Instructions:

- Turn in your results in some kind of clear form (handwritten, typed, pdf).
- Feel free to work in groups.
- I have tried to be consistent in my language in my prompts if I want something specific.
  - *Sketch:* Hand draw a plot. Axes should be labeled conceptually, with key features indicated and explained.
  - *Generate a plot:* Plot using software. Label axes, use appropriate significant figures, etc.
  - *Calculate:* Actually calculate numbers using math. Report units and uncertainty, as appropriate.
  - *Describe/Argue:* Use any combination of writing, sketching, plotting, and calculation to argue for an interpretation.
  - Finally, I will sometimes specify the sort of explanation I want.
    - *Molecular*: Describe what the atoms and molecules are doing in space and time. Depending on the context of the question, this might also involve explaining the result in terms of atomic properties like hydrophobicity.
    - *Energetic*: Describe in energetic terms (entropy, free energy, statistics).
    - *Mathematical*: Answer in terms of how the functions behave. For example, if I asked "mathematically, why does $Kx/(1+Kx)$ saturate with increasing x" the answer would be: "because as $x \to \infty$, $Kx \gg 1$ and the function tends to 1."
- Some of the questions may require you to play with math and/or ideas we did not explicitly discuss in class. This is intentional. Many times in science you will be faced with a paper that uses an approach you are not familiar with. Learning how to gather enough information to critically evaluate their findings, as well as understand any mathematical models employed, is important.
- Some questions are listed as **GRAD STUDENT** questions. Undergrads are free to do those questions, but it is not required.

7 Diffusion and phase transitions

1. How long, on average, would it take a 60 kDa protein to diffuse from the center of a cell to a point on the edge, assuming that this distance is 12 μm, the protein is an sphere with a density of 1.41 $g \cdot cm^{-3}$, the temperature is 298 K, the viscosity of the cytoplasm is 0.5 $g \cdot cm^{-1} \cdot s^{-1}$, and the cell is relatively flat so diffusion can be assumed to be 2 dimensional. Express your answer in seconds.

2. You are studying the formation of a homodimer. The protein monomer is 16 kDa and can be viewed as a sphere with a radius of 1.5 nm. At 298 K, in water ($\eta = 0.01\ kg \cdot m^{-1} \cdot s^{-1}$), you measure the rate of dimer formation as $1 \times 10^6\ M^{-1} \cdot s^{-1}$. Does this reaction appear to be diffusion limited?

3. You are studying the polymerization of actin filaments from ATP-actin monomers at 298 K. Actin filaments grow from a single, fast-growing end. After a small amount of time under your experimental conditions, actin filaments (and thus elongating ends) are 0.1 nM and actin monomers are 1 μM. You find that the elongation rate for each filament at this time point is 0.9 $nM \cdot s^{-1}$. Using a separate experiment, you find that the dissociation rate constant for actin monomers under these conditions is from the filament end is 1.4 s^{-1}.

   (a) What is the association rate constant of actin monomers for the filament end under these conditions?

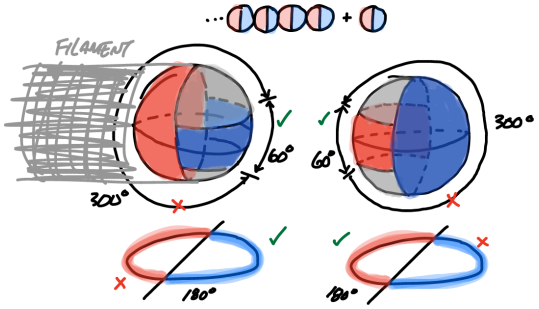
   (b) Is the association of monomers and actin filaments diffusion limited or not?

      i. Filaments (and thus elongating ends) are huge and therefore moving much, much slower than individual actin monomers.

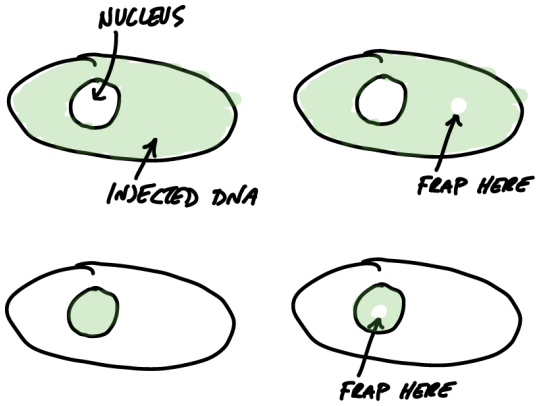
      ii. Each actin monomer can be viewed as a sphere of mass 42 kDa with a density of 1.41 $g \cdot cm^{-3}$.

      iii. The reaction occurs at 298 K in water, which has $\eta = 0.1\ g \cdot cm^{-1} \cdot s^{-1}$.

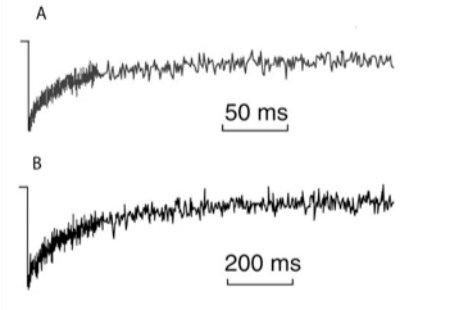
      iv. The expression for k_{coll} we discussed in class ($k_{coll} = 4\pi(D_A + D_B)(r_A + r_B) \times 6.022 \times 10^{20}$) assumes that every collision is productive. For actin, an interaction is productive only if the blue patch on the filament lines up with the red patch on the incoming monomer (denoted by the bands below). This is a patch 60° by 180° on both the incoming actin monomer and the elongating end.



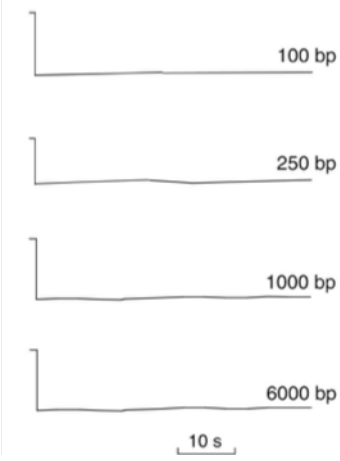
4. Shown below are traces from a Fluorescence Recovery After Photobleaching (FRAP) experiment. The researchers injected fluorescently labeled DNA of two different lengths (A and B) into the cytoplasm of a living cell. They then subjected a small area within the cytoplasm to a laser burst that bleached (e.g. destroyed) any fluorophores. They then followed the recovery of fluorescence within the monitored area, thus measuring the diffusion of new fluorophores into the area. (See top row of the schematic). Note: For all of the FRAP plots below, only the fluorescence trace is shown, and not the axes. Assume that for each plot the y-axis is the fluorescence and the x-axis is time.



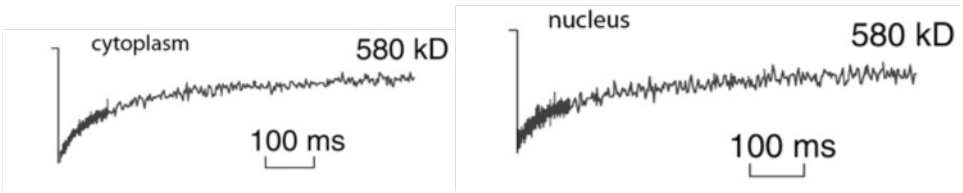
(a) Based on the data below, which of the two experiments, A or B, uses the longer DNA? Explain you reasoning. (Note that the scale of the x-axis (time) is different in each plot – also note that the y-axis shows fluorescence).



(b) The experiment in a is repeated, except the DNA is injected into the nucleus and a FRAP experiment performed in the nucleus (see bottom row of schematic above). What conclusions can be drawn from this experiment?



(c) Fluorescent dextran with a MW of 580 kDa is injected into the cytoplasm and the nucleus and the recovery times measured. Based on the results shown below, provide a molecular explanation for the results shown above.



5. Explain how "bulk" experiments (i.e. those averaging over millions of molecules in a cuvette) can be deceptive. How can single-molecule experiments overcome this difficulty?

6. You would like to monitor a protein's conformational change using FRET by attaching fluorescein and tetramethylrhodamine to cysteines on the protein's surface. The protein is thought to undergo motions such that the distance between the two cysteines change from 42 to 57 Å. Calculate the change in FRET efficiency that you would expect to observe by monitoring this change directly.