please do not copy and paste.
 2. FJC treats polymers as discrete segments that are independent of each other. Thus it is an uncorrelated chain and the behavior of each segment can be modeled like a random walk distribution. It only accounts for the entropic contribution. In the limit of low force, FJC actually behaves like a Gaussian entropic spring. WLC treats polymers as an isotropic flexible continuous rod. Thus it considers both entropic and energetic contributions. After trying to apply the FJC model, this paper discovers that it is not sufficient to model DNA and recognize that they need to consider the energetic contributions.
 3. The DNA is attached to a magnetic bead in flow and the glass slide. Thus, there are two forces at play: magnetic and hydrodynamic forces. These forces extended the DNA by some angle and depending on the position of the particle, the angle would be different. All of these positions formed an ellipse to give a complete idea of the total force. In order to truly measure the force, they unleashed the DNA from its tether and measured the velocity. This velocity is used to determine the maximum force via Stokes relation but it needed a correction since beads near the glass would exhibit more viscous drag.