DNA spring 2: Protein folding

Protein (Un)Folding in the Optical Tweezer

Motivation. Proteins are composed of amino acids, of which there are 20 forms (http://www.elmhurst.edu/~chm/vchembook/561aminostructure.html). As you probably know, the three-dimensional structure that a protein adopts is crucial to its functioning in the cell, and even slight structural changes to a protein's shape, perhaps caused by a single amino acid mutation, can have devastating effects on the organism's ability to survive. How, then, does the linear sequence of amino acids comprising a protein (its "primary" structure) determine its three-dimensional shape (its "tertiary" structure)? Put another way, how do we know what three-dimensional form a protein will take if all we are given is its linear sequence of amino acids? As important as this question is, professional scientists don't know the answer! The protein folding problem – the problem of going from amino acid sequence to folded three-dimensional protein – is one of the most pressing mysteries in molecular biology. In this problem, we'll explore one aspect of the folding problem via the optical tweezer apparatus we've seen before.

Question 1: Can you think of reasons for why the protein folding problem is so hard to solve? What are some of the factors that make it hard to predict three-dimensional structure when all you know is the linear sequence of amino acids?

Experimental Set-up. Recall the optical tweezer set-up that we employed in studying the spring-like properties of DNA in the previous problem. Now imagine that we have two such DNA springs, each acting as a "linker" or "handle," and that a protein such as RNase H is placed between the two, as shown in Figure 1.

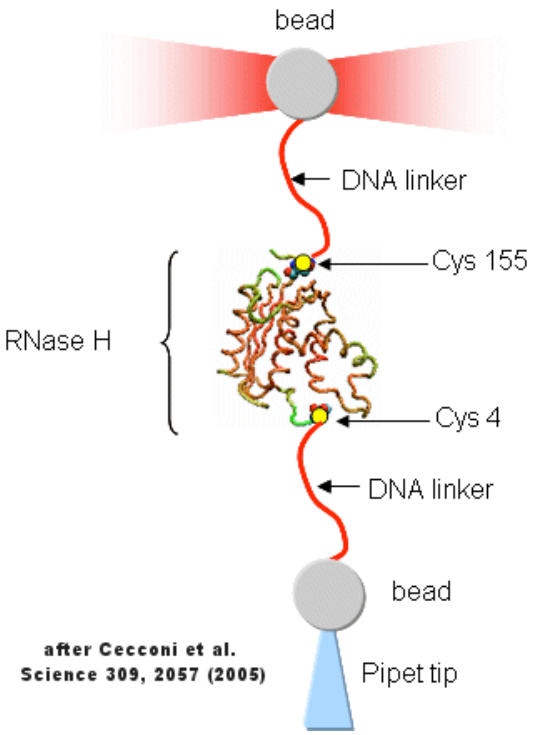

Figure 1. Optical tweezer set-up for protein folding experiments.

As in the previous problem, the polystyrene beads to which the DNA linkers are attached are held in place, one by a laser beam and the other (in this case) by the suction of a pipette tip. The protein RNase H is chemically attached to the DNA linkers via bonds involving cysteine amino acids at the two termini of the protein. Under normal physiological conditions, and under the conditions of this experiment, RNase H spends most of its time in its folded form, the form illustrated in Figure 1. By moving the focus of the laser beam slightly, we can begin to exert small forces on the DNA, and in turn on the protein to which it is attached, eventually supplying enough force to "unfold" the protein.

Estimating the work required to unfold RNase H. The data obtained when force is applied to the system shown in Figure 1 is given in Figure 2 below.

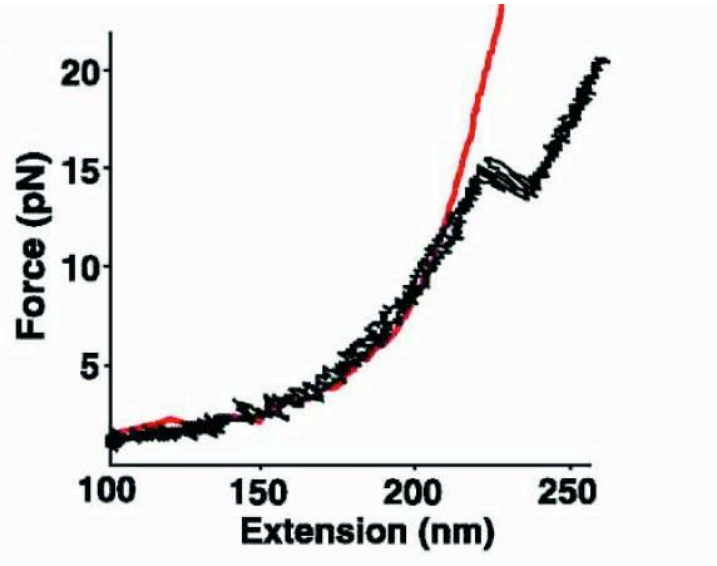

Figure 2. Data obtained when RNase H is explored via the optical tweezer set-up described above.

The "extension" in Figure 2 refers to the distance between the two beads in the experimental set-up, and the red line represents a theoretical prediction of what would be seen if no protein were present. Focus on the black curve, which shows what happens when RNase H is attached to the DNA linkers as in Figure 1.

Question 2: Focusing on the black curve in Figure 2, describe what might be happening (a) when the applied force is between 0 and 15 pN, (b) when the applied force is about 15 pN, and (c) when the applied force is greater than 15 pN.

The really interesting behavior occurs when the tweezer has applied a force of about 15 pN. Here, we see a sudden increase in the distance between the two beads, and we interpret that event to be the unfolding of the RNase H protein. Protein unfolding events often occur in multiple steps and can be far more complicated than the single-step unfolding process shown in Figure 2. Sometimes multiple "rips" are seen as different domains of the protein unfold sequentially when the force increases, and sometimes the protein momentarily re-folds. All of this information can be useful in elucidating the specific protein folding process, but for the case at hand let's look a little more closely at the single-step rip that occurs at around 15 pN.

Question 3: About how much work did the tweezer device do on the protein in order to unfold it? (Hint: remember that work is defined as a force applied across a distance....)

Question 4: Is the energy of the unfolded RNase H protein smaller or larger than the energy of the folded RNase H protein? By how much?

Mutations. Now imagine that we mutate the amino acid sequence of RNase H, so that one alanine somewhere in its sequence is replaced by a lysine. This change, while seemingly small, serves to *destabilize* the folded protein structure, i.e., it makes its folded form *higher* in energy than the un-mutated species. Let's think for a moment about how that destabilized, mutated protein will behave under the conditions imposed by the optical tweezer.

Question 5: Do you expect the force at which the unfolding "rip" occurs to be smaller or larger in the mutated case than it is in the wild-type (un-mutated) RNase H?

Question 6: Do you expect the work required to unfold the mutated RNase H to be less or more than that required to unfold the wild-type (un-mutated) RNase H?

Question 7: Do you think it is possible to mutate the RNase H in a way that stabilizes the protein relative to its wild-type form, i.e., in a way which lowers its energy? If so, what effects would this have on the plot of force-vs-extension in the tweezer experiment?

Clearly, experiments of the sort described in this problem do not "solve" the protein folding problem. However, by performing such experiments under a whole host of conditions and with a whole host of differently mutated protein species, it is possible to learn a tremendous amount about the pathways that particular proteins take in going back and forth between linear amino acid sequence and folded tertiary structure. A number of interesting papers describing this work can be found here, for anyone interested: http://zebra.berkeley.edu/publications.php