Development of a Theoretical Model for Proton-Coupled Electron Transfer Reactions Catalyzed by Amino Acids in Enzymes

Background: Proton-coupled electron transfer (PCET) reactions refer to the sequential or concerted transfer of both a proton and an electron. PCET reactions are indispensable to life, occurring throughout cellular respiration and photosynthesis, and they even drive the photocycles of many light-sensing photoreceptor proteins. These proteins often accomplish this chemistry through the use of tyrosine and tryptophan residues, which are oxidized to their corresponding radical species that subsequently play a role in the reaction mechanism. PCET reactions involving tyrosine and tryptophan are of particular importance to the biological processes mentioned above.

Motivation: Despite the emerging prevalence of tyrosine (Y) and tryptophan (W) mediated PCET reactions, much of the basic science remains unknown. The local environment of these residues varies from coordination to a metal ion, such as iron or manganese, to being buried in a hydrophobic core. How does the local protein environment stabilize and direct the reactions of these radicals without causing oxidative damage to the protein? Are there any common trends in the existing enzyme systems that utilize tyrosine or tryptophan residues for facilitating catalysis? These questions are difficult to study experimentally due to turnover times dictated by conformational changes, high redox potentials, and the reactive nature of the radical intermediates. To overcome these limitations and isolate a single, well-defined active site, a small α-helical protein with a buried Y or W residue (denoted α_3X, where X refers to the catalytic amino acid), has been created. Without significant perturbation of the protein structure, the native Y/W residues can be reversibly oxidized, and the redox potential can be tuned by incorporating Y/W chemical derivatives.[2]

Proposed Study: *I will use the well-characterized α_3X systems of our experimental collaborators as a base to build an extensive computational model, with the goal of understanding the role of the solvated protein environment and electronic effects on amino acid mediated PCET reactions.* To address these issues, I will use two main approaches, which will provide the necessary information for the culminating study on PCET kinetics. In my first approach, I will investigate the effect of the protein environment by computing the redox potential of Y/W derivatives using density functional theory (DFT) in both implicit solvent and with electrostatic embedding to include the α_3X protein environment. In my second approach, I will use molecular dynamics (MD) to probe the role of water and protein conformational changes in this system. These two approaches will provide the information needed to compute the PCET rate constant.[3]

Aim 1: I will assess how the protein environment influences the redox potential by computing the redox potentials of a series of amino acid derivatives with implicit solvent and in the protein environment through electrostatic embedding. The partial charges representing the protein environment will be obtained from the MD simulations in Aim 2. The experimentally measured relative redox potentials from our collaborators on tyrosine and its derivatives in the α_3X scaffold will be used to benchmark our methods. The influence of the protein has been viewed as a constant shift compared to aqueous values, and finding theoretical evidence to support or disprove this assumption, as well as an explanation, would be valuable. Preliminary data (Table 1) for implicit solvation studies reproduces the ordering and magnitude of the experimental redox potentials in the protein

Solvent	MUE (mV)	MUE (kcal/mol)
gas phase	13	0.3
aqueous	18	0.4

Table 1. Mean unsigned errors (MUE) of the relative redox potentials with respect to *p*-cresol. As shown, the mean unsigned error (MUE) is below the accepted error threshold of 1 kcal/mol. Data includes a series of fluorinated tyrosine derivatives. The calculations were performed at the DFT B3LYP/6-31++G** level with the PCM solvation model.[1]

very well, suggesting that the protein is uniformly affecting each chemical species and cancels out when computing relative redox potentials.

Aim 2: I will characterize the role of protein conformational motions and water accessibility in radical formation and decay. Water serves as the proton acceptor in many PCET systems, and access to the often-buried redox-active residues may be gated by conformational changes. MD studies will be used to build a hypothesis of the mechanism of water entrance into the hydrophobic interior of the α_3X proteins. The water exchange dynamics and probability distribution will be assessed from calculation of the radial distribution function and the average residence time of water in the hydrophobic pocket. In addition, the protein conformational changes associated with water entering and leaving the pocket will be analyzed. If water is found at a high occupancy, the representative conformations for water will be examined in Aim 1.

Aim 3: I will predict the rate constants for these PCET reactions using a vibronically nonadiabatic PCET theory and will probe the electronic effects as the substituent on the Y or W is changed. The information calculated from the previous aims will be used as input to the analytical rate constant expression for vibronically nonadiabatic PCET. The underlying PCET theory is related to Marcus theory for electron transfer reactions,[4] but the transferring proton is also treated quantum mechanically to include hydrogen tunneling.[3] In the past, this theory has been applied to enzymes such as soybean lipoxygenase,[5] illuminating the role of protein motions on the large kinetic isotope effect observed experimentally. Application to the α_3X system will provide an unprecedented opportunity to conduct a systematic study on a series of substituted Y and W residues in a controlled protein environment. These results will allow a much deeper understanding of biological PCET reactions.

Intellectual Merit: The proposed work aims at advancing our fundamental understanding of biologically relevant PCET reactions through theoretical examination. The α_3X protein system will allow the study of the protein environment and electronic effects in tyrosine and tryptophan residues, which can serve as a model for understanding important radical chemistry in DNA synthesis, cellular respiration, and photosynthesis. The knowledge gained from a well-constructed computational model can motivate the synthesis and incorporation of unnatural tyrosine and tryptophan analogs into biological systems, thereby tuning their energy transfer capabilities. Additionally, the incorporation of unnatural amino acids into proteins for use as fluorophores or mechanistic probes is growing rapidly. With these advancements, an understanding of these probes and their chemical reactivity in the protein environment will be crucial to successful protein design.

Broader Impacts: To aid experimental collaborators and non-specialists in understanding this work, I will develop a biological PCET module for our *web*PCET Java server.[6] The *web*PCET server is freely accessible and provides an overview of PCET and related research by illustrating the theoretical underpinnings through interactive calculations. By construction of this module, I will be improving the accessibility of theoretical chemistry to biochemists and enzymologists, who may otherwise shy away from mechanistic and chemical studies.

1a) Becke, A. D. *Phys. Rev.* 1988, *38* (6), 3098-3100; **1b)** Miertuš, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* 1981, *55* (1), 117-129; (c) Becke, A. D. *J. Chem. Phys.* 1993, *98* (7), 5648-5652. **2)** Martínez-Rivera, M. C.; Berry, B. W.; Valentine, K. G.; Westerlund, K.; Hay, S.; Tommos, C., *J. Am. Chem. Soc.* 2011, *133* (44), 17786-17795. **3)** Soudackov, A.; Hammes-Schiffer, S., *J. Chem. Phys.* 2000, *113* (6), 2385-2396. **4)** Marcus, R. A. *J. Chem. Phys.* 1956, *24* (5), 966-978. **5)** Li, P.; Soudackov, A. V.; Hammes-Schiffer, S. *J. Am. Chem. Soc.* 2018, *140* (8), 3068-3076. **6)** *web*PCET Application Server, Yale University, http://webpcet.chem.yale.edu (2009); Hammes-Schiffer, S.; Soudackov, A. V.