L.9: Polarized Raman investigations on ordering of hemoglobin molecules inside red blood cells

Under physiological conditions the intracellular concentration of Hemoglobin (Hb), the main constituent of red blood cells (RBCs) is very high ~34%. While this high concentration ensures a large oxygen storage capacity of RBCs, the resulting interactions between neighbouring molecules strongly decrease the Hb mobility. This reduction in Hb mobility inside the cells can lead to a reduction in the oxygen transport through the cell. It has therefore been hypothesized that intracellular Hb may be ordered to facilitate diffusion of oxygen. Indeed, studies on low angle scattering of X-rays from RBCs and calculations based on size and intracellular concentration of Hb molecules provided some evidence of ordering of Hb. However, there are no confirmatory studies showing the ordering of Hb within the RBCs. Polarized Raman spectroscopy (PRS) is a powerful technique to investigate the orientation of molecules in organized environments and thus can be useful to investigate the intracellular hemoglobin ordering. At LBAID of RRCAT, Raman optical tweezers have been used to probe Hb ordering by investigating the dependence of polarized Raman spectra of a RBC on its orientation relative to the Raman excitation beam polarization direction. For these studies the equatorial plane of RBC was oriented at angles ranging from 0 to 180° with respect to the polarization direction of the excitation beam using a dual trap generated by holographic optical tweezers.

A RBC, when subjected to dual traps, separated by less than the RBC diameter, orients itself such that its equatorial plane becomes parallel to the plane containing the two trap beams as this maximizes its overlap with highest intensity region of the trap beams. RBC so trapped could be rotated in a controlled manner about the axis of the central Raman excitation beam. Raman spectrum of RBC was recorded by changing the angle of its equatorial plane with respect to the Raman excitation beam polarization direction in steps of 45°, from 0 to 180°.

Fig. L.9.1 shows the parallel polarized mean Raman spectra at different orientation angles of the trapped RBCs. The most prominent orientation dependent intensity changes were observed to occur in the parallel polarized spectra for the band at ~ 754 cm^{-1} and for bands in the spectral region 1500-1700 cm^{-1}. The intensities of these bands decreased as the cell was rotated from 0 to 45° orientation and was lowest at 90°. Upon further rotation, these changes reversed and the intensity of the bands again became highest at 180° orientation. However, no significant orientation dependent intensity changes were observed in the perpendicularly polarized Raman spectra.

To comprehend these observations, theoretical simulations based on a hypothetical RBC packed with haemoglobin (Hb) molecules such that planes of all the heme groups were parallel to the RBC equatorial plane, were carried out. Considering D4h point group symmetry of the heme group, predictions were made about the dependence of intensity of bands belonging to different symmetry species on the cell orientation and the results were found to be in good agreement with the experimental results (Fig. L.9.2). Thus our findings suggest that the Hb arrangement in RBCs may be such that the heme planes are preferentially orientated parallel to the RBCs' equatorial plane.

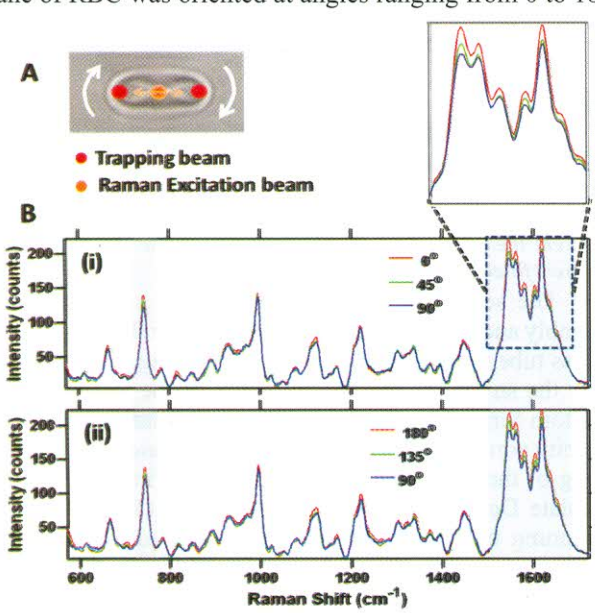

Fig. L.9.1: (A) CCD snapshot of a trapped RBC. Raman excitation beam polarization direction is shown by orange color arrow. (B) Parallel polarized mean Raman spectra at different orientation angles of the trapped RBCs. The shaded regions highlight the spectral region which showed most significant orientation dependent intensity changes.

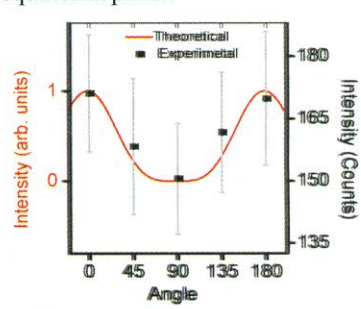

Fig. L.9.2: Theoretical (normalized intensity) and experimental observed dependence of the intensity of the band at 1547 cm^{-1} on the orientation of RBC relative to the excitation beam polarization direction.

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