SUMMER 2024 – SC INBRE RET PROJECT DESCRIPTION FORM

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Research Subject Area	Investigation of the Reaction Mechanisms of Enzymes

A. Briefly describe overall research program at your laboratory:

Across the scope of cellular metabolism, enzymes frequently employ an iron cofactor as an integral part of their chemistry. While typical biochemical and organic reactions occur via Lewis acid-base (two-electron) chemistry, nature has taken advantage of iron in performing much needed one-electron reactions due to the relative ease of switching between the Fe^{2+} and Fe^{3+} states. These "iron-dependent" enzymes can perform a large variety of potential reactions and nature has tried them all! One of the big questions in the field of enzymology is how does each enzyme tailor the environment at and around the iron cofactor to drive a single specific reaction.

The research in our lab is focused on understanding the underlying principles that drive the reaction of the iron-dependent enzyme, 2,4'-dihydroxyacetophenone dioxygenase (DAD). This enzyme catalyzes the oxygen-dependent cleavage of a specific C–C bond in the substrate, 2,4'-dihydroxyacetophenone (DHA). This reaction is quite unique as it is a rare example of an iron-dependent enzyme involved in the cleavage of a non-aromatic C-C bond. Our long term goal is to understand the underlying principles that drive this particular reaction and compare and contrast against homologs that perform a different reaction employing the iron cofactor. This will lead us to a better understanding of how the environment provided by an enzyme moderates the reactivity of the iron cofactor to a specific need.

Our focus for summer 2024 is two-fold. First, we will be doing the final rounds of experiments looking at the formation of the enzyme-substrate complex (i.e. DAD-DHA complex). We have synthesized several substrate analogs that are expected to disrupt (or possibly enhance) complex formation. During Summer 2024, we will be synthesizing the last couple-few substrate analogs and performing concentration-dependent assays to evaluate the steady-state kinetics of these substrate analogs and compare the results to the wild-type reaction.

Our second project is focused on the role of a highly-conserved active-site tyrosine (Tyr-94). For Summer 2024, we will be expressing and purifying two specific mutations of Tyr-94 and then evaluating the steady-state kinetics of these mutants. We will also be employing our stopped-flow system in an attempt to "see" individual steps of the reaction. Experiments may extend to looking at the O_2-dependence of the kinetics as well.

B. Briefly describe specific project(s) for your teacher:

Experiments will include performing activity assays using our UV-absorbance spectrometer and/or our stopped-flow system in which enzyme and substrate are mixed at a variety of concentrations with kinetic data determined both initial rate fits and/or full-trace fitting; spectrometric assays are performed in-cuvette, collecting data as a change in absorbance due to consumption of the substrate; stopped-flow assays involve rapid-mixing of enzyme and substrate samples, monitoring the change in absorbance on sub-second timescales with the potential to observe reaction intermediates; analysis of the data using multiple fitting methods including single-trace fitting and global fitting of multiple traces and experiments; expression, purification, and kinetic analysis of mutant enzymes.

The teacher will gain experience in relatively basic enzymology and biochemical techniques that are generally transferable to the high-school setting. Many of the techniques we use in the lab are low-cost with typically entry-level equipment. We're not "swimming in cash" and we still get good work done!

C. Will any other people (post docs, grad students, undergraduate students, colleagues, etc.) be involved directly with your teacher?

The Roberts lab currently has four undergraduates working in the lab (two Junior chemistry majors, a Senior biology major, and a Senior Exercise Sport Science major). Two of these students are expected to continue this summer. As I expect both projects described above to be active this summer, I expect that each project will "enlist' one of these two students who will work directly with the teacher on their project. The PI will also be fully active in the lab during the summer term.

D. Will you require any advanced reading/preparation for the teacher?

Some advanced reading will be expected before start of the experiments. But TBH, there isn't a whole lot known about DAD so the literature burden is pretty light. Those articles will be provided at the first introductory meeting.