

## The Reaction Mechanism of the Enzyme Salicylate Dioxygenase Revealed by QM/MM Simulations

Salicylate 1,2-Dioxygenase (SDO) is the first enzyme discovered to catalyze the oxidative cleavage of a monohydroxylated aromatic compound, salicylate, in contrast to the well-known electron-rich substrates. We have investigated the mechanism of dioxygen activation in SDO by QM/MM calculations. Our study reveals that the non-heme Fe<sup>II</sup> center in SDO activates salicylate and O<sub>2</sub> synergistically by a strong covalent interaction to facilitate the reductive cleavage of O<sub>2</sub>. A covalent Salicylate-Fe<sup>II</sup>-O<sub>2</sub> complex is the reactive oxygen species in this case, where the electronic structure is best described as between the two limiting cases, Fe<sup>II</sup>-O<sub>2</sub> and Fe<sup>II</sup>-O<sub>2</sub><sup>•-</sup> with partial electron transfer from the activated salicylate to O<sub>2</sub> via the Fe center. Thus, SDO employs a synergistic strategy of 'substrate activation' and 'oxygen activation' to carry out the catalytic reaction, which is unprecedented in the family of iron dioxygenases. Moreover, O<sub>2</sub> activation in SDO happens without the assistance of a proton source. Our study essentially opens up a new window in the mechanism of O<sub>2</sub> activation.