

## E0010

**Structural Basis for O<sub>2</sub> Activation in Extradiol Ring Cleaving Dioxygenases.** E. G. Kovaleva, and J. D. Lipscomb, Dept. of Biochemistry, Molecular Biology, and Biophysics, Univ. of Minnesota, Minneapolis, MN 55455.

Homoprotocatechuate (HPCA) 2,3-dioxygenase (2,3-HPCD) catalyzes proximal extradiol ring-cleavage of catecholic substrates. Substrate binding to the active site Fe(II) promotes binding and activation of O<sub>2</sub> as well as the subsequent insertion of both oxygen atoms to yield the product. Here, we use structural approaches to explore the mechanistic consequences of: (1) replacing an electron supplying substrate ring substituent with an electron withdrawing group, and (2) making subtle changes in a 2<sup>nd</sup> sphere amino acid residue. High-resolution crystal structures and optical spectra in combination with kinetic studies show that 4-NO<sub>2</sub>-catechol (4NC) binds in the same site as HPCA and undergoes either ring oxidation to the quinone or ring cleavage depending on the active site amino acid composition. A residue capable of acid-base chemistry is required near the O<sub>2</sub> binding site in order to observe the ring-cleaved 4-NC product. When HPCA is bound instead of 4-NC, or when the normal acid-base residue is present, ring cleavage is always observed, suggesting that both the substrate and the active site acid-base contribute to reaction fidelity. The techniques developed here are being used to structurally characterize reaction cycle intermediates for the first time.