# A study of distance dependence on vibrational energy transfer in proteins taking advantage of the periodic character of α helices

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We investigated vibrational energy transfer from heme in heme proteins by observing intensity changes of anti-Stokes Raman bands of a tryptophan (Trp) residue.[1-2] In our previous study, we observed energy transfer using Trp residues at different distances from heme in globular proteins.[1] However, it is impossible to observe distance dependence without altering orientation between heme and Trp because globular protein has complex folding structure. In this study, we systematically observed distance dependence on energy transfer in protein taking advantage of the periodic character of α helices.

Cytochrome $b_{562}$ has four anti-parallel helices (Figure 1a). Taking advantage of the periodic character of α helices, distance between heme and Trp can be changed with equal intervals by introducing a Trp residue to one-turn separated positions of the same helix. The schematic structures of the cytochrome $b_{562}$ mutants are shown in Figure 1b.

In time-resolved anti-Stokes spectra of five mutants, W18, W17, and W16 bands due to the introduced Trp residue were observed at 770, 877, and 1010 $cm^{-1}$, respectively. We compared the temporal changes in the anti-Stokes W18 band intensities among five Trp residues (Figure 1c). The intensity changes of W18 band decreased and the rise of W18 band intensity became slower as the heme-Trp distance increased. These results are qualitatively consistent with the prediction from the classical thermal diffusion. This indicates that, due to highly dense nature of protein structure, vibrational energy flows in protein moiety mainly through atomic contacts. However, the intensity changes of W18 band was not quantitatively reproduced by those calculated on the basis of a one-dimensional classical diffusion model. Moreover, the Trp residues in the different helices showed different intensity changes even though their distances from heme are almost the same (Figure 1c, light and dark blue curves). This result means that the protein moiety is not uniform as a thermal conductor and/or that contacts of the residues to solvent water affect the energy relaxation.

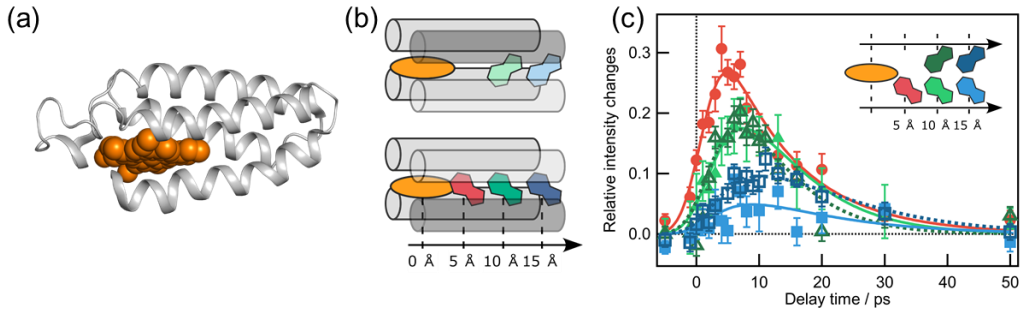


**Figure 1** (a) Crystal structure of the cytochrome $b_{562}$. Orange spheres represent heme. (b) Position of five introduced Trp residues into cytochrome $b_{562}$.mutants depicted as a schematic structure. Trp is represented by a polygon. (c) Temporal changes of relative intensity in the W18 bands.

1) N. Fujii, et al, *J. Phys. Chem. B* **2011**, *115*, 13057. 2) S. Yamashita, et al, *J. Phys. Chem. B* **2018**, *122*, 5877.