Biophysics Final Exam

You may consult any notes, texts or articles, but you may not discuss the exam with anyone, with the exception of normal generic exam griping. Please contact me if any question seems unclear.

Variation on the MWC model for hemoglobin

Adapt the MWC model for hemoglobin (p. 29 of the Binding lecture) for a hemoglobin that has one defective subunit. That is, suppose someone has engineered a mutant hemoglobin subunit with the heme binding site effectively permanently occupied.

I. Write down the partition function for a hemoglobin that contains a 1:3 ratio of mutant:wildtype subunits. Use the same variables as in lecture (ε_T, ε_R and ε) for the tight and relaxed binding energies and the tight-relaxed transition energy.

II. Calculate the binding curve (fraction of sites occupied) as a function of concentration. You can use the nondimensionalized concentrations x and y in this expression if you prefer.

III. Plot the binding curves for both normal Hb and this hybrid Hb with a reasonable set of binding coefficients ($Kx = 55 / mmO_2$, $Ky = 0.63 / mmO_2$, $\varepsilon = 10\ k_BT$). Give a brief explanation of the difference between the two curves.

Nonmotility

There are many ways to knock out cell motility, including (1) deleting the peptidoglycan binding motif in MotB (2) deleting MotA (3) abolishing the protonmotive force (PMF) using FCCP or similar poisons, or (4) weakening or dissolving the cell wall (peptidoglycan layer). From the outside of the cell, the effects of these interventions are indistinguishable: they all produce an immobile but intact flagellum. However, they rely on two distinct mechanisms to abolish motility: one knocks out rotation of the motor itself, while the other decouples motor rotation from flagellum rotation.

I. Which of (1)-(4) relies on which mechanism?

II. How would you experimentally demonstrate which mechanism was at work, assuming you had access to all the biophysical techniques we've talked about this semester? For each mechanism, write a short paragraph proposing a technique or two that would convince a skeptical reviewer, with specific details if possible.

Flashing ratchet model for kinesin

In lecture (pp. 38-39 of Motors I), I presented position versus time data for a one-headed kinesin and a two-headed kinesin. Fitting to the data gives speeds and effective 1D diffusion coefficients of

motor	v	D	t_{step}	L_{step}
one-headed kinesin	140 nm/s	44000 nm^2/s	10 ms	8 nm
two-headed kinesin	710 nm/s	2200 nm^2/s	1 ms	4 nm

My model of the stepping behavior gives the stepping times in the last column. L_{step} is the step length for the center of the kinesin molecule; for two-headed kinesin, this is half the head step distance (8 nm).

I. *Random walk analysis.*
   A. Using the biased random walk model from lecture (p.19 of Diffusion I), calculate the forward and backward rate constants k_+ and k_- using the above parameters.
      1. Show that you get nonsensical rates if you use an 8 nm step size for two-headed kinesin
   B. Using t_{step} and the rates from part 1, compute the forward and backward probabilities (per step) for both kinesins.
      1. Show that you get nonsensical probabilities (greater than 1) for one-headed kinesin.

II. *Flashing ratchet analysis*. To fix the $p > 1$ problem for one-headed kinesin, we need to invoke a more sophisticated model than the simple single step random walk. In the flashing ratchet model, one-headed kinesin diffuses freely (it's in the "loose binding state") with diffusion coefficient $D_{1\text{d}}$ for a time t_{step}. It then rebinds and is quickly pulled into the minimum of the tight-binding curve. The figure from lecture has a distribution of tight-binding locations (see P(x) in b2), but for simplicity we'll treat the motor as being *exactly* at one of the locations (...,-L,0,L,...) when tightly bound. Asymmetry is produced by the peak of the tight-binding free energy (U(x) in a2) being displaced a distance δ from the midpoint between binding sites. Take δ = 1.4 nm here.
   A. Starting from x = 0, and using $D_{1\text{d}}$ = 43,700 nm^2/s, write the probability distribution p(x) for positions of a motor after it has diffused for a time t_{step}.
   B. Binding causes all motors in the range {–L/2–δ, L/2-δ} to be pulled to x=0, and similarly for the other binding sites at –2L, –L, L, 2L and so on. Calculate the probability that the motor will rebind at positions –10 L, –9L, ... -L, 0, +L, ... + 10L. You'll need to do this numerically in Mathematica or something similar. Plot

$p(\Delta L)$. Your distribution should be pretty broad, which means there's a significant probability that the one-headed kinesin will take step of $2L$, $3L$, or even $4L$ in either forward or backward directions. (The equivalent distribution for two-headed kinesin is much less broad, which is why we can treat two-headed kinesin as only taking steps of $\pm L$).

C. The effective speed is

$$v = \frac{\langle L \rangle}{t_{\text{step}}} = \frac{\sum p(\Delta L)\Delta L}{t_{\text{step}}}$$

Calculate this and verify that it's close the quoted mean speed for one-headed kinesin.

D. The effective diffusion coefficient is.

$$D = \frac{\text{var}(x)}{2t_{\text{step}}} = \frac{\langle L^2 \rangle - \langle L \rangle^2}{2t_{\text{step}}} = \frac{\sum p(\Delta L)\Delta L^2 - \langle L \rangle^2}{2t_{\text{step}}}$$

Calculate this and verify that it's close the the quoted effective diffusion coefficient for one-headed kinesin.

Figuring out t_{step} and D_1 from the original data for D and v is the inverse of the procedure you just followed, which is considerably more complicated since it involvs fitting through the non-analytic process in step IIB. Note that you can do a similar analysis for the twoheaded ratchet; this gives D_{1d} = 1,577 nm²/s and a very narrow distribution for $p(\Delta L)$, (essentially no probability other than at -L, 0, and +L), justifying *a posteriori* the single-step random walk model in part I.

Flagellar motor

This section refers to the minireview “Flagellar movement driven by proton translocation” by David Blair in *FEBS Letters* 545:86-95 (2003), which is available in the “E-reserves” section of the course website. Please answer the following questions about the paper:

p. 86. “The fuel ... *Vibrio* species”: Why couldn’t a “neutrophile” use a Na^+ gradient too?

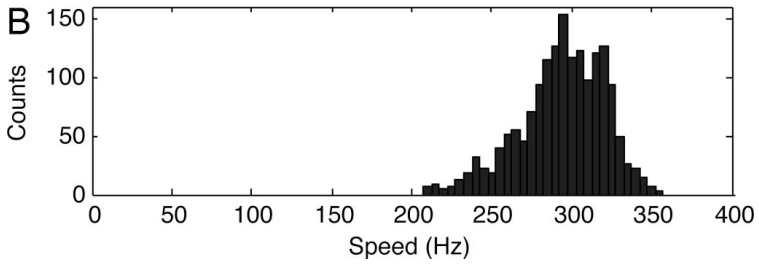
p. 88, “The rotor-stator interface ... C-ring”: How would you expect the torque-speed curve (Figure 3) to change depending on the location of the rotor-stator contract? In particular, what would you expect to happen to the x-intercept, y-intercept and knee of the plot? Assume that everything else about the motor is unchanged: only the contact radius changes.
p. 88, “(1) For a given ... constant torque”: Explain why this implies constant torque.

p. 88, “In an extension ... generation of torque”: Give a brief explanation of how symmetry mismatch can lead to a mechanism for rotation.

p. 88, “The lack ... motor components)”: Explain

p. 89, Figure 3.

I. We would like to probe the two portions of the torque-speed curve both below and above the ~200 Hz “knee”. The easiest way to do this is to shear off the flagellum, attach a bead to the stub, and let the motor turn the bead at some speed. Assume beads spin on axis; i.e. ignore the possibility that the bead is whipped around the circumference of a circle.
   A. What bead size would cause the motor to operate exactly at the knee?
   B. You can buy latex beads with diameters ranging from 0.5 microns to 10 microns. On a printout of Figure 3, indicate much of the torque-speed curve can you probe with this range of bead sizes. You will probably need to solve this problem graphically.
   C. In contrast, a recent paper (Yuan & Berg, *PNAS* 105:1182 (2008)) attached 60 nm diameter colloidal gold particles to the flagellar stub and measured the rotation rates below. Locate this point on Figure 3 above. Is this measurement consistent with Figure 3?



II. Panel B has idealized sketches of torque-speed curves for a “powerstroke” and “thermal ratchet” mechanism.
   A. In a few sentences, explain what “power-stroke” and “thermal ratchet” mean.
   B. The idealized curves in Fig. 3B are distinctly different around speed=0. Explain the difference, with particular attention to the question of backwards rotation.
   C. At high speed, it appears that the “thermal ratchet” curve is asymptoting to zero.
      1. Under what conditions would this happen?
      2. Under other conditions the “thermal ratchet” curve will go *through* zero at high speed. What conditions, and what is the significance of the zero crossing point?

p. 89, “Lithium ... load is light”: Does this agree with the H^+ motor mechanism as outlines in the previous paragraphs?

p. 90, “550 H^+ per revolution”: Blair skips a couple of steps in deriving the 550 proton number. Fill in the calculation explicitly.

p. 90, “Steps in the rotation ... smooth the motion”. Explain why this makes it hard to observe steps.

p. 92, “Titratable groups ... rapid rates needed”. Why would having titratable groups have any effect on kinetics?

p. 92, “Gly-rich linker .. flexible”. Why would a Gly-rich linker be flexible?