QM-Cluster Model Study of Enzymatic Reaction between Cytochrome P450 Enzyme GcoA and Various Substrates

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Recently, a promising *Amycolatopsis* cytochrome P450-reductase pair -- a two component P450 class composed of GcoA and GcoB has been discovered.[1] GcoA was found to efficiently demethylate several lignin-derived monomers,[2] suggesting a broad substrate scope for lignin degradation and valorization. The key step of the O-demethylation of guaiacol by GcoA was studied by our group with Density Functional Theory using various sized quantum mechanical (QM)-cluster models.[3] The computed free energy of activation is in good agreement with experimentally measured k_{cat} of demethylation of guaiacol by GcoA (6.8 ± 0.5 s^{-1} at 25°C). Hence, the enzymatic reactions of seven different substrates catalyzed by GcoA were studied by a similar approach, starting from Molecular Dynamics simulations of GcoA in complex with each substrate. QM-cluster models were generated by the toolkit *Residue Interaction Network-based ResidUe Selector* (RINRUS). This study will provide details for better understanding enzymatic O-demethylation of lignins to form catechol derivatives by GcoA and shine a light on structure-activity relationship models for future ligand design and enzyme bioengineering.

Reference:

(1) Tumen-Velasquez, M.; Johnson, C. W.; Ahmed, A.; Dominick, G.; Fulk, E. M.; Khanna, P.; Lee, S. A.; Schmidt, A. L.; Linger, J. G.; Eiteman, M. A.; et al. Accelerating Pathway Evolution by Increasing the Gene Dosage of Chromosomal Segments. *Proc. Natl. Acad. Sci.* **2018**, *115* (27), 7105–7110. https://doi.org/10.1073/pnas.1803745115.

(2) Mallinson, S. J. B.; Machovina, M. M.; Silveira, R. L.; Garcia-Borràs, M.; Gallup, N.; Johnson, C. W.; Allen, M. D.; Skaf, M. S.; Crowley, M. F.; Neidle, E. L.; et al. A Promiscuous Cytochrome P450 Aromatic O-Demethylase for Lignin Bioconversion. *Nat. Commun.* **2018**, *9* (1), 2487. https://doi.org/10.1038/s41467-018-04878-2.

(3) Cheng, Q.; DeYonker, N. J. QM-Cluster Model Study of the Guaiacol Hydrogen Atom Transfer and Oxygen Rebound with Cytochrome P450 Enzyme GcoA. *J. Phys. Chem. B* **2021**, acs.jpcb.0c10761. https://doi.org/10.1021/acs.jpcb.0c10761.