

MOLECULAR, CELLULAR, & TISSUE BIOMECHANICS

Spring 2015

Problem Set #2

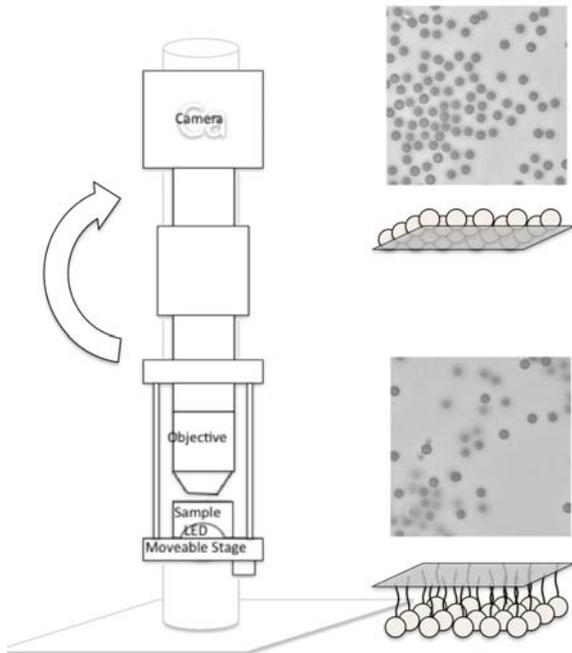
Distributed: Tuesday, February 10, 2015

Due: Wednesday, March 4, 2015

Problem 1: The Flipscope

In lecture we discussed a few different methods to obtain single molecule force extension curves or unbinding kinetics, including two that were capable of obtaining data on large numbers of molecules simultaneously (recall the centrifugal force scope and the “flipscope”, both from Wesley Wong’s lab at Harvard). In the second case, molecules are tethered to a bead at one end, and to a planar surface at the other. Force is applied by gravity when the microscope is rotated 180 degrees due to the difference in density between the bead and the surrounding fluid when the scope is inverted (see figure below).

Given values: $l_p = 0.4 \text{ nm}$, $L_c = 40 \text{ nm}$, $k_B T = 4.1 \text{ pN nm}$



a) First, treating each protein as a random coil (no internal bonds) under a constant force, $F = 10 \text{ pN}$, use the Gaussian chain model to calculate the most probable end-to-end length of the protein. Would you expect the Gaussian chain model to provide an accurate estimate under this level of force? Why or why not?

b) Now, assume that the molecule remains tethered to the surface, but has two well-defined conformations, which we refer to as states 1 and 2. Given that we know the applied force, and assuming that we have the capability to measure the distance of each tethered bead from the surface (i.e., the end-to-end length of the molecule) show how you would calculate the free energy difference, ΔG , between the two states. That is, provide the equation that you would use to compute ΔG and explain how you would determine each of the other parameters in the equation.

You may assume that in this experiment each of the molecules remains attached to both the surface and the bead.

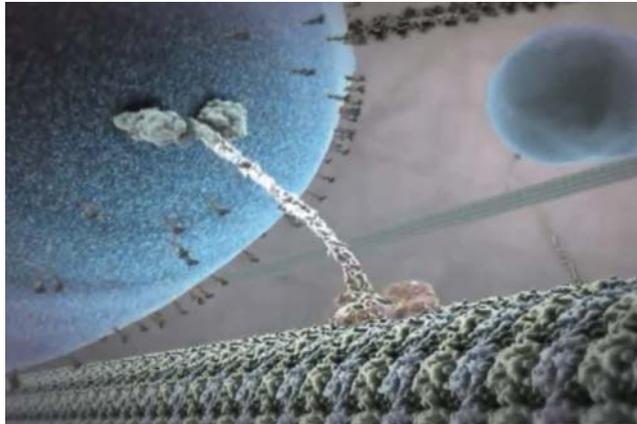
c) Sketch, on a graph of p_2 vs. time, the evolution of extended states at two different levels of force. You don't need to provide numbers, but be careful to capture the relative slopes and magnitudes of the two curves and indicate which is at the higher force.

Problem 2 – Motor walking

One mode of intracellular transport is by means of molecular motors, which carry cargo contained in lipid vesicles along the filaments that span the intracellular space. Motors such as kinesin (shown in the image), walk along these filaments (microtubules) in a hand-over-hand movement in which one hand remains fixed to the filament while the other reaches over, binds to the filament and causes release of the one behind. This can be likened to the hand-over-hand motion of someone climbing a rope.

In this problem we ask how far a motor can travel along a filament before it breaks loose. You can assume the following:

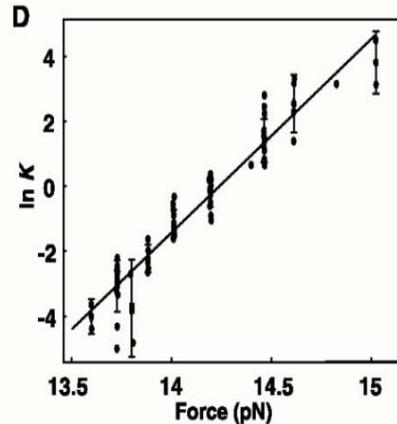
- During the time that one “hand” of the motor is attached to the filament, it exerts a constant force $F = 2 \text{ pN}$ in order to drag its cargo (the vesicle) along.
- That the unbinding of the motor from the filament can be viewed as a transition between two states, one bound and one free.
- That each step that the motor takes is 8 nm in length and that the rate of stepping is such that the time during which the motor is exerting the (constant) force F is 0.2 s during each step.
- That the difference in free energy between the bound and free states ($G_2 - G_1$) = $2 \text{ pN}\cdot\text{nm}$, that the distance from the bound state to the unbound state is $x_2 - x_1 = 3 \text{ nm}$, and the distance from state 1 to the transition state is 1 nm .



- a) What is the ratio of probabilities at equilibrium between the bound (“1”) and free (“2”) states when a force of 2 pN is applied?
- b) Obtain an algebraic equation that can be used to calculate the rate of change of the probability of unbinding during the period of time, Δt , that the motor arm is attached to the filament. (For parts b-c of the problem, assume that once unbound, the motor cannot rebind to the filament and that the value of the unforced rate constant $k_+^0 = 0.1 \text{ s}^{-1}$.)
- c) Solve the equation in (b) to estimate the probability that the motor will unbind during a single step. You can assume that once the bond is ruptured, it cannot reform, and that the initial probability of being bound (state “1”) is $p_1 = 1$ at $t = 0$.
- d) Given your result in (c), and assuming that unbinding is an independent event on each step, how far on average will the motor walk along the filament before it has a 50% probability of unbinding?

Problem 3:

Consider the RNA unfolding experiments by Liphardt et al. (Science 2001) where a RNA hairpin unfolds under force. The experiment can be modeled as a two state system of RNA transitioning between an unfolded and a folded state with a force dependent equilibrium constant $K_{eq}(F)$. Plotting the logarithm of K_{eq} , i.e. $\ln(K_{eq})$, vs. the applied force F yields the following result:



- Based on the plot $\ln(K_{eq})$ vs. F , calculate the distance Dx between the folded state and the unfolded state.
- Using your result from (a) and the information from the graph, determine the equilibrium constant in the absence of force, K_{eq}^0 , and the unfolding free energy, DG , between the folded and the unfolded state. Express in terms of $k_B T$ and also in kJ/mol . Does this amount of free energy, DG , seem reasonable for a RNA hairpin of ~ 20 nucleotide pairs? Assume that experiments are done at room temperature, i.e. $k_B T \sim 4.1$ pN \cdot nm.
- Derive an expression for the fraction of unfolded molecules (to the total number of molecules) as a function of force. What fraction of molecules is in the unfolded state when no force is applied? At what force are 50% of the molecules unfolded?
- Assuming that the transition state is approximately equidistant between the folded and unfolded state, sketch the energy landscape of the system (using protein extension as the reaction coordinate) (i) in the absence of force and (ii) with ~ 15 pN force applied.

Problem 4

Certain pathological conditions are associated with changes that occur only intermittently, and are therefore sometimes difficult to observe during infrequent visits to the clinic. In this case, assume that the disease is associated with infrequent increases in blood flow velocity to a particular organ. The proposed solution to capture a record of this event is to inject “flow probes” which consist of a microsphere tethered to a protein that forms a strong and persistent bond with one of the receptors expressed on the surface of endothelial cells (that line the inside wall of the blood vessels) of a particular organ. These probes, when subjected to high flow creating a drag force on the bead, produce a conformational change in the protein bridging between the bead and the receptor, switching its conformation so that the protein becomes fluorescent. (There are a number of ways to do this, but for now we are interested in the mechanics of the change in conformation rather than in the imaging details.)

- a) As a first approximation, the protein used can be modeled as a Gaussian chain having a persistence length of 0.4 nm and a total contour length of 400 nm. What is the radius of gyration of the protein?
- b) Actually, none of the three models discussed in class (Gaussian chain, FJC, WLC) does a very good job in describing the behavior of protein unfolding. Why?
- c) When the probe becomes tethered to the wall (under zero flow conditions), the configuration is as shown schematically in Fig. 1a. The protein is engineered so that it can be characterized by the two-state energy landscape shown in Fig. 1b. What is the difference between the two free energies, $G_1 - G_2$ if you want 99.9% of the protein sensors to reside in state “1” under zero force?
- d) How much force must be exerted on the microbead by the flow in order to switch 50% of the protein into state “2”?
- e) If the elevated flow state is short-lived, then it is important that a large fraction of the proteins shift into the extended (fluorescent) state during this short period. What is the ratio of rate constants k_+/k_- under a force of 5 pN? Given that you could calculate the individual values of k_+ and k_- for this condition, what equation would you solve to determine the rate at which state “1” becomes repopulated? (We want an equation here for dP_1/dt in terms of the rate constants and probabilities, P_1 and P_2).
- f) Once the protein is switched into state “2”, it must remain there for some period of time (e.g., remain fluorescent), under zero force, until the patient returns to the clinic for observation. Considering the energy landscape of Fig. 1b, describe one way in which you might redesign the protein to slow the rate at which it reverts to state “1” without changing the ratios of P_1 and P_2 under the forced or unforced conditions. Only a qualitative answer is expected.

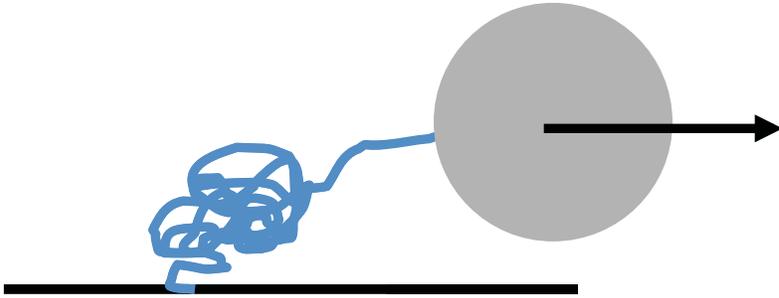


Fig. 1a

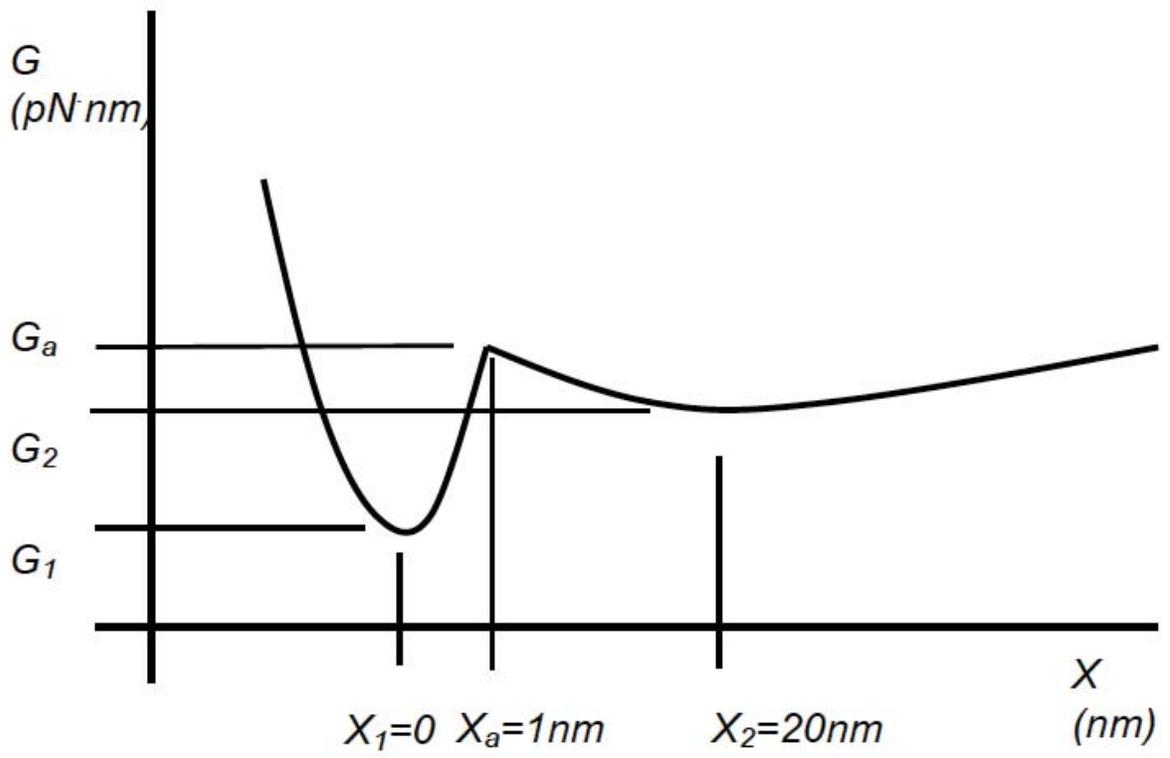


Fig. 1b

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