



*University of Utah*

UNDERGRADUATE RESEARCH JOURNAL

## DEVELOPING SPECTROSCOPIC METHODOLOGY TO UNRAVEL RESPIRATION

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While methods characterizing protein structure are well-developed, yet, a significant gap in current methodologies exists with respect to elucidating a causative relationship between protein motion and function. Although it may be known as to “what” a protein or enzyme does but as to exactly “how” remains to be unknown with current methodologies. The main reason for this is that much of what is known about protein dynamics is based solely on computational simulations of protein behavior. One approach to address this challenge is by developing a structure and function correlation methodology using spectral vibrational probes. Spectral vibrational probes, also known as Unnatural Amino Acids (UAA), measure electric field changes produced by the protein under Infrared (IR) spectroscopy. First, the use of UAAs in studying protein function will be tested on a model protein, and later applied to study the function of an enzyme that plays a major role in a key process in almost every organism -- respiration.

Proteins are composed of bonds between C, N, H, O, and S, which yield overlapping and congested spectral features under Infrared (IR) spectroscopy. However, there is a window between  $\sim 1800$   $\text{cm}^{-1}$  and  $\sim 2500$   $\text{cm}^{-1}$  where proteins do not normally show peaks. Unnatural amino acid (UAA) residues that show spectral peaks in this advantageous transparent window are engineered into the protein while largely preserving the protein's function, which makes it possible to use Infrared (IR) spectroscopy to study the protein's dynamics. The UAAs essentially act as probes (hence the alternative name “spectral vibrational probes”) that pick up electric field changes produced by the protein or enzyme. In the Kieber-Emmons Lab, a modified phenylalanine derivative UAA is used to develop and test the methodology on a simple well-understood model protein, Photoactive Yellow Protein (PYP). Afterwards, the methodology was applied to a mitochondrial enzyme Ubiquinol Oxidase (UbO). UbO plays a major role in generating the energy currency in the cell (ATP), but little is known how exactly this enzyme catalyzes the conversion. Thus, this project has two aims: to improve the capability of spectral vibrational probes in studying protein dynamics, and to understand more about the dynamics of UbO.