ELUCIDATING REACTION MECHANISMS OF COUPLED BINUCLEAR COPPER ENZYMES BY CORRELATING QM/MM CALCULATIONS AND SPECTROSCOPY

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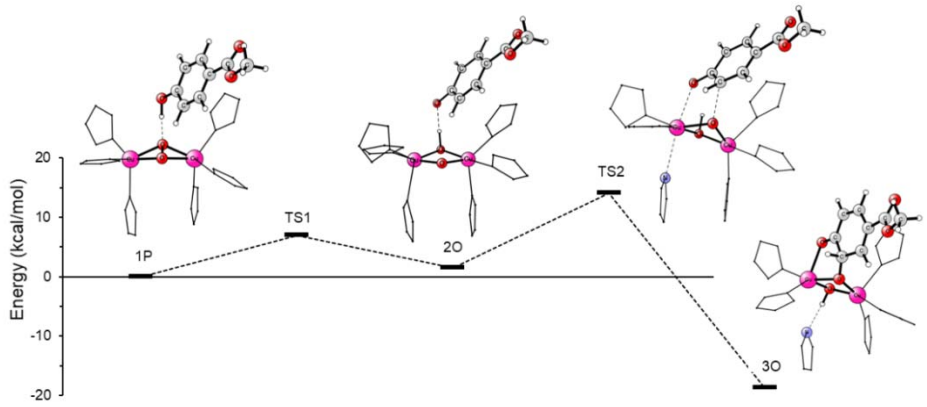
Coupled binuclear copper (CBC) enzymes are used by nature to catalyze variety of chemical conversions (e.g., hydroxylations, oxidations).[1] One such example is tyrosinase (Ty), the ubiquitous enzyme responsible for O_2-dependent *ortho*-hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), as well as the subsequent two-electron oxidation of L-DOPA to L-dopaquinone. These elementary reactions are the initial and rate-limiting steps in melanogenesis.[1] Due to its role in the biosynthesis of melanin, Ty is of an interest in the detection, prevention, and treatment of complex human diseases, including skin cancer [2] and Parkinson's disease.[3] By combining theoretical and experimental methods we investigated H-bonding interactions in oxy-Ty [Ty/O_2] active site, [4] and predicted structure of the elusive ternary complex [Ty/O_2/monophenol] intermediate.[5] Next, we examined monooxygenation reaction of Ty with the analogue of native substrate, that is with methyl 4-hydroxybenzoate.[5] Subsequently, we investigated the ortho-hydroxylation step employing series of substrates with different electron donating/withdrawing group in phenol para-position revealing biphasic substrate dependence of the monophenol monooxygenation reaction of tyrosinase. This biphasic nature is ascertained mainly by correlating experimental and theoretical energy barrier/k_2, and solvent KIE.



[1] E. I. Solomon et al., *Chem. Rev.* **2014**, *114*, 3659–3853.

[2] B. Ciui et al., *Adv. Healthcare Mater.* **2018**, *7*, 1701264.

[3] I. Carballo-Carbajal et al., *Nat. Commun.* **2019**, *10*, 973.

[4] I. Kipouros, A. Stańczak, L. Rulíšek, E. I. Solomon et al., *Chem. Commun.* **2022**, 58, 3913–3916.

[5] I. Kipouros, A. Stańczak, L. Rulíšek, E. I. Solomon et al., *Proc. Natl. Acad. Sci. U. S. A.* **2022**, 119, e2205619119.