

# **Capturing Protein Dynamics with Time-Resolved Luminescence Spectroscopy**

Thesis by

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# Abstract

The presented doctoral research utilizes time-resolved spectroscopy to characterize protein dynamics and folding mechanisms. We resolve millisecond-timescale folding by coupling time-resolved fluorescence energy transfer (trFRET) to a continuous flow microfluidic mixer to obtain intramolecular distance distributions throughout the folding process. We have elucidated the folding mechanisms of two cytochromes—one that exhibits two-state folding (cytochrome  $cb_{562}$ ) and one that has both a kinetic refolding intermediate ensemble and a distinct equilibrium unfolding intermediate (cytochrome  $c_{552}$ ). Our data reveal that the distinct structural features of cytochrome  $c_{552}$  contribute to its thermostability.

We have also investigated intrachain contact dynamics in unfolded cytochrome  $cb_{562}$  by monitoring electron transfer, which occurs as the heme collides with a ruthenium photosensitizer, covalently bound to residues along the polypeptide. Intrachain diffusion for chemically denatured proteins proceeds on the microsecond timescale with an upper limit of  $0.1 \mu\text{s}$ . The power-law dependence (slope = -1.5) of the rate constants on the number of peptide bonds between the heme and Ru complex indicate that cytochrome  $cb_{562}$  is minimally frustrated.

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In addition, we have explored the pathway dependence of electron tunneling rates between metal sites in proteins. Our research group has converted cytochrome  $b_{562}$  to a *c*-type cytochrome with the porphyrin covalently bound to cysteine sidechains. We have investigated the effects of the changes to the protein structure (i.e., increased rigidity and potential new equatorial tunneling pathways) on the electron transfer rates, measured by transient absorption, in a series of ruthenium photosensitizer-modified proteins.

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