Probing Single Biosystem Dynamics using Optical Tweezers and Spectroscopy

Satish Rao
ICFO, Barcelona, ES

The use of optical tweezers in biology is well established. Due to their intrinsic ability to trap micron sized objects in a liquid suspension, there is a natural application to trapping single cells, such as yeast cells or red blood cells, which are in this size range. Additionally, whole new fields of biophysics have developed where single biomolecules, through attachment to trappable micron sized dielectric handles, can be manipulated and studied. Optical tweezers provides one with the ability to hold a single biosystem, apply and measure forces from picoNewton to femtoNewton scales.

However, it is well known that biosystems, such as cells, DNA, and proteins, function through an interplay between chemical and mechanical properties. The chemical changes of a protein or the internal effects of a cell are often not accessible in a measure of mechanical properties alone. Alternatively, Raman spectroscopy is sensitive to such internal changes, both structural and chemical, but is not an active probing method. The goal of this work is to obtain a complete picture of these mechanochemical relationships by combining the force probing of optical tweezers with Raman spectroscopy.

The Raman tweezers setup utilizes separate beams for the optical trapping and Raman excitation. For the former, a 1064 nm beam is passed through an interferometer with movable mirrors in order to create two independently controlled traps at the sample. Cells or biomolecules are then trapped at each end thus stopping the inherent rotation that would occur in a single trap. A 785 nm beam is passed through the center of the object and the backscattered light is collected to a spectrometer for the Raman scattering detection.

In the first experiment, we demonstrate a static method for monitoring the chemical processes inside a cell. We trapped a single yeast cell with the 785 nm beam that also excited the Raman scattering, and monitored the growth of the cell until budding. Distinct Raman bands that are indicative to the presence of proteins and lipids appear whose intensities are tracked over time. The trends of the intensities follow a similar path for the known growth cycle. Thus, the Raman bands of the cell can be used to observe the growth cycle of the cell.

The second experiment demonstrates the dynamic method where mechanical forces are applied to a cell and their response is observed using Raman spectroscopy. In this instance, we trapped a single red blood cell at atmospheric conditions. The cell was stretched by moving one of the 1064 nm beam traps while the backscattered light from the 785 nm beam was collected. A depiction of the configuration is given in figure 1 along with a video camera image of the relaxed and stretched states of the cell.

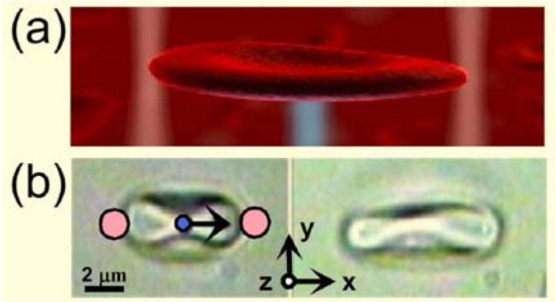

Figure 1: (a) Drawing of the configuration of the red blood cell trapped and studied by Raman tweezers. (b) Video camera image of the red blood cell in a relaxed (left) and stretched state (right). The pink and blue dots indicate the positions of the 1064 nm trapping and 785 nm Raman excitation beams, respectively, which are both directed in to the plane. The arrow indicates the direction of polarization of the excitation beam.

The main Raman bands that appear are those of the heme groups that are the oxygen carriers of the hemoglobin protein. Oxygenated and deoxygenated hemoglobin have distinct Raman spectra that are well established. A Raman spectrum was recorded for the cell in a relaxed state and then another while the cell was held in an extended or stretched state. The stretched state was about 40% bigger than the normal size of the cell which corresponded to a range of about 10 pN of force. The results show that when a red blood cell had high oxygen content in the relaxed state, indicated through its Raman spectrum, the hemoglobin experienced a transition to a deoxygenated state when the cell was stretched. This was determined by observing Raman spectra that agreed with previous studies of artificially oxygenated and deoxygenated red blood cells. The increased deformation enhances interaction of hemoglobin with the membrane and with neighboring hemoglobin. For the former, more acidifying and O_2 / CO_2 exchange occur at the membrane thus increasing the probability of oxygen dissociation. For the later, increase contact between hemoglobin proteins constrains the movement of the heme groups which is essential for stabilizing the bound oxygen.

This idea is taken one step further by repeating the previous measurement but collecting the separate polarized components of the Raman scattering; polarization parallel and perpendicular to the excitation beam, in order to obtain depolarization ratios of certain bands. The results show that when the red blood cell is stretched, the hemoglobin proteins become more packed together and seem to orient themselves in an ordered way. This suggests that the deformation of the cell not only affects the oxygenated state of the hemoglobin, but may order the proteins as well, thus possibly playing a role in intracellular oxygen transport.

Finally, we applied this concept to DNA molecules. Due to their low scattering cross section, we utilize surface enhanced Raman scattering (SERS) in order obtain measurable Raman spectra of DNA. Silver colloids, 70 nm in diameter, are passively attached to a DNA molecule with micron sized dielectric beads at each end. The beads are trapped and the DNA is moved until the characteristic Raman signal is observed, thus indicating that the DNA is in the focus of the excitation beam. The results show Raman bands that agree with previous studies on DNA solutions which demonstrate our ability to detect single DNA molecules using SERS.