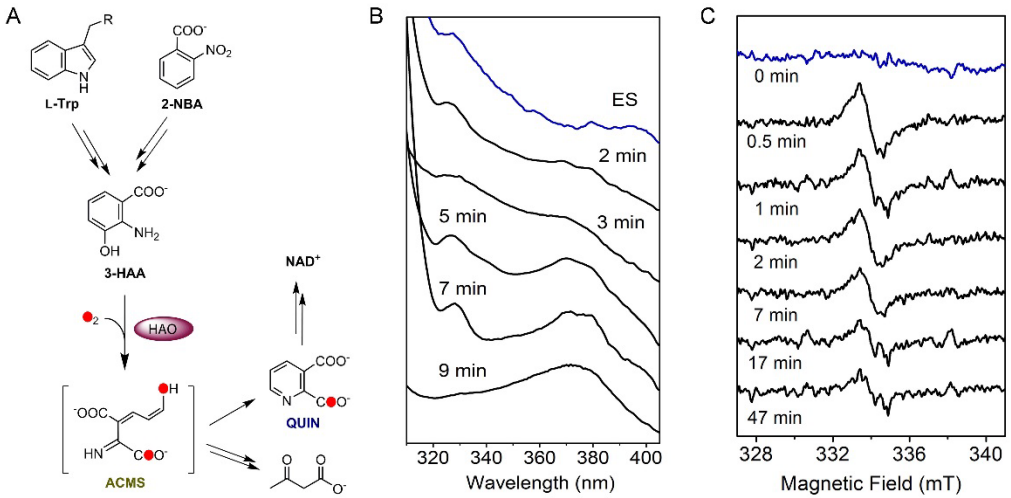
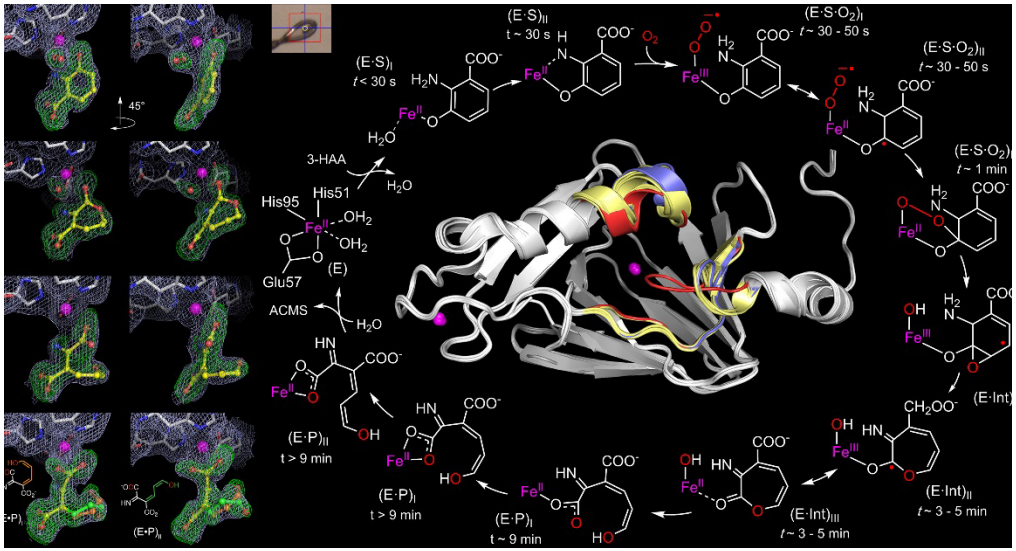
Aimin Liu, University of Texas at San Antonio, CHE-1623856: Structure-Function Correlations in a Type III Extradiol Dioxygenase

Enzyme-in-Action Movie: Observing 3-hydroxyanthranilate-3,4-dioxygenase in action through a crystalline lens





Top: (A) HAO plays a central role in QUIN production, (B) Single-crystal UV-vis spectral of the HAO reaction shows several spectroscopically distinct intermediates, and (C) EPR of a slurry of single crystals as a function of reaction shows a radical intermediate. **Bottom**: seven intermediate crystal structures were determined during *in crystallo* reaction, enabling the construction of an enzyme-in-action movie for understanding its catalysis mechanism.

E-mail the Highlight to the Division of Chemistry at: chemhighlights@nsf.gov

This completion of this project made 3-hydroxyanthranilate-3,4-dioxygenase (HAO) one of the best understood enzymes. Specifically, a thorough and rigorous understanding of the enzyme in action has been achieved by observing how oxygen is activated and transfers oxygen atoms to the organic substrate (doi:10.1073/pnas.2005327117), and how HAO employs loop dynamics to accommodate two substrates with disparate polarities for substrate recognition and oxygen binding to the catalytic iron center (doi:10.1074/jbc.RA118.002698), Moreover, how the enzyme preserves metal essential metal iron for activity by making an iron reservoir (doi:10.1074/jbc.M115.650259).

The synthesis of quinolinic acid from tryptophan is a critical step in the *de novo* biosynthesis of nicotinamide adenine dinucleotide (NAD^+) in mammals. This project solves the missing piece of the information by investigating a dioxygenase that regulates quinolinic acid levels. The results from this project provide a comprehensive view of the dioxygenase mechanism by enabling step-by-step visualization of the catalytic cycle and the protein dynamics during catalysis. The results also reveal how the enzyme regulates metabolic pathway product distributions, including the nonenzymatic product of biologically significant compounds. This knowledge will help to understand NAD^+ hemostasis, immune regulation, and oxygen activation and utilization.

This project was funded by the Chemistry of Life Processes (CLP) Program in the Division of Chemistry, primarily by the award CHE-1623856 and at the later stage partially by CHE-1808637; both awards were administrated by Program Officer Dr. Catalina Achim.

The development of single-crystal EPR spectroscopy for characterizing catalytic intermediates in enzymes is technically innovative.

The funding of this project also enabled the PI to run an EPR/ENDOR facility in the deep South region.

An African-American student, Kednerlin Dornevil, graduated with a Ph.D. degree in December 2017 with training from this project. Three undergraduate students (Ms. Elizabeth Fritz, Ms. Uyen Ha, and Mr. Gerardo Virgen) also participated in the CLP-funded dioxygenase mechanism project, and two of them co-authored publications.