Graduate Research Plan

Research Title: Characterizing the Impact of Spin Intersystem Crossings on Cytochrome P450 Reactivity

Cytochromes P450 (P450s) are a superfamily of monooxygenase enzymes that utilize a unique cysteine-ligated heme cofactor to activate molecular oxygen for the efficient and selective functionalization of aliphatic C–H bonds. These enzymes are most well-known for their critical metabolic roles in hepatic drug detoxification, but also they also play pivotal roles in the biosynthesis of steroids and variety of natural products [1]. Their ability to selectively activate thermodynamically challenging C–H bonds (ΔG_{C-H}: ~100 kcal/mol) has garnered much attention from a wide array of disciplines including inorganic catalysis and *de novo* enzyme design [2]. Fig. 1 shows the P450 catalytic cycle, highlighting changes in the first coordination sphere of the iron atom. While the intermediates involved in this cycle and overall biochemical functions are agreed upon, the mechanisms by which it accomplishes reactivity are still an area of active debate. The complexity of this mechanism can be primarily attributed to the complex electronic ground state of the heme. The unpaired spins of the iron center strongly interact with the heavily delocalized porphyrin macrocycle, giving rise to several possible electronic configurations and thermodynamically accessible spin states [3]. There has been a resurgence of interest in invoking multiple spin-states in inorganic reactions, which is partially a result of rapid advancements in *ab initio* computational methods and the development of advanced spectroscopic capabilities [4]. The underlying motivation is that excited spin states can exhibit low-energy transition states which accelerate reactivity. However, the phenomena and conditions required to access excited spin states via spin intersystem crossings (SICs) remain poorly understood.

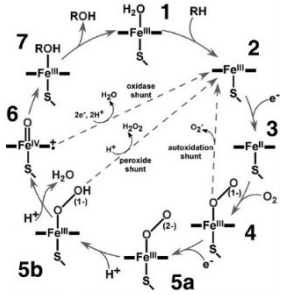

Fig. 1: P450 catalytic cycle from [1]

There are several factors that can mediate SICs in complexes containing transition metals. As shown in Fig. 2, large spin-orbit couplings (SOCs) tend to dominate the mixing of spin states, enabling transitions between states of different multiplicities when the energy gap is sufficiently small. This strong SOC facilitates SICs in systems like heme with heavy atoms and complex ligand fields because the electronic structure is highly sensitive to geometric and environmental perturbations [5]. Step 6 of the P450 catalytic cycle in Fig. 1, known as compound I (Cpd-I), is of particular importance because it is the highly reactive and oxidized intermediate that catalyzes hydroxylation. The ground state of Cpd-I consists of an S=1 iron center exchange-coupled to a porphyrin-based cationic radical ligand. However, recent molecular modeling suggests a role for a low-lying S=2 excited state with reduced activation energy, complicating our understanding of which states are involved [3]. Recent experiments have supported these models and shown hydroxylation rates depend on the composition of the ligand [2,4]. If the mechanisms of these effects can be elucidated in detail, they could be used as powerful tools to manipulate the properties of P450s as novel drug targets in medicine and for the design of catalysts in selective bond functionalization. In this research plan, I propose a combination of simulation and experiment to test how variations in the local electromagnetic environment of molecular orbitals may be a primary driver of SICs that impact reactivity.

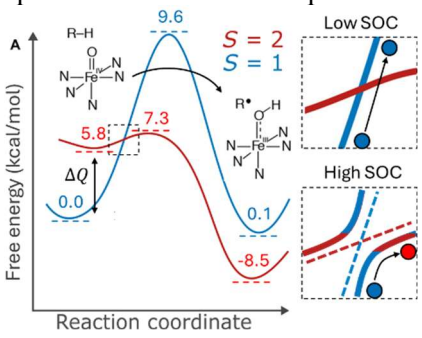

Fig. 2: Diagram of spin-dependent reactivity, adapted from [2-5]

Intellectual Merit:

As described in my personal statement, I have considerable experience in experimental and computational physics from many years of collaborative work in both academic and industry research. While much of my experience has been in solid-state and quantum systems, I believe my expertise is directly transferable to biochemical physics. For instance, the Hamiltonians used to describe electron spin qubits are analogous to those used in chemistry for modeling electronic dynamics, leveraged here to develop a computational model spin-dependent reactivity [6]. To guide my current skill transfer and future growth, I have sought mentorship from subject matter experts. In particular, I have regularly met with Prof. Alec Follmer shaping this research plan. The Follmer Lab's expertise in biochemistry, electron paramagnetic resonance (EPR), and X-ray absorption spectroscopy (XAS) will be vital for the execution of this plan.

Aim 1: Modeling the Quantum Dynamics of Hydroxylation by Compound I

An outline of the hydroxylation reaction pathways is given in Fig. 2. The primary aspect to model is the interaction between the ground triplet and the low-lying excited quintet state, split in energy by ΔQ and influenced by the exchange coupling of a secondary radical. My approach will be to model the dynamics of the overall system, which can be described by the general Hamiltonian:

$$H_{tot} = H_{zee} + H_{zfs} + H_{ex} \quad (1)$$

H_{tot}, H_{zee}, H_{zfs}, H_{ex} represent the total Hamiltonian, Zeeman interaction with an external magnetic field, zero-field splitting of the iron center where the SOC is expressed, and exchange coupling between the catalytic iron center and the secondary radical, respectively. Electronic relaxation and hydroxylation are dissipative processes which either decay the excited state to the ground state or destroy the state, respectively. The dynamics of the system described by H_{tot} with dissipators is governed by the Lindblad master equation, a generalized form of the Schrödinger equation.

As an example mechanism, I examine the dynamics of a simplified two-level model, depicted in Fig. 3(a). The ground singlet can be excited via a thermal photon or phonon, $\hbar\omega$. These electrons interact with an iron-57 nucleus with coupling proportional to their spatial wavefunction overlap. This overlap changes between orbitals and drives singlet-triplet SICs during the electronic relaxation time. This system is governed by the Hamiltonian:

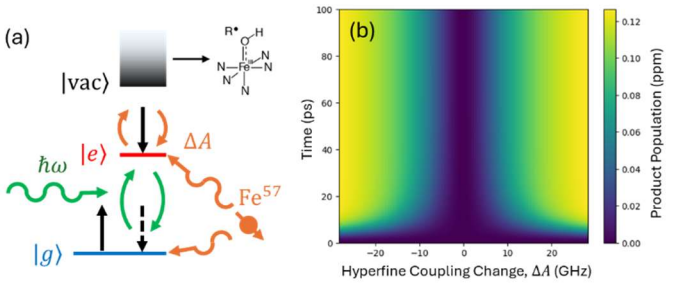

Fig. 3: (a) Schematic of spin-dependent model (b) Hyperfine coupling change vs. product accumulation

$$H_{tot} = \gamma_e B_z \Sigma_{i,\alpha} S_z^{(i,\alpha)} + \gamma_n B_Z I_Z + \Delta Q |e\rangle\langle e| + \gamma_n \Delta A \left(S_x^{(2,e)} I_x + S_y^{(2,e)} I_y \right) \quad (2)$$

B_z, γ_e, γ_n, ΔA are the external magnetic field, electron and nuclear gyromagnetic ratios, and change in transverse hyperfine coupling, respectively. $S_i^{(\sigma,\alpha)}$ are the Pauli spin matrices for electron σ in orbital α, and I_i are spin matrices for the nucleus. Using the electronic relaxation rate measured in [2] and spin-dependent hydroxylation rates from [3], I simulated the time evolution of the density matrix, giving the population of electronic configurations captured by the model. In aggregate, these lead to a ΔA-dependent accumulation of products, as shown in Fig. 3(b). As ΔA increases, the SICs allow access to an accelerated reaction pathway, increasing product accumulation. Although this result is based on a toy model, it suggests a measurable effect and motivates further investigation using a more comprehensive Hamiltonian.

Aim 2: Measuring Magnetic Field Dependent Intermediate Formation and Hydroxylation Rates

With a theoretical understanding of the relevant pheomena, I will explore and validate their impacts experimentally by measuring the magnetic field dependence of intermediate formation and hydroxylation rates. To achieve this, I will learn to perform protein purification and expression of a model P450, P450cam, and employ a combination of stopped-flow, EPR, and XAS to characterize the magnetic behavior of the enzyme. These techniques will enable me to analyze the effects of magnetic fields on intermediate formation kinetics, ground-state electronic structures, and time-resolved valence configurations.

Broader Impacts:

Experimentally measuring and theoretically describing the SOCs and SICs in P450s is the next step in uncovering the intricate mechanisms of these powerful molecular machines, unlocking their applications as novel therapeutic targets in medicine and designer biocatalysts in biosynthetic chemistry. The complex electronic dynamics of the active metal-center combined with functional control from surrounding amino acids place P450s at the intersection of all chemistry disciplines and pushes the boundaries of the entire field. Therefore, this work could more broadly define a framework for measurements and computations necessary to characterize bioinorganic molecules, advancing the field towards leveraging the unique spin properties of metal-centered molecules for novel biomedical and industrial applications.

References: [1] Denisov, et al., *ACS Chem. Rev.*, 105 (6), 2005. [2] Onderko, et al., *J. Am. Chem. Soc.*, 147 (11), 2025. [3] Shaik, et.al., *Nat. Chem.*, 3, 2011. [4] Rice, et al., *Sci. Adv.*, 10, 2024. [5] Swart & Costas, *Wiley*, pp. 103-129, 2015. [6] Bimstefer, "NSFGRFP_2025," *gbimGit GitHub Repo.*, 2025.