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Unfolding Proteins: Fast versus Slow

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Abstract:

As the structure of the protein is essential to its function, a better understanding of protein folding is fundamental to understanding protein function. [1] This comprehension of the function and folding of proteins will enable medicine to synthesize proteins in a way currently not possible. One of the major hurdles is that the protein has the capability of folding in different ways and speeds. The goal of our experiment is the examination of the hYAP protein, and to see the different speeds of folding that it undergoes. Our specific goal was to find instances of fast folding of the protein - folding occurring within less than a millisecond. The methodology behind this experimentation is presented, along with results for fast folding of the protein, and slow folding of the protein.

1. INTRODUCTION

Current engineering of proteins is difficult, because of the direct relationship of function and folding. Furthermore, a greater understanding of protein folding can provide a possible method of combating prions - proteins that have folded in a manner different than their native structure. At times a function detrimental in comparison to the standard function will arise as a result of this abnormal folding. This abnormal folding can result in other proteins folding abnormally as well, leading to diseases such as bovine spongiform encephalopathy in certain animals, Creutzfeldt-Jakob disease, Alzheimer's disease or Gerstmann-Straussler syndrome in humans for example. [2] [3] By understanding how proteins fold, the possibilities of chemical synthesis of proteins for individuals with unique dietary needs becomes possible. [4] Consequently, a greater understanding of diseases thought to be caused by prions will lead towards more efficient treatment of these diseases. In this paper, we use the single molecule trapping technique to study protein folding. This technique is useful in this matter as it allows us to build a stochastic model of the protein, one molecule at a time. This is in contrast to other methods that result in building a model of the protein that is an average of the behavior. In this experiment, we utilize an optical trapping method in which we capture two "beads" that when placed in the optical traps form a DNA tether with each other.

2. EXPERIMENTAL

2.1 Overview of Trapping Method

The sample chamber and experimental set up is contained in a room of its own, with baffling on the walls to prevent noise pollution, and the lights off to prevent light pollution from affecting the experiment. With the beads captured in the optical trap, the motion of the beads can be controlled to a certain degree. The trap itself is conical in shape and creates a restoring force upon the bead trapped within it. If the trap is moved to the left, the bead will be pulled to the left as well as it re-centers itself in the trap. This is what allows us to directly apply a force to an individual protein. A calibration is found for every bead pair that tells us the trap stiffness and the conversion constant for determining the position of the beads. Once this is done, the beads begin fishing for a tether. This entails one of the optical traps remaining stationary, while the other oscillates closer and further from the stationary optical trap. The streptavidin beads have a DNA strand on them, followed by the protein, followed by another strand of DNA. The strand of DNA not directly on the streptavidin bead forms a digoxygenin with anti-digoxygenin bond with the anti-digoxygenin bead. The successful formation of this bond is what is called a tether. Once a peak force is measured during the movement further from the stationary trap, this is indicative that the DNA from the streptavidin bead has formed a tether with the anti-digoxygenin bead. At this point, the bead pair is able to be used to exert force on the protein such that it will unfold, as seen in Figure 1.

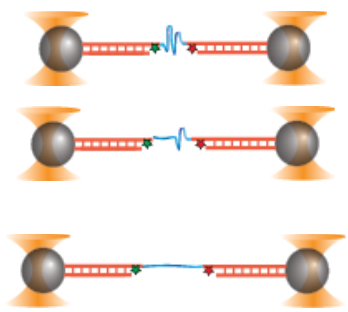

Figure 1: Starting from the top, the beads are tethered together by the DNA. Left to right, it is an anti-digoxygenin bead, DNA, the protein under examination, DNA, and finally the streptavidin bead. As the beads are moved further apart, the protein experiences a force and eventually unfolds.

The beads are caught in the optical trap by the platform the sample chamber is mounted on being moved. This movement is controlled outside of the room the sample chamber is contained in.

2.2 Optical Trap Set-up

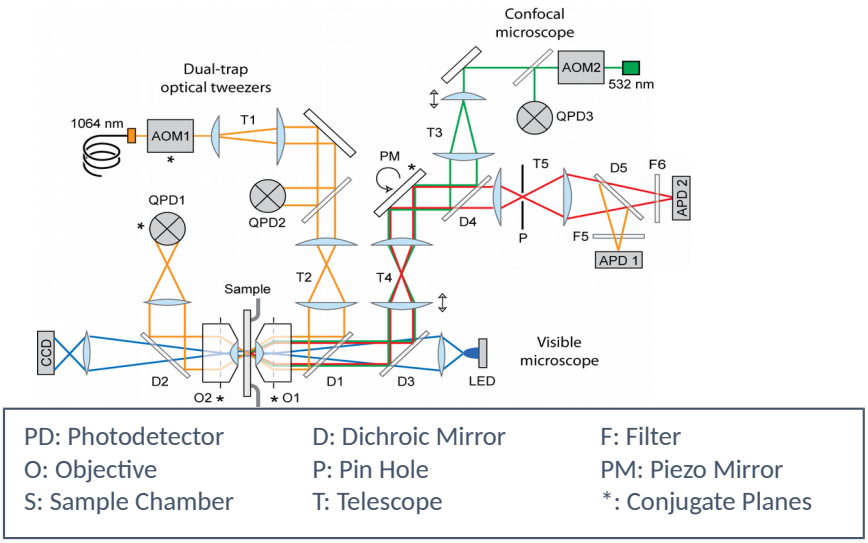

Figure 2: The experimental setup. [5]

The laser used in this experiment is in the infrared spectrum at 1064 nm and is a 5W laser. One of the objectives is held fixed, while the other is allowed to be moved perpendicularly towards and away from the sample chamber (see figure 2). This movement changes the diameter of the laser beam, and provides better collimation of the laser. The accuracy of the collimation is checked with the aid of an infrared fluorescent card. The camera focused on the experiment has two lenses in front of it, an ultraviolet lens and an infrared lens. When the infrared lens is filtering light, the laser can be seen on a monitor. This allows a visual check of the laser to be conducted. The experimentalist looks for a shape that is as near a uniform circle as can be obtained visually. If the circle appears to be more elliptical, this is indicative that the sample chamber itself is not presenting a vertical surface for the laser to pass through, and as such the laser is coming out angled rather than straight. This is adjusted by physically rotating the chamber to achieve a uniform circle rather than an ellipse. This is a measure taken to ensure that before the sample is being experimented with, the optical trap will successfully capture the beads. If the chamber is not presenting a vertical surface for the laser to pass through, this can lead to issues during the experiment. As the sample chamber is moved, the angling can result in the captured bead being removed from the optical trap. This occurs because if the sample chamber is angled, as it is moved the odds of the optical trap causing a bead to collide with the surface of the sample chamber increase.

2.3 Preparation of Sample Chamber

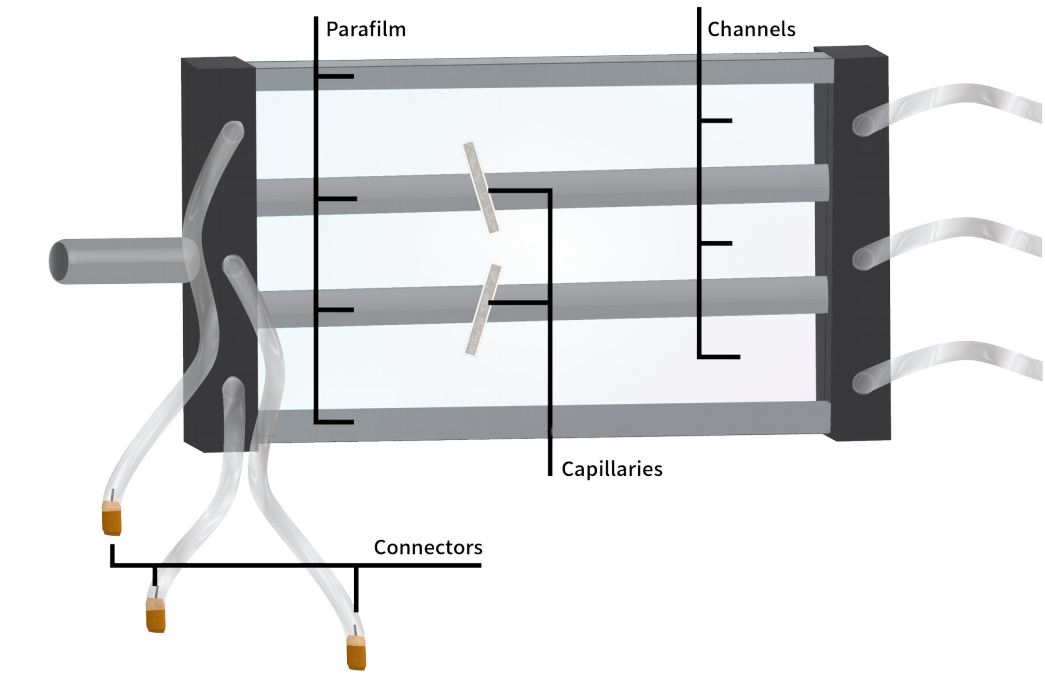

Figure 3: The sample chamber. Syringes are connected to the connectors, which have a needle on them attaching them to a tube. Through these tubes, the buffer, streptavidin and anti-digoxygenin beads flow through the channels. The capillaries allow the beads in the top and bottom channels to flow into the middle channel where the optical trap is then used to capture the beads and perform the experiment. The fluids then leave the chamber through the tubes on the left. Image is not to scale. Image credit: Fallyn Stieglitz

Before the experiment can be conducted, the sample chamber must be prepared for the optical trap. The chamber used in this experiment is constructed out of cover glass and parafilm (see figure 3). The parafilm is used to create three separate channels on the cover glass: the bottom channel is where the streptavidin beads with the protein construct flow through, the middle channel is where the buffer solution flows through, and the top channel is where the anti-digoxygenin beads flow through. On the middle channel there are two capillaries connecting the channel to the top and bottom channels. These capillaries are what allow the beads to flow through to the middle channel and be captured by the optical trap. The chamber is initially prepared for a trapping session by first pushing milli-Q water (filtered 0.2 microns) through the channels using syringes. This is done to ensure that no excess of experimental materials is wasted. The purpose of pushing the milli-Q through the channels is to ensure that there are no air bubbles covering, or in, the capillaries before the experimental materials are added. The beads are

on the order of a micron in size and would not be able to flow into or through the capillary if an air bubble is blocking the capillary in any way. The chamber is then placed between the objectives of the experimental setup and visually verified for the clear openings on the capillaries by the experimentalist. These syringes containing the milli-Q are left connected to the sample chamber until the syringes containing the sample to be used are connected to the chamber. This is another step taken to ensure that no air gets into the chamber and causes bubbles that might interfere with a capillary.

2.4 Sample Preparation

The preparation of the sample involves making the buffer solution. The buffer solution is a combination of Triss-HCl, Glucose, NaCl, and water in a centrifuge tube. Three separate centrifuge tubes are then used to portion out the buffer solution. After the buffer has been portioned, the beads are then added to the tubes. Before adding the anti-digoxygenin beads to a tube, the beads are first vortexed. This is to ensure that upon pipetting the beads, the container has a uniform concentration of beads as over time the beads will settle at the bottom of the container. The streptavidin beads are then added to a separate tube, after being mixed by inverting or flicking the container. As the streptavidin beads have DNA attached to them, they cannot be vortexed as this would shred the DNA. Finally, pyranose oxidase (poxy) is added to all three tubes. The purpose of the addition of the poxy is to prevent oxygen from interacting with the solution as best as possible. Oxygen in solution can get converted into “radical oxygen”. Radical oxygen is highly reactive and will react with the molecules, possibly breaking the DNA tethers or damaging the protein under study. The poxy is an oxygen scavenger, and will keep the radical oxygen from reacting within the solution. The three separate mixtures are then transferred into separate airtight syringes. The airtight syringes and the poxy are implementations put into place to mitigate waste of material and ensure that oxygen is not a reason for lack of success during the experiment. However, at some point the poxy will become saturated with oxygen, and unable to scavenge any more oxygen from the solution. This will typically end the experiment, as the oxygen will now be able to interact with the DNA and prevent tethers from being formed successfully. To begin the experiment, the airtight syringes replace the syringes currently attached to the sample chamber. During this replacement, it is imperative that air not be able to enter the tubes of the sample chamber. To prevent this, the experimentalist adds milli-Q to the connection location, to ensure that once the airtight syringe is connected, there is no air between the solution contained within the syringe and the milli-Q currently in the tubes of the sample chamber. The airtight syringes are then placed in specific positions that correspond to controls used by the setup to control the flow of the beads (anti-dixoxygenin or streptavidin) and the buffer. There are

motorized syringe pumps at these positions that push the solution out of the syringes at speeds ranging in the hundreds of nanoliters per second.

2.5 Application of Force to Protein Construct

Once this is done, the goal is to trap an anti-digoxygenin bead in one optical trap, a streptavidin bead in the other optical trap, and then form a tether between the two beads using the DNA. Once the beads are both successfully caught in the optical trap, a calibration of the trap for this bead pair is done. This will determine the trap stiffness constant and a conversion constant that gives the bead position from the optical method. [5] The calibration also gives the experimentalist a control over data collection. For bead pairs of a sample protein, the calibration for a bead pair should not be vastly different in comparison to other bead pairs of the same sample protein. If a bead pair presents a calibration that is outside of the standard calibration for a sample protein, it is immediately apparent to the experimentalist that this bead pair cannot be used for data collection. This can happen for a variety of reasons: the beads vary in size, there could be more than one bead in the optical trap, there could be multiple tethers formed between the beads, the sample protein could possibly be bad. If the calibration is within the standard calibration for the sample, the beads then form the tether. Once the tether has been formed, a force can be applied to the protein. This is done quickly at first, to test whether or not the tether is stable. One of the optical traps is held stationary, and the other trap moves away from it (see figure 1) which cause the force to be applied to the protein. At a certain force the protein will unfold, and this force is measurable. After this fast scan has been performed, a slow scan is performed. The slow scan involves the trap moving in discrete jumps as it moves away from the stationary trap. The slow scan is performed in order to see the fast folding occur. The fast folding occurs rapidly, in times less than a millisecond; scanning too quickly risks missing these fast folding occurrences. By scanning slowly, we keep the protein at a certain force for a longer time. Due to the statistical mechanical nature of folding, at certain forces the unfolded state will be just as likely to occur as the folded state. This slow scan allows us to see this happen, as shown in the following results.

3. RESULTS AND DISCUSSION

3.1 Slow Folding Proteins

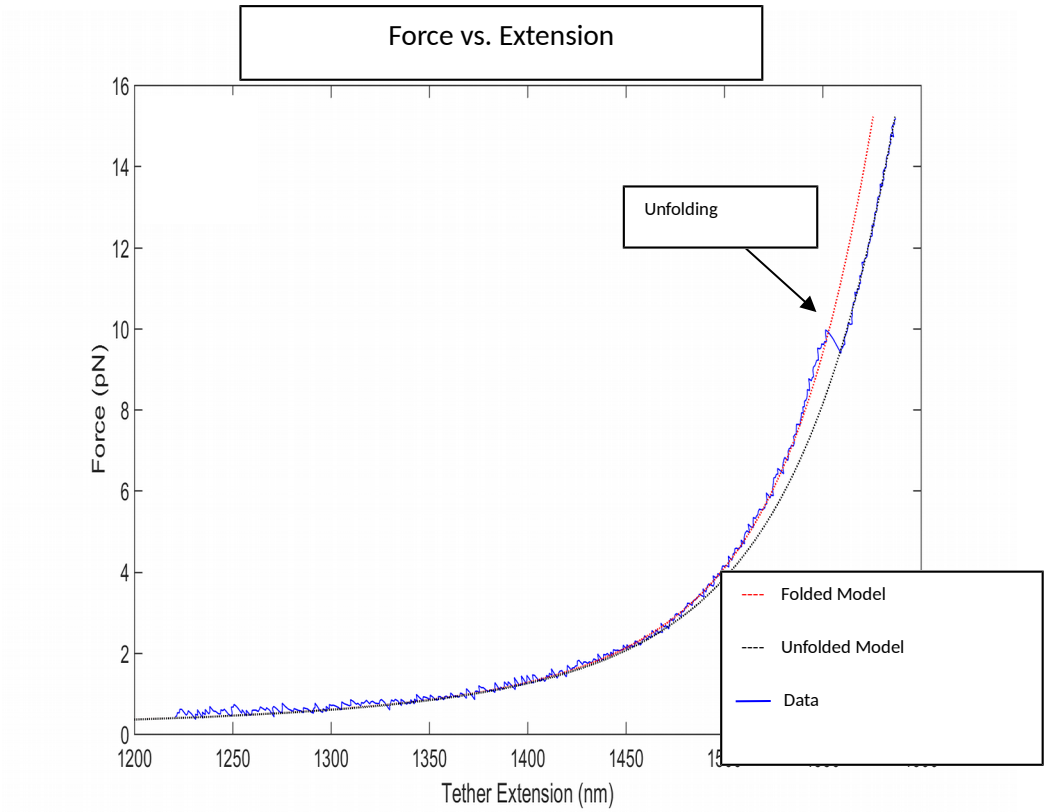

Figure 4: An example of slow folding. In comparison to fast folding, slow folding takes place in half to full seconds.

Shown in figure 4 is an example of slow folding. There are two different models to describe the dependence of the tether extension on the force applied: a model for the protein folded, and a model for the protein unfolded. The molecules are modelled using the statistical mechanics of polymers. One of these models includes the unfolded protein polymer, the other does not. At low forces, the models are indistinguishable from one another, but as the force increases, the models separate. This separation between models is used to identify when and where the protein unfolds. In the example presented, the protein extended slightly less than 1550 nm, at which point the protein then unfolded (indicated by the arrow), at a force of approximately 10 pN. The data is interpreted as a representation of slow folding because as can be seen at the point of unfolding, there is a single distinct jump; the blue line moves from the folded model to

the unfolded model and stays there.

3.2 Fast Folding Proteins

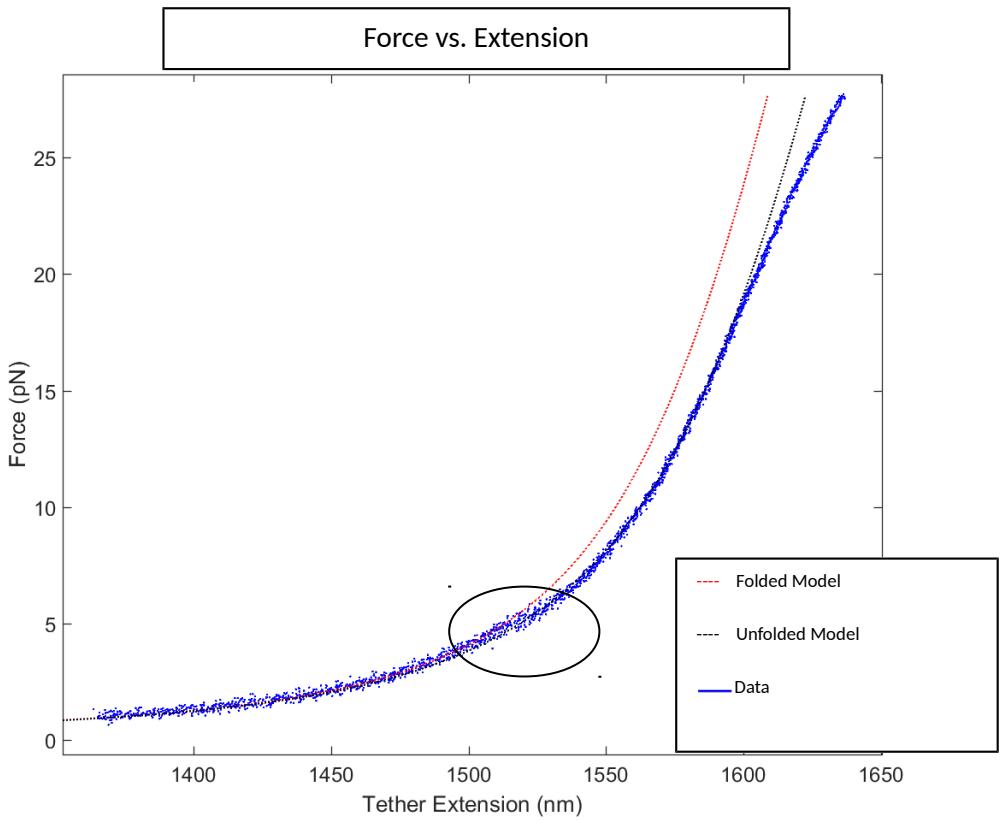

Figure 5: An example of fast folding. The expected time for fast folding to occur is in the millisecond time range. As seen in the circled area, the data is not just following one model. The data points, if followed sequentially, would alternate between the folded model and the unfolded model as the protein switches between the two states rapidly.

Shown in figure 5 is an example of fast folding. The difference here being that rather than there being a single distinct jump, there is more of a smooth transition between the folded and unfolded models that occurs over an extension range. This is due to how the data is taken; as the data is being taken, it is being averaged to avoid files of an excessive data size. The above is said to be fast folding because it is not folding just once, but jumping between models rapidly. The result is that as the protein is jumping between the models, at the lower force it spends a greater amount of time folded than it does unfolded. As the force increases, the time spent folded lessens and it is more likely

to find the protein in an unfolded state, until finally the protein is unfolded and following the unfolded model.

3.3 Conclusion

Based upon the data taken during these experiments it was determined that fast folding was possible for the protein construct. However, these instances of this different folding were few and far between. Over the course of 8 weeks of experimentation, we found 4 instances that were believed to be indicative of fast folding. However, we have shown that the methodology presented is useful for examining different folding procedures for a given protein. As a result of this, future studies will be made involving a protein construct Protein G, as it has also shown possibilities of there being different types of folding occurring. Whereas before it seemed to have two distinct states, the folded and unfolded state, through the use of this method there is evidence to suggest that there might be a state in between the folded and unfolded states. This in between state of Protein G is the focus of future studies using this method.

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