Technical Report: Defining the Mechanism of Proton Pumping in Heme-Copper Oxidases

During this award, progress was made in two distinct areas of science, one was within the context of bioinorganic chemistry and another in the context of biomimetic, small molecule chemistry. The overarching goals of this work was to understand the fundamental mechanistic drivers for biological reactions specifically, proton transfer reactions. In the biochemical sphere, this goal was investigated through investigation of the bacterial proton pump, ubiquinol oxidase (UbO). Further work in the bioinorganic space was completed in collaboration with the Bandarian group on a novel natural peptide product. While in the biomimetic space, this work was explored through a number of smaller studies relating to proton coupled electron transfers in iron complexes as relating to the heme active site in proton pumping enzymes.

Proton pumping in ubiquinol oxidase: Proton transfer reactions play a vital role in biological processes including driving ATP synthesis during aerobic respiration. The scope of this reaction is considerable with global implications including metabolic respiration and biological energy conversion. It is of additional importance as the reverse reaction is catalyzed by photosystem II during photosynthesis. In respiration, the four-electron reduction of dioxygen to generate water represents the terminal step of the electron transport chain, pumping protons vectorally against their chemical gradient. While this reaction is vital to biological function, major incongruencies exists in both the location of vital proton transfer sites, the mechanism of mechanical coupling of protein dynamics to proton pumping, and the role of electrostatics in driving the vectoral proton translocation. Work under this grant sought to investigate the latter unknown, the role of electrostatics in proton pumping in UbO. This was accomplished using unnatural amino acid vibrational probes incorporated into UbO. Unnatural amino acid vibrational probes are known to report on local electrostatic and structural variations in proteins. The vibrational frequencies of probes are modulated by changes in the local environment if either or both the anharmonicity in the vibration and/or the force constant of the vibration are sensitive to the local external electric field which is attributed to a Stark effect. The magnitude of the energy dependence on an external electric field is described by the Stark tuning rate ($\Delta\mu$).

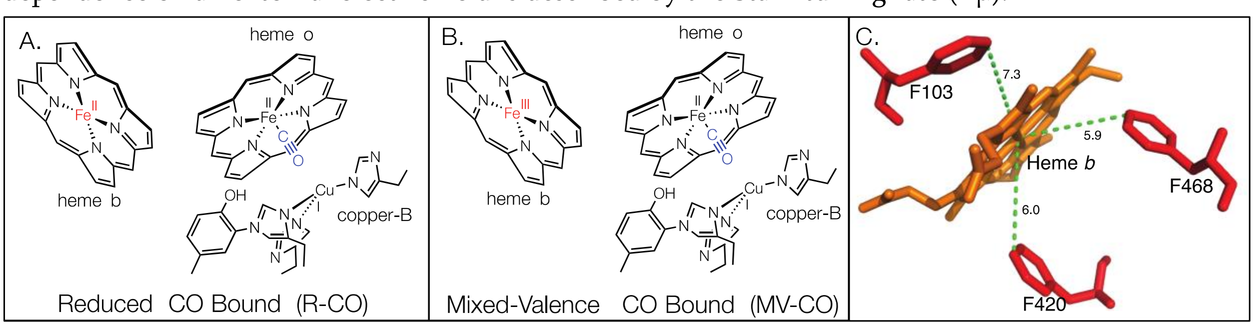

Figure 1: A. Ubiquinol oxidases heme-copper active site in the the fully reduced (R) state. B. The same active site in the mixed-valent (MV) state. C. Enzyme residues that were mutated in near one of the active site heme complexes.

For the purpose of the following work, measurements were conducted via Fourier transform infrared (FTIR) spectroscopy using the p-cyanophenylalanine (F*) vibrational probe. F* containing UbO was measured in two, well defined states, the mixed valent state (MV) and fully reduced (R) which have a one electron difference at the electron transfer heme, heme *b* (Figure 1 **A** and **B** respectively). F* was genetically incorporated into UbO at three key positions near the active site creating the F103F*, F420F*, and F468F* variants (Figure 1 **C**). Peak vibrational shifts as measured from FTIR spectra were surprisingly small indicating low electrostatic variation between the MV and R states. Under the auspices of this grant, the phenomenon was probed further using MD simulations with DFT parameterized force field modification for the bimetallic active site. Given that current mechanisms implicate electrostatic forces as drivers for proton pumping, understanding the attenuation of electronic charge is critical to understanding the driving forces of proton pumping. Small electrostatic variation may indicate a strong dependence of pumping on mechanical coupling (or dynamical changes) in addition to potentially supporting the role of a conformation switch in E286 at a literature proposed proton gate. Overall, this work highlights the intricate, fine-tuned nature of biological reactions and the delicate balance between protein structure and motion that is required for function and energy transfers.

Spectroscopic and computational investigation of a thio(seleno)oxazole RiPP natural product: A small portion of work during this granting period went towards spectroscopic investigation of a novel thiooxazole product in a ribosomally synthesized post-translationally modified peptide (RiPP) natural product. This work was completed in a collaboration with the Bandarian group at the University of Utah [*Biochemistry* **2021**, 60 3347-3361]. The work funded by this project, was able to spectroscopically and computationally characterize the formation of a thiooxazole product which to our knowledge is a novel reaction catalyzed by a radical SAM enzyme. Notably, this is also a likely product involving a SeCys-contaning peptide.

Electronic structure investigations of thioether – iron complexes: Work within this space ultimately resulted in a publication in collaboration with the Riordan group at the University of Delaware [*Inorg. Chem.* **2021**, 60, 6480-6491]. The role funded by this grant included the computational investigations of the electronic structure of tris(thioether)-iron complexes using density functional theory (DFT), time-dependent DFT, and complete active-space (CAS) calculations. The computations completed for this work were significant in their support for spectroscopic analysis performed by EPR spectroscopy and Squid magnetometry. Results revealed the ground state of Fe(II) – radical configuration has significant ionic contributions weighted towards the Fe(I) – neutral configuration. The significance of these findings include a potential effective strategy to leverage the slow magnetic relaxation identified in the Fe(I) – neutral species for preparing single-molecule magnets.