Towards Crystallization using Optical Tweezers

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ABSTRACT

Recently we have shown that protein crystals could be grown while they were three-dimensionally trapped by optical tweezers. This permitted studies of modifications of single crystals while gradually changing the conditions in the growing solution. Furthermore it allowed the crystals to grow far away from container walls favoring high quality crystal growth. Many protein crystals themselves consist of fairly large molecules, with sizes up to tens of nanometers. Here we present experiments studying the effect of optical trapping potentials on large molecules, with the aim to explore ways to further enhance crystal growth. For this purpose we extended our tweezers setup with a specially developed detection system allowing us to monitor changes in the molecule concentration of a solution. Using polyethylene oxide (PEO) molecule solutions we were able to demonstrate that the trapping potential of an optical trap is sufficient to collect large single molecules. Our results show that the optical trap induces an increase in the molecule concentration in the focal region. As expected only molecules above a certain molecular weight could be manipulated, and the concentration in the focal region depended on the power of the trapping laser. The ability to locally increase the concentration of molecules may be useful in assisting nucleation of crystals.

Keywords: laser tweezers, nucleation, crystallization, lysozyme crystals, PEO molecules

1. INTRODUCTION

1.1. OPTICAL TWEEZERS

Optical tweezers can trap and move dielectric materials non-invasively at length scales ranging from tens of nanometers to tens of micrometers, and so have provided unprecedented access to physical, chemical and biological processes on the (sub)micrometer-scale[1-4]. The optical forces acting to trap particles result from the transfer of linear momentum from the trapping beam to the trapped object. The single beam gradient optical trap, often referred to as optical tweezers, consists of a tightly focused laser beam, in which the same objective is usually used for focusing the laser beam as well as for observing the trapped object[5]. For particles with an index of refraction higher than that of the surrounding liquid the gradient of the electric field intensity creates a net force towards the focal point of the beam in three dimensions. To date, many experiments with biological specimens, such as living cells and viruses, have been performed. Examples are the study of the interaction forces between different motor molecules[6,7], the use of optical tweezers to study the dynamic of fusion pores[8] and the determination of the elastic properties of single DNA strands[9]. For the handling of biological specimens a wavelength of the trapping beam in the near-infrared (NIR) is a necessary requirement to minimize heating and avoid damaging the trapped sample.

Recently we have shown that protein crystals can also be held in an optical trap without measurable degradation of the crystal[10]. This permits studies of modifications of single crystals while gradually changing the conditions in the growth solution. Studies performed included increasing the protein concentration and thermal cycling[11]. The latter method is based on temperature changes induced by the laser trap itself (by changing the trapping power) and/or by external thermo-electric elements. The independent control of the temperatures of the object chamber and of the microscope

Photonics: Design, Technology, and Packaging II, edited by Derek Abbott, Yuri S. Kivshar, Halina H. Rubinsztein-Dunlop, Shanhui Fan, Proc. of SPIE Vol. 6038, 60380B, (2006) · 0277-786X/06/$15 · doi: 10.1117/12.651755

objective allows the production of a fairly large temperature gradient within the growing solution. In this way crystals with a non-perfect morphology could be trapped, shrunk to diameters of the order of a micrometer by increasing the temperature, and re-grown by a subsequent cooling of the environment. The re-grown crystals in almost all cases have a well-defined shape[11]. This can be attributed to the fact that these crystals were grown in free space, away from interfering container walls. The temperature difference between the object chamber and the objective can also be utilized to favor the growth of the trapped crystal at the expense of the surrounding crystals. Also in this case the growth of a well-defined crystal shape is encouraged by the free-space trapped crystal.
The ability to control the temperature of the solution together with having means to locally increase the temperature in the focal spot – by increasing the laser power - can also be used to locally solidify materials displaying a lower critical solution temperature (LCST), as we demonstrate in section 3.3. However, this effect is solely thermal, and is not induced by the trapping potential of the laser beam. To be able to identify if large molecules show a response to the trapping potential created by a laser trap we used dilute solutions of PEO molecules with different molecular weight. The effect of the trapping potential on the PEO molecules was monitored by the scattering of a second low power laser at the higher refractive index region which is associated with a higher molecule concentration in the focal region. To further increase the sensitivity of the system the detection laser beam was used in a kind of dark-field-geometry, allowing only rays that are scattered at an angle larger than the incident angle to reach the detector.

1.2. PROTEIN CRYSTALS

In general, crystals are formed when a chemical compound solidifies in such a way that its atoms or molecules are arranged in a symmetrical, three dimensional pattern which is composed of regular, repeating geometrical units. The crystallization of proteins is an important technique in biochemistry. Protein crystals can be analyzed using X-ray diffraction, which takes advantage of a crystal's ability to scatter X-rays into a regular pattern. Analysis of X-ray diffraction by a protein crystal helps determine the three dimensional structure of the folded protein. In addition to giving insights about the structure and function of proteins, structural biology then allows the development of drugs which can interact with specific regions of the protein.
Lysozyme crystals are a widely used model to study nucleation and growth of protein crystals to establish the fundamental understanding that can be applied to the crystallization of other proteins. In its most common (tetragonal) form, lysozyme forms a positive uniaxial birefringent crystal, with a well-characterized morphology. Images of lysozyme crystals we have grown in our lab can be seen in Figure 1.

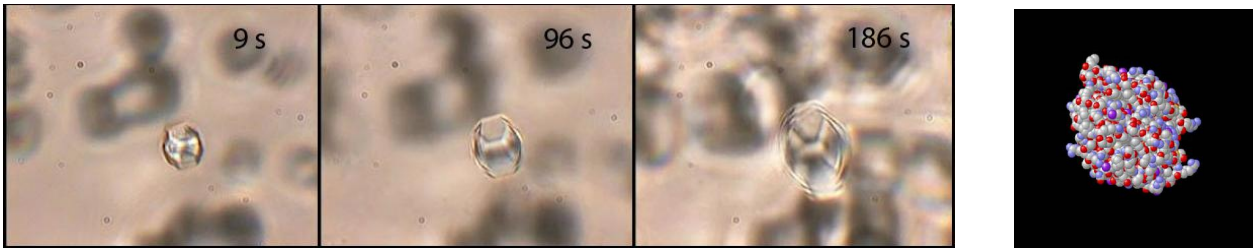

Figure 1: Left: Series showing the growth of a lysozyme crystal that is three-dimensionally trapped[10]. Even though the crystal was grown extremely fast the crystal still maintains its well-defined morphology. Right: Image of a single lysozyme molecule (from NIH Protein Database).

In order to produce crystals the appropriate growing solution needs to be "supersaturated". This is usually achieved by changing the condition of the whole solution (e.g by vapor diffusion or temperature changes). As a result many nucleation sites across the entire solution are generated. Thus the aim of generating one large crystal from an often limited amount of growing solution is replaced by the competitive growth of many crystals.

1.3. POLYETHYLENE OXIDE

For our studies we used PEO molecules of different molecular weight (m_w =100 kDa, 300 kDa, 900 kDa, Sigma Aldrich, USA). PEO (also known as polyethylene glycol (PEG) in lower molecular weight ranges) is used as a precipitating agent for the crystallization of proteins, with the best results obtained for molecular weights in the range of 6–20 kDa, at low ionic strengths[12]. PEO has an index of refraction of n = 1.45, thus its trapping ability is comparable with biological samples, which often have indices of refraction in this range. Polyethylene oxide becomes insoluble in water at high temperatures, displaying a lower critical solution temperature (LCST, cloud point) near 100ºC. Addition of salts reduces the LCST, as does an increase in molecular weight[13]. The radius of gyration of PEO molecules in water can be calculated[14] and the appropriate values are r_g=17.6 nm for m_w=100 kDa, r_g=33.5 nm for m_w=300 kDa and r_g=63.6 nm for m_w=900kDa.

2. EXPERIMENT

2.1. SETUP

An optical tweezers setup, based on an inverted microscope, was used to create the trapping potential for the molecules. Laser light from a 5W CW fiber laser (IPG, USA), operating at 1070 nm, was coupled into a 100×, NA = 1.25 objective (Olympus, Japan) and brought to a focus in the sample chamber. The power of the trapping laser was varied between 0 and 0.7W. The effects of the trapping laser on the molecules was monitored using a low power He-Ne laser (632.8 nm, JDS Uniphase, USA), which was aligned collinearly with the trapping laser. This allowed the observation of particles smaller than the resolution limit of the microscope, in our case the observation of the local increase in the molecule concentration in the focal volume.

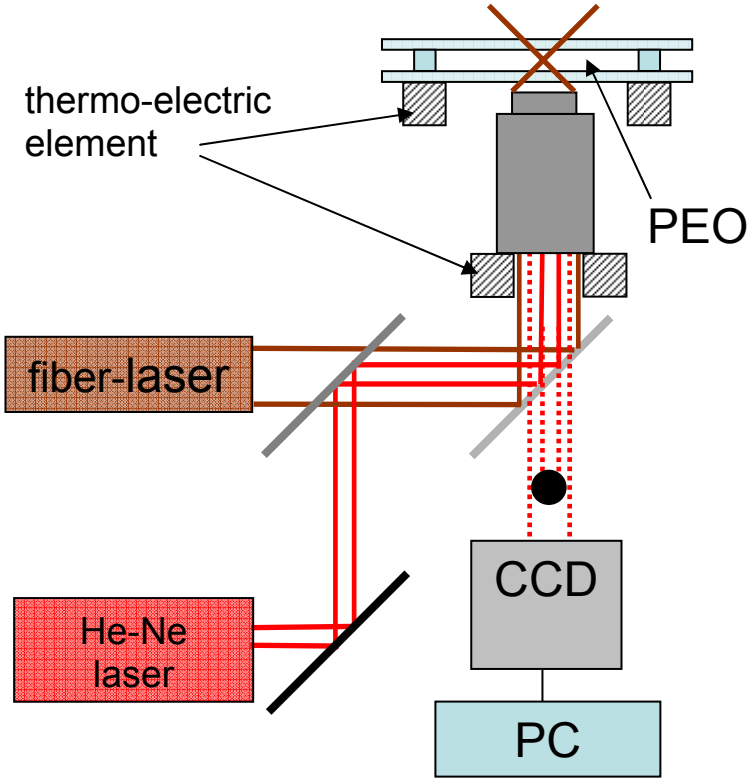

Figure 2: Schematic diagram of the experimental setup. The fiber laser was used to manipulate the PEO molecules, while the local increase in the molecule concentration was monitored collecting the scattered light of the collinear He-Ne laser. A beam block in the detection beam path increased the sensitivity of the detection system. The thermo-electric elements were used to independently control the temperature of the objective and the sample chamber.

The increased concentration of the initially homogeneously dispersed molecules in the focal region is associated with a higher index of refraction—as compared to the surrounding water—and thus resulted in scattering of the He-Ne light. The scattered light was imaged with a CCD camera, and its intensity was quantified using a MATLAB program evaluating the corresponding pixels. To further increase the sensitivity of the setup we blocked the central portion of the He-Ne reflection, allowing only rays which are scattered at angles larger than those of the incident beam to reach the CCD camera (dark-field geometry). For this purpose the incident He-Ne laser beam did not fill the back aperture of the objective, thus a beam block between the dichroic mirror and the CCD camera could be used to discriminate between light reflected from the cover slips and the light scattered from the probe volume (see figure 2).

2.2. POLYETHYLENE OXIDE SAMPLE PREPARATION

The PEO was dissolved in deionized water and the whole sample was heated up to 50°C for several days prior to use to ensure that the molecules were completely dissolved. The concentrations we used were all well below the respective overlap concentration [14-16], thus the PEO molecules could be treated as single particles. To be able to compare the trapping effects for different molecular weight PEO molecules we used the same number density of dissolved PEO molecules for all solutions (approx. 300 molecules per μm^3). This number density correspondes to concentrations c of 0.01, 0.027 and 0.077 wt% for PEO of molecular weights m_w=100, 300 and 900 kDa. For the measurements we have put 40µl of each sample between two cover slips, which were separated by 0.5mm using a silicone gasket.

2.3. PEO DETECTION METHOD

The left graph in figure 3 shows a typical time series of the scattered He-Ne light intensity after switching on of the trapping laser. The potential well created caused an increase of the molecule concentration in the focal region, which resulted in an increase of the intensity of the scattered light from the He-Ne laser. Typically the intensity of the scattered light increased until it eventually saturated. The saturated level of scattered light intensity represented the steady-state equilibrium concentration for the given trapping potential. Since the power of the detection laser was kept constant for all measurements, the scattered light intensity was a measure for the increase of the concentration in the focal region. The typical timescales were in the order of minutes, depending on trapping power.

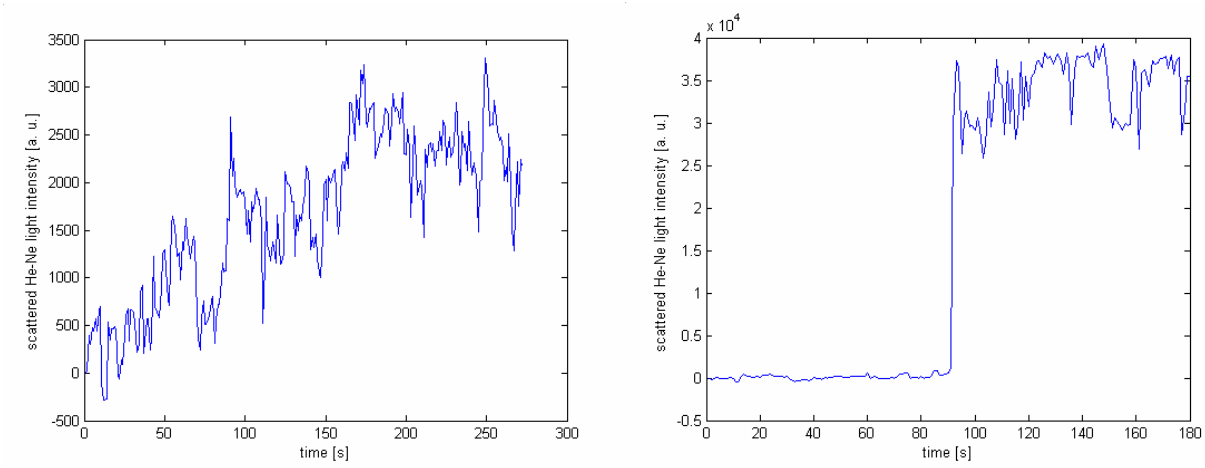

Figure 3: Left: Increase of the scattered He-Ne light intensity as a result of a concentration increase due to a trapping potential created by the trapping laser. Right: Occasional trapping of a "PEO aggregate", even though not visible in the microscope, resulted in a huge unsteady increase of the intensity of the scattered light.

By recording the whole time course of the scattered light intensity we were able to clearly distinguish between a continuous increase in the concentration of single molecules and the collection of "PEO aggregates", which were occasionally assembled prior to trapping. A continuous increase of the light intensity also always led to a reduction of the scattered light intensity as soon as the trapping power was decreased. Occasional trapping of "aggregates" always resulted in an unsteady increase of the scattered light intensity (see right graph in figure 3), and a subsequent reduction of the trapping laser power did not reduce the intensity of the scattered light. Although in these cases the intensity of the scattered light was huge, the trapped aggregate was still not visible on the CCD camera in brightfield mode. This demonstrates the sensitivity of the detection method used. Since the intensity of the scattered light depends on many unknown parameters we do not try to quantify the actual concentration in the focal region, nor do we give a relation between the intensity of the scattered light and the locally increased molecule concentration. Nevertheless, the scattered light is a measure for the relative concentration increase, and the determination of the threshold trapping power as a function of the molecular weight, does not depend on the knowledge of this relation.

3. RESULTS

3.1. TRAPPING OF PEO MOLECULES

As can be seen in figure 3 a certain power of the trapping laser caused an increase in the intensity of the scattered light of the detection laser. This intensity eventually reached a steady-state value. In figure 4 we have plotted the intensity of the scattered He-Ne light for a stepwise change of the trapping power. In between the trapping intervals the power of the trapping laser power was decreased to almost zero (intervals 2, 4 and 6 in figure 4). The intensity of the trapping laser was not completely reduced to zero to prevent the region of increased concentration to diffuse out of the detection laser. Decreasing the laser power to values below the threshold power for trapping of the respective PEO molecule resulted in a decrease of the scattered light, showing that the molecules diffuse out of the focal region.

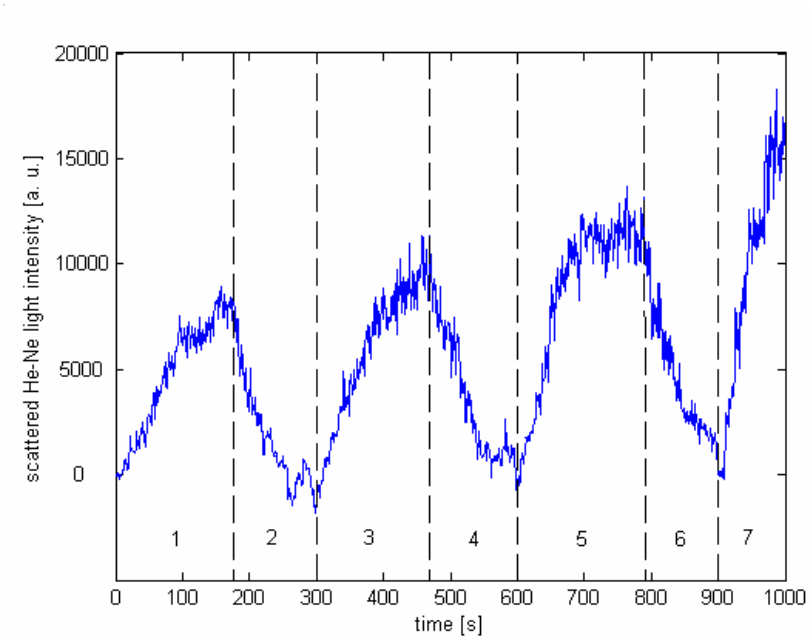

Figure 4: Intensity of the scattered light as a response to the variation of the trapping power. The graph is for PEO m_w = 300 kDa and an initial concentration of 300 molecules per μm^3. The adjusted values of the trapping laser power were: in interval 1: 0.58W, in 3: 0.60W, in 5: 0.64W and in 7: 0.7W. In the intervals 2, 4, 6 the power was adjusted to 0.16W.

As can be seen in figure 4, each trapping power corresponds to a certain steady-state value of the scattered light intensity. The steady-state values for the scattered light intensity increase with the trapping power. This can be attributed to the fact that a larger trapping potential causes a higher concentration in the focal spot. From the steady-state value and the corresponding trapping laser powers we can extrapolate the threshold trapping powers for the confinement of molecules of different molecular weight. The threshold powers for PEO with molecular weight m_w = 900 kDa was 0.21W and for m_w = 300 kDa it was 0.43W. As expected the trapping powers needed to confine the molecules decreases with molecular weight. PEO molecules with a molecular weight of 100kDa could not be confined at all (for laser powers up to 0.7W).

3.2. TRAPPING POTENTIAL OF AN OPTICAL TRAP

The trapping potential of an optical trap depends on several parameters. For a given laser beam profile and a given numerical aperture of the focusing lens the polarizability of a trapped object is the most important parameter for determining the trapping potential. For small particles (diameter << wavelength) the polarizability α scales with the volume of a particle. Because for stable trapping the trapping potential has to overcome the thermal energy there is a lower size limit of particles that can be trapped.

The quasi-static polarizability α of a non-magnetic dielectric sphere of radius r and refractive index n, immersed in a medium of refractive index n_m, is given by (Eq. 1) $\alpha = 4\pi\varepsilon_0 n^2 n_m^3 (n_r^2-1)/(n_r^2+2)$, where $n_r = n/n_m$ denotes the relative refractive index[17]. A comparison of the gyration radii of PEO in water with the stretched chain lengths (lc = 2.4μm for 300 kDa, and lc = 7.2μm for 900 kDa) of the PEO molecules shows that the individual molecules can be approximated as spheres with gyration radii r_g. Since the individual molecules are much smaller than the wavelength of the trapping laser the gradient force (Eq. 2) $F_{grad} = \alpha / (2\, c_0\, nm\, \varepsilon_0)\, \nabla S$ can be calculated using Rayleigh scattering. A subsequent integration over the Gaussian beam profile gives the trapping potential.

Using the experimentally obtained threshold trapping powers to calculate the actual trapping potential (using equation Eq. 1 and 2) we found that the trapping potential was 7×10^{-20} J, independent from molecular weights of the molecules. This value is approximately 10 times the thermal energy.

These findings are consistent with previous publications[5,7] showing that in practice the trapping potential must be significantly larger than the thermal energy of the particle to achieve stable trapping. This is due to the fact that in order to achieve stable confinement the time for the particle to diffuse out of the trap must be a lot larger than the time required to pull a displaced particle back to the center of the trap.

3.3. THERMALLY INDUCED SOLIDIFICATION

As can be seen in figure 2 our setup also incorporated two external thermo-electric elements, which could be independently controlled. Using those thermo-electric elements we were able to adjust the temperature of the solution by ± 7°C around the ambient temperature. Polyethylene oxide becomes insoluble in water at high temperatures. Addition of salts reduces the LCST, as does an increase in molecular weight[13]. For a saturated salt solution containing 1.93mg/ml 1MDa PEO clouding occurred at room temperature[11].

Cooling the solution to temperatures below room temperature moved it away from the cloud temperature. Thus we could study the effect of localized heating due to absorption of laser light in the solution. In figure 5 the laser powers necessary to create clouding in the focal spot of solutions with different temperatures are displayed. One can see that the lower the solution temperature, the higher the laser power has to be to induce clouding in the focal region. In the case of clouding the temperature in the focal volume has reached the critical (cloud) temperature, in our case room temperature. Regardless of the specific temperatures or laser powers used, we always created only one cloud point in the entire solution.

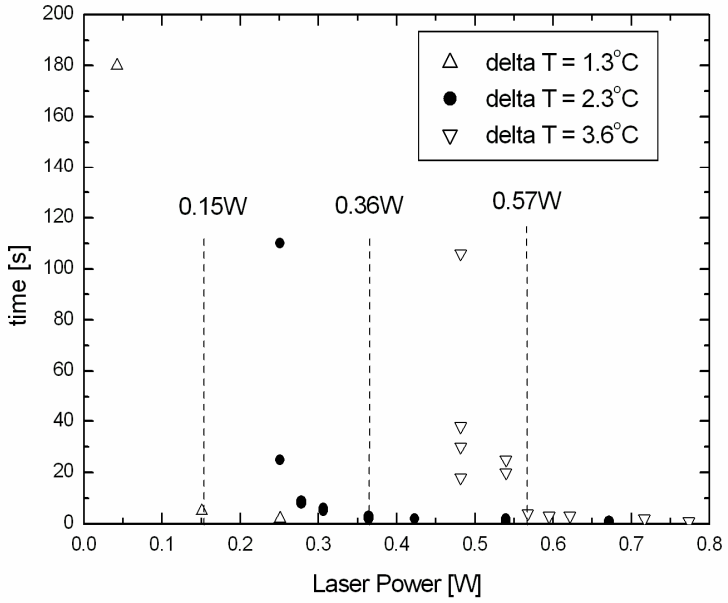

Figure 5: Graph showing the time until thermally induced solidification (clouding) occurs. The threshold laser powers stated are for 3 different solution temperatures are shown (delta T is the temperature difference between the temperature of the solution and the cloud temperature). The larger the temperature difference, the higher the laser power needed to achieve the necessary heating.

4. CONCLUSIONS AND SUMMARY

We have shown that large single molecules can be manipulated using optical tweezers. An optical trapping potential causes an increase of the molecule concentration in the focal region. The increase in molecule concentration can be adjusted by the power of the trapping laser. A subsequent reduction of the trapping laser always caused a decrease of the molecule concentration again. We propose that this method can be used to selectively trigger nucleation of protein crystals. In contrast to conventional methods for nucleation, where the properties of the entire growing solutions are changed, this method would favor the creation of only one nucleation site in the entire solution. As shown the method only works for large molecules. However, using high molecular weight precipitant molecules this method could also be extended towards smaller molecules as well.

We have also shown that for solutions showing a lower critical solution temperature the same effect can be triggered solely by thermal effects. Consequently, for LCST solutions this method is not restricted to large molecules.

5. ACKNOWLEDGEMENTS

We would like to acknowledge the support of NASA grant NAG8-1590, the University of Queensland and the Australian Research Council. We are indebted to Gregor Knoener and Simon Parkin for their contributions.

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