Terahertz time-domain spectroscopy study of functional configurations of photoactive yellow protein in aqueous solution.

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***Abstract**. The structure of a protein under physiological conditions is closely linked to its biological function, hence there is great demand for techniques to monitor conformational changes of proteins in such conditions. Here we show that terahertz spectroscopy can be used as a convenient probe of conformational changes in proteins suspended in physiological buffer solution. We have observed an increase in terahertz absorption of the partially unfolded "photo-intermediate" state of photoactive yellow protein in comparison to its fully folded "dark" state. Normal mode and molecular dynamics simulations indicated that this increase in absorption is related to an increase in the density of delocalised vibrational modes in the more flexible photo-intermediate state.*

Photoactive yellow protein (PYP) from *Ectothiorhodospira halophila* has recently become a model system for the study of conformational changes in proteins owing to its relatively small size (125 amino acids) and the structural reconfiguration it presents, which is conveniently induced by illumination with blue (~446nm) light. The reconfiguration involves an isomerisation of the chromophore p-coumaric acid followed by the destabilisation of a hydrogen-bond network involving residues 42, 46, 50 and 69 which leads to partial unfolding of the structure (see Fig. 1 pB and pG). Recently the novel technique of terahertz (THz) time-domain spectroscopy (TDS) has given spectroscopists unprecedented access to the far-infrared region of the spectrum (100GHz-10THz; 3-300cm^{-1}). THz spectroscopy is sensitive to delocalised "low frequency" vibrational modes of secondary substructures of macromolecules such as proteins; in contrast mid- and near-infrared spectroscopy probes localised atom-atom stretches. The possibility of performing terahertz spectroscopy of proteins in aqueous solution has proven to be challenging owing to the density of broad and strong absorption modes of water in this spectroscopic range, yet the potential of these studies has recently generated much interest both in the biochemical and terahertz spectroscopy communities.

A solution of the A5C mutant of photoactive yellow protein was prepared with a concentration of ~1 mM and kept in Tris–HCl buffer, the solution was encapsulated between two z-cut quartz windows using a ~25μm teflon spacer. A standard THz-TDS spectrometer was used to measure transmission through the protein solution while four high power blue LEDs were used trigger the reconfiguration of the protein [1].

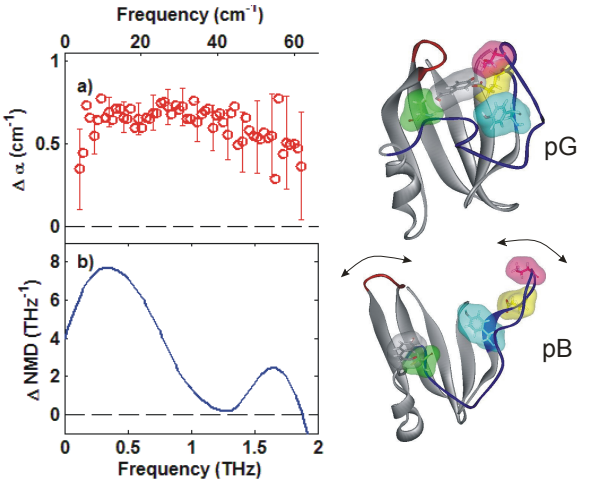

Fig. 1. *(a) The difference in absorption between the "illuminated" and "dark" state of PYP, as measured by terahertz time domain spectroscopy; (b) Shows a normal mode density difference calculated using Molecular Modelling Tool Kit [2]. On the right the "ground state" (pG) and the photo-intermediate (pB) configurations of PYP are shown, residues 42, 46, 50 and 69, as well as the chromophore p-coumaric acid, are highlighted in cyan, yellow, magenta, green and grey respectively. (Structures PDB: 1XFN and 1XFQ[3])*

The THz absorption shows an increase for the illuminated state in comparison to the dark state (Fig. 1a). Normal mode analysis [1,2] was used to calculate the normal mode density as function of frequency for each conformation. The difference (Fig. 1b) of normal mode densities is positive showing an increase in the number of low frequency modes for the 0-2THz range for the photo-intermediate (pB) structure when compared with the ground (pG) structure. Therefore the increase in absorption correlates well with this increase of normal mode density. Furthermore simulations on both structures show that after thermal relaxation in water, the root-mean-square-displacement of almost all residues is higher. This demonstrates that the entire structure becomes more flexible and therefore free to vibrate in the photo-intermediate state, which is in good agreement with the measured increase in absorption.

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