

Probing the Elasticity of Short Proteins with Optical Tweezers

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Abstract: Probing relatively short molecules, such as the protein elastin (~200 nm), with optical tweezers requires manipulating trapped polystyrene beads at separations small compared to their micron dimensions. We discuss experimental complications arising from this proximity, such as hydrodynamic and optical interactions, and our efforts to minimize them.

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1. Introduction

Stretching and relaxing single proteins provides quantitative information on their elasticity and other mechanical properties [1]. While this has most commonly been done with the atomic force microscope (AFM), optical tweezers provide lower loading rates and access to different dynamical regimes. Optical tweezers stretching requires the ends of the protein to be chemically tethered to polystyrene beads that are used both to manipulate the molecule and measure its response. Our goal is to use this method to study human structural proteins, such as collagen and elastin, whose mechanical properties are directly related to their physiological function. These proteins have contour lengths of approximately 300 nm and 170 nm, respectively, making them short molecules compared to others whose elasticity has been studied quantitatively with optical tweezers [2]. This introduces some specific difficulties which must be addressed. In protein unfolding experiments, where the features of interest are conformational changes, many of these issues can be avoided by using DNA handles to effectively extend the molecule [3]. However, artifacts introduced by such handles may be significant in quantitative elasticity measurements. We wish to compare elasticity measurements of proteins with and without handles, and so must be able to pull on short molecules. Since the molecular extensions are low they must be measured with high precision, requiring both precise position detection of the polystyrene beads and minimization of drift in the system. In addition there are physical interactions between the beads whose effects are negligible at micron length separations but which must be considered when the beads are only tens of nanometers apart. For sufficiently short molecules, significant stretching takes place within this range so bead interactions must be addressed. These interactions are described below along with our ongoing efforts to minimize their effects.

2. Preliminary Results with Elastin

In our instrument, described in more detail in [4], one bead is held in an optical trap and a second on the tip of a micropipette. Tethered molecules can then be stretched between these beads, as shown schematically in Figure 1. The optical trap is produced by a single focused beam from an 835 nm, 200 mW diode laser. The micropipette is mounted in the sample chamber and a bead is held to it by suction. The sample chamber is mounted on a high-precision two-axis piezo-electric stage (Mad City Labs, Nano H-50) allowing it to be moved relative to the trap with nanometer-scale accuracy in the plane perpendicular to the optical axis. Transmitted laser light is directed onto a duo-lateral photodiode (UDT Sensors, DL-10) so the deflection of the laser by the trapped particle can be measured. Both beads are imaged by video microscopy. The chamber is designed for buffer flow permitting delivery of beads with different chemical labels to the trap and pipette, facilitating tethering of a molecule between the two beads.

We have had some preliminary success using our system to determine the mechanical properties of elastin, the protein responsible for the elastic response of many human tissues. Molecular level mechanical properties of elastin are highly relevant to its physiological function [5]. Previous single-molecule experiments have used AFM to characterize chemically synthesized elastin-like polypeptides [6], but not proteins corresponding to full-length native elastin. Figure 2 shows force-extension curves recorded in our lab for a single elastin molecule.

These plots highlight difficulties we have encountered in image analysis. Since the molecule may be attached to the pipette-mounted bead at a point out of the focal plane, at large extensions the trapped bead tends to be slightly displaced along the optical axis, resulting in an unfocussed image. This effect is significant in short molecules, where the distance from the tether point to the focal plane may be a considerable fraction of the molecule's length.

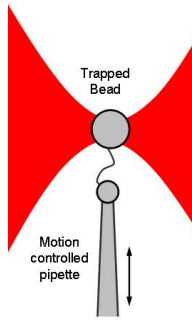


Figure 1: Schematic of a stretching experiment

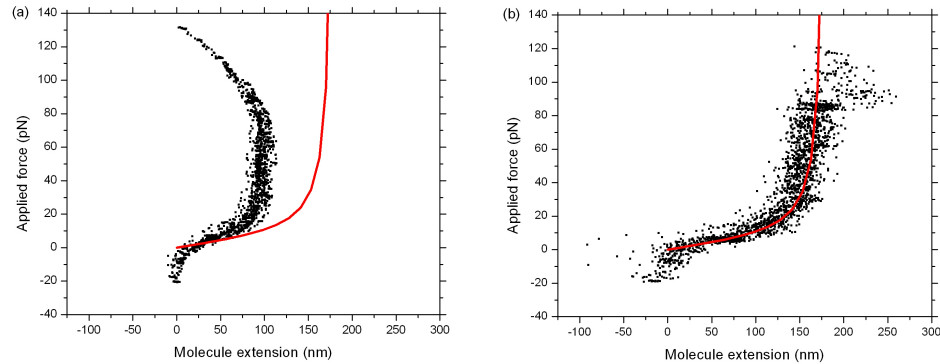


Figure 2: Force-extension curves for multiple stretching and relaxation trajectories of a single elastin molecule (points). Plots are produced from analysis of video images, using a correlation algorithm (a), or a circle-fitting algorithm (b). Also plotted is the theoretically predicted behaviour of a freely jointed chain with a Kuhn length of 0.4 nm (red line), as suggested by the results of [6].

Accurate tracking of an out-of-focus bead is challenging. Figure 2(a) was produced using an image analysis algorithm based on correlation with a template image of a bead, as used in [7], while Figure 2(b) was produced from the same images using an algorithm which fits a circle to the edge of the bead image (Labview 8.5, IMAQ Find Circular Edge). For a focused image the correlation-based algorithm is preferred for its higher resolution. However, it is more drastically affected by changes in focus; at higher extensions Figure 2(a) shows unphysical behaviour. Figure 2(b) shows behaviour consistent with previous results, but the spatial resolution is insufficient for quantitative analysis. We are continuing work on improving the tracking of out-of-focus beads.

3. Hydrodynamic Interactions

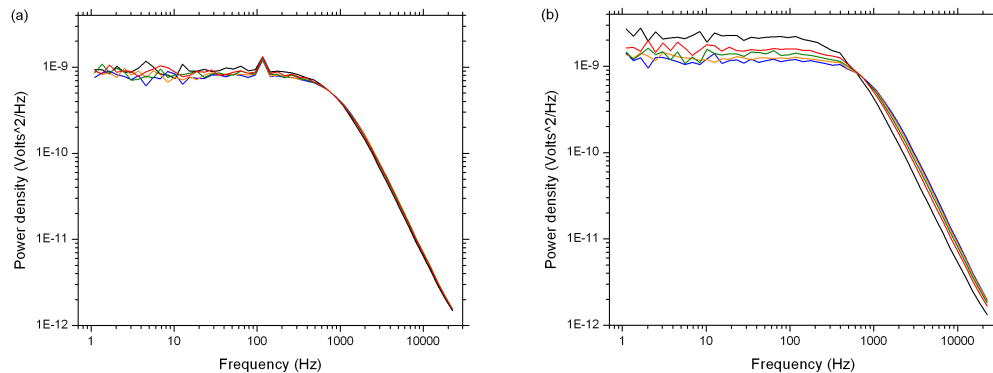


Figure 3: Power spectrum of a 2.1 μm polystyrene bead optically trapped in distilled water, as measured from the deflection of the laser on the PSD. Motion perpendicular to the trap-pipette axis is shown in (a), and parallel motion in (b). Data sets correspond to a bead separation of 100 nm (black), 500 nm (red), 1 μm (green), 2 μm (orange), and 5 μm (blue).

At small bead separations it is necessary to consider the effects of hydrodynamic interactions, which could exert additional forces on the trapped bead. It has been shown that the close proximity of a surface to a bead held in an optical trap can dramatically alter its drag coefficient, and also shift the trapped bead's equilibrium position [8]. These effects are complex, and depend on solution and surface conditions. Similar effects on a trapped particle are expected to result from the proximity of a second bead. To evaluate the impact of dynamic effects, we measured power spectra of the Brownian motion of a trapped bead with the pipette-mounted bead held at a number of separations, as shown in Figure 3. The motion perpendicular to the direction of the pipette remained practically unchanged by the position of the pipette. However, as the pipette-mounted bead was brought to within 2 μm of the trapped bead the power spectrum of the parallel motion showed a shift over all frequencies, with increased power at frequencies below the corner frequency of the trap and decreased power at higher frequencies. The corner frequency also appeared to decrease. These changes became more pronounced with reduced bead separation. In addition we used video microscopy to measure the equilibrium position of the trapped bead, finding that at the position of closest approach (100 nm separation) it shifted by approximately 10 nm. This shift was toward the pipette, implying an attractive force between the beads. These effects will have to be taken into account for quantitative results to be extracted from our stretch-relaxation measurements.

4. Optical Effects

In addition to hydrodynamic effects, optical interactions between the pipette-mounted bead and optical trap are possible at small separations. The high numerical aperture required to produce a trap means that the beam diverges rapidly away from the focal plane, as shown in Figure 1. Undesired in-plane and out-of-plane interactions of a pipette bead with the focused laser beam are possible, if bead sizes are not chosen appropriately. These would result in trap distortion and likely alteration of trap stiffness, complicating quantitative determination of forces. We wish to use small beads to localize the tethering point on the pulling axis; however, a sufficiently large bead must be used in the optical trap to isolate the trap from interactions with the pipette bead at short molecular extensions. To determine the range of in-plane motion over which different sizes of pipette beads interact with our trapping laser, we used the piezo stage to move the pipette bead through the optical trap, while the laser deflection was monitored with the photodiode (Figure 4). This was done for 1.27 μm and 2.1 μm diameter beads. It can be seen that the 1.27 μm bead starts to interact with the trapping laser at in-plane separations of $\sim 1.1 \mu\text{m}$. Therefore, a 2.1 μm bead in the trap and a 1.27 μm bead on the pipette easily allow the two bead surfaces to be brought into contact without the pipette bead interacting with the optical trap. This configuration was used for taking the data in Figure 2.

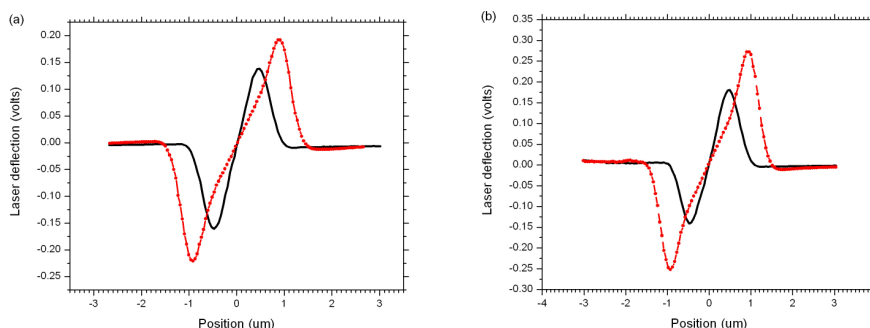


Figure 4: Plots of laser deflection measured by PSD as a function of pipette-mounted bead position relative to optical trap centre. The horizontal deflection of the laser as the pipette moves through trap horizontally is shown in (a), and the vertical deflection of the laser as the bead moves through trap vertically is shown in (b). Solid curves correspond to a 1.27 μm diameter bead and dotted curves to a 2.1 μm diameter bead.

5. Conclusion

The application of optical tweezers to elasticity measurements on molecules with lengths of the order of a hundred nanometers requires the consideration of several effects which are less significant for the longer molecules usually studied with this technique. We have identified some of these effects in our apparatus and are engaged in ongoing attempts to minimize them. This should help us to achieve quantitative measurements on elastin, with which we have had some preliminary success.

Acknowledgments

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