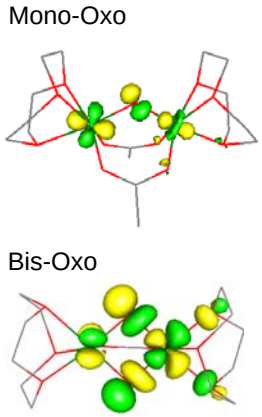
Spectroscopic and Computational Characterization of Intermediate X of *E. coli* Class I Ribonucleotide Reductase

Michael Dean Clay[1], Nataša Mitić[1], Lana Saleh[2], J. Martin Bollinger, Jr.[2], and Edward I. Solomon[1]

[1]*Department of Chemistry, Stanford University and* [2]*Department of Biochemistry and Molecular Biology, The Pennsylvania State University*

Ribonucleotide reductases (RR) catalyze the rate-limiting step in DNA biosynthesis through the reduction of ribonucleotides to deoxyribonucleotides. This unique family of enzymes is further divided into three classes. Class I RR is composed of two subunits, R1 and R2. The R1 subunit comprises the site of ribonucleotide binding and reduction, whereas the R2 subunit uniquely contains a binuclear non-heme iron site for tyrosyl radical generation. While a general mechanism has been proposed for tyrosyl radical generation, relatively little is known about the geometric and electronic structure of the diiron-oxygen intermediates that are involved in this pathway. However, recent spectroscopic and computational results have helped elucidate the geometric and electronic of the peroxo (P) intermediate and high-valent (X) diiron-oxygen intermediates (1, 2). This study focuses on understanding the geometric and electronic structure of intermediate X, through the recently developed rapid freeze-quench magnetic circular dichroism (RFQ-MCD) methodology and density functional theory (DFT) calculations (3). Furthermore, DFT was used to explore the relative energetics and corresponding reaction coordinate pathway from resting diferrous to dioxygen activation and subsequent tyrosyl radical generation.



1. Skulan, A. J.; Brunold, T. C.; Baldwin, J.; Saleh, L.; Bollinger, J. M., Jr.; Solomon, E. I. *J. Am. Chem. Soc.* **2004**, *126*, 8842-8855.
2. Mitić, N.; Saleh, L.; Schenk, G.; Bollinger, J. M., Jr.; Solomon, E. I. *J. Am. Chem. Soc.* **2003**, *125*, 11200-112001.
3. Mitić, N.; Clay, M. D.; Saleh, L.; Bollinger, J. M., Jr.; Solomon, E. I., in preparation.

This research is supported by NSF-MCB-9214214 (E.I.S.), NIH-Kirschstein postdoctoral fellowship (M.D.C) and NIH GM55365 (J.M.B.).