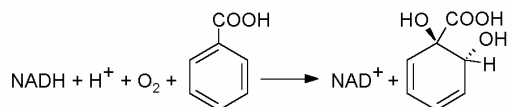


## Structural Effects on O<sub>2</sub> Activation by Benzoate Dioxygenase

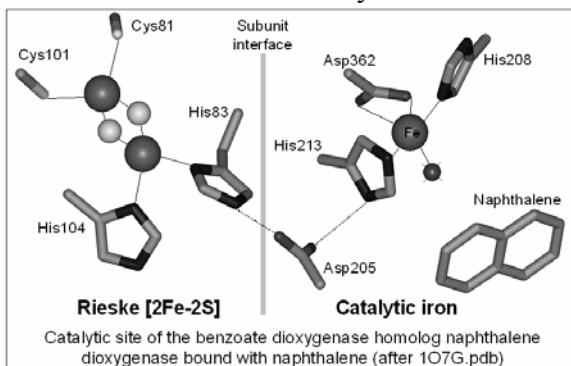
Melanie Rogers, Sarmistha Chakrabarty,  
Matt Neibergall, Mike Mbughuni, and John D. Lipscomb

*Department of Biochemistry, Molecular Biology and Biophysics; and Center for Metals in Biocatalysis, University of Minnesota, Minneapolis, MN 55455.*

The Rieske dioxygenase benzoate 1,2 dioxygenase catalyses the *cis*-dihydroxylation of benzoate, incorporating both atoms from O<sub>2</sub>. The two electrons required for this process are transferred from NADH



via a reductase component to the Rieske [2Fe2S] cluster and catalytic mononuclear iron center of the oxygenase component. Oxygen could, in principle, bind to the reduced iron center and become activated, but this does not occur unless substrate is first bound in the active site and the Rieske center is also reduced. The structural basis for this complex regulation of O<sub>2</sub> activation appears to involve the unusual  $\alpha_3\beta_3$  architecture of the enzyme. By analogy with the structurally characterized naphthalene 1,2-dioxygenase oxygenase component (NDO, see figure), the catalytically relevant Rieske cluster/mononuclear Fe pair appear to be located across an  $\alpha$ - $\alpha$  subunit interface. Our past ENDOR studies<sup>1</sup> with NDO indicate that the relative separation of the mononuclear iron and substrate is sensitive to the oxidation state of the Rieske cluster located 12 Å away across the subunit interface. Given the regulatory mechanism



described above, substrate is also likely to cause structural changes in the iron vicinity that facilitate O<sub>2</sub> binding. The structural basis for these effects and the synergy that rigorously couples them together to allow the O<sub>2</sub> activation process to commence are being investigated using spectroscopic, kinetic, and crystallographic techniques. Supported by NIH GM024689.

<sup>1</sup>Yang, T.-C.; Wolfe, M. D.; Neibergall, M. B.; Mekmouche, Y.; Lipscomb, J. D.; Hoffman, B. M. *J. Am. Chem. Soc.* **2003**, *125*, 2034-2035 and 7056-7066.