BE/Bi 101: Order-of-Magnitude Biology
Homework 6
Due date: Friday, February 20, 2015

"Life is the sum of trifling motions."

—Joseph Brodsky (1987 Nobel Prize in Literature, 1991 US Poet Laureate)

1. Entopy and viral packing.

In the very first lecture, we learned how to do approximate integration by approximating curves as lines. We estimated how much energy it takes to pack the φ29 genome. As a reminder, the experimental setup is shown in Fig. 1a. A bead in an optical trap is connected to the φ29 DNA. The viral capsid is attached to an immobilized bead. The packing motor works to pack the genome into the capsid. The optical trap is calibrated such that the force counteracting packing can be measured. The resulting measurement is the force-packing curve in Fig. 1b. The area under this curve is the energy required to pack the ≈20,000 bp genome.

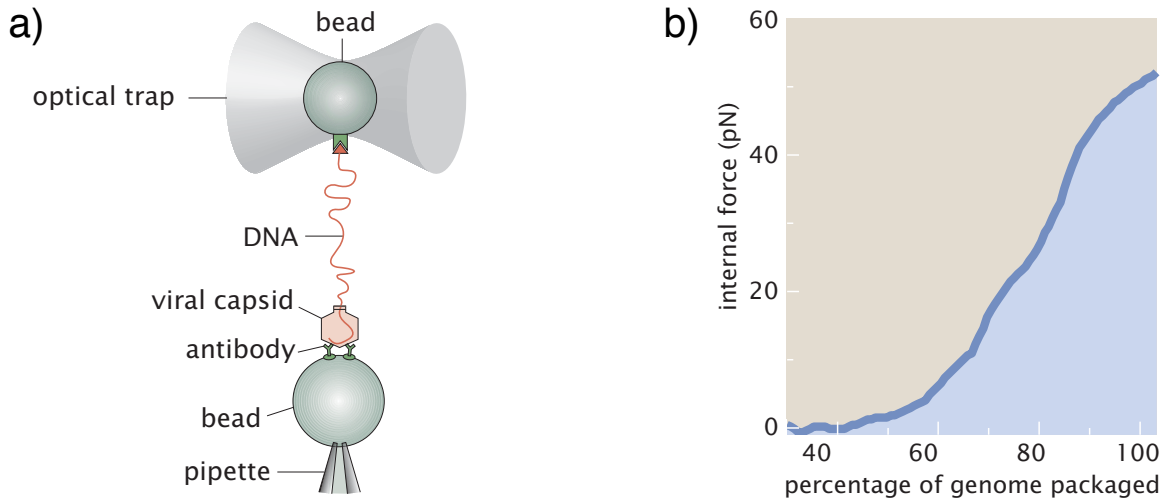

Figure 1: Force versus fraction of genome packed for the φ29 virus. Adapted from *Physical Biology of the Cell, 2nd Ed.*, by Phillips, Kondev, Theriot, and Garcia, who adapted it from Smith, et al., *Nature*, **413**, 748–752, 2001.

In this problem, we will estimate that energy (in terms of pN-nm this time, and not units of ATP) and compare it to the energy required to overcome the loss of entropy incurred by packing a free chain into a tiny virus. Presumably, the rest of the energy required to pack the DNA comes from charge interactions and bending and twisting the DNA to fit into the tiny virus. As you work through the problem, remember that we gave you some key numbers to remember in lecture, including the persistence length of DNA, which is about 50 nm, or 150 bp.

a) Prior to packing, we will assume that the DNA can be modeled as a random walk. For simplicity, you can model your random walk on a 3D lattice (this choice does not strongly

affect the results). After packing, the DNA has almost crystalline order inside the virus. We will assume that the packed DNA has only one configurational state. Our goal is now to compute the entropy change, ΔS for packing. For now, express your answer in units of the Boltzmann constant. *Hint*: Remember what is written on Boltzmann's tomb: $S = k_B \log W$, where S is the entropy, k_B is the Boltzmann constant (on the tomb, it's actually written only as k), W is the total number of configurations available to the system (in our case the DNA polymer), and the logarithm is base e.

b) The contribution to the energy required for packing as a result of limiting the configurations of the DNA polymer is $T\Delta S$. When computing this contribution to the packing energy, remember a key number that we gave you in the first lecture, $kT \approx 4$ pN-nm.

c) Use Fig. 1b to estimate the energy necessary to pack the φ29 genome into the virus. How does this compare to the entropic energy you computed in part (b)? Is the entropy of confinement an important contribution to the energetics of packing?

d) We assumed that we could model the DNA as a random walk and that there was only one spatial configuration of DNA when packed. Under these assumptions, do you think we would overestimate or underestimate the entropic contributions to the packing energy? What other contributions to the entropy of packing should we consider?

2. Thermal diffusion versus gravity.

In our discussions of random walks modeling diffusion, we neglected the influence of gravity. Imagine we have a particle of size a that is diffusing in a solvent. The difference in density between the particle and solvent is $\Delta\rho$; for simplicity in thinking about this problem, we will assume the particle is more dense than the solvent. You can imagine that if the particle is in a very tall tank of solvent, the particle will tend to settle, having higher probability of being toward the bottom of the tank. Conversely, if the tank of solvent is short, the particle can be anywhere within the tank with essentially equal probability. There is then some length scale, ℓ_{sed}, for which the thermal forces balance the gravitational forces. Write an expression for ℓ_{sed} in terms of $\Delta\rho$, a, and of course the gravitational constant, g, and the thermal energy, k_BT.

Feric and Brangwynne (*Nat. Cell Biol.*, **15**, 1253–1259, 2013) found that nucleoli in *Xenopus* eggs are of higher density of the nucleoplasm, with $\Delta\rho \approx 0.03$ g/mL. The nucleus of *Xenopus* eggs is gigantic, about 450 µm across. How big would the nucleoli have to be to start to see sedimentation? Incidentally, nucleoli in the *Xenopus* egg can be over 2 µm in size.

3. Biased active random walks and diffusion to capture.

During *Drosophila* oogenesis, droplets of RNA and protein (RNPs) are actively transported as cargo of kinesin motors walking on microtubules from the anterior of the oocyte to the posterior. This is a distance of about $l_{\text{oocyte}} \approx 70$ µm. Interestingly, the microtubules are more or less randomly oriented throughout the cytoplasm. This system is depicted in Fig. 2.

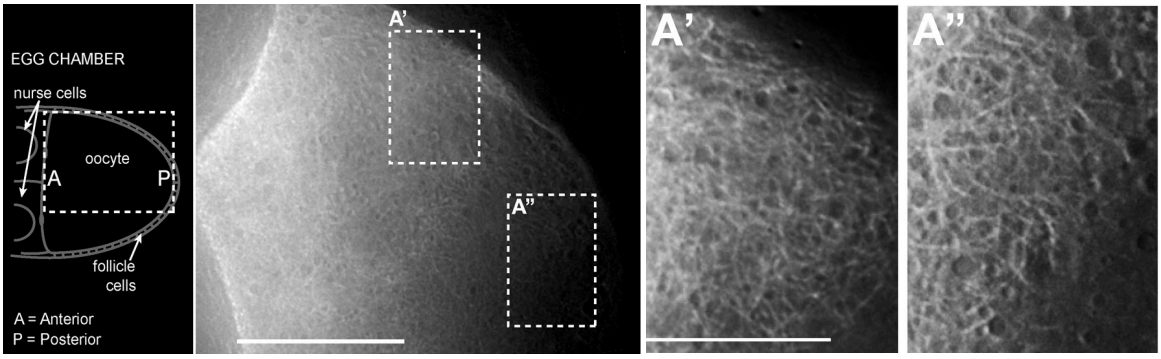

Figure 2: A *Drosophila* oocyte. Left, schematic with anterior and posterior labeled. Second from left, Tau-GFP live imaging, showing microtubules. Scale bar: 25 µm. A′ and A″ depict zoomed regions. Scale bar: 10 µm. Adapted from Parton, et al., *J. Cell Biol.*, **194**, 121–135, 2011.

In a series of careful and painstaking experiments, Zimyanin and coworkers (*Cell*, **134**, 843–853, 2008) observed that the kinesin motors move the RNPs along the microtubules at a speed of about 400 nm/s. They also observe that the kinesin motors travel about 2.5 µm before detaching and reattaching to a neighboring microtubule.

a) This process can be described as active diffusion. What is the diffusion coefficient?

b) The posterior has a sticky goo that traps the RNPs when they arrive there. So, one may think of the process of moving the RNP from the anterior to the posterior as a diffusion/capture process. Derive a scaling relation relating the diffusion coefficient you computed in part (a) to the typical time to capture at the posterior. Is this timing consistent with typical developmental time scales?

c) A typical RNP is about 100 nm in diameter. One of us (JB) has measured the viscosity of the cytoplasm in the oocyte to be about 1000× that of water. On average, how long would it take the RNP move from the anterior to capture at the posterior by thermal diffusion? How does this timing compare with active diffusion? Is thermal diffusion consistent with developmental time scales? *Hint:* Remember the Stokes-Einstein-Sutherland relation, $D = k_B T/6\pi\eta a$, and some of the key numbers from the first day of class, $k_B T \approx 4$ pN-nm and $\eta_{\text{water}} \approx 0.001$ Pa-s $= 10^{-9}$ pN-s/nm^2.

d) Interestingly, Zimyanin and coworkers also measured that the microtubules have a 57% bias in their alignment pointing toward the posterior. This exact number was later verified by other methods by Parton and coworkers (*J. Cell Biol.*, **194**, 121–135, 2011). So, the process is actually an active diffusion plus drift to capture. What is the drift velocity? Use the drift velocity to determine the time to capture of an RNP starting at the anterior. How does this compare to the active diffusion to capture estimate from part (b)?