ENGINEERING OF A SPECIFIC CYP450 FOR AN INDUSTRIAL PROCESS SHOWS 700-FOLD INCREASE IN ACTIVITY WITH K_{CAT} OF 6.2 S^{-1} – RESIDUES CAUSING HYDROGEN MIGRATION AND DOUBLE HYDROGEN ABSTRACTION AT Δ^X CARBON IDENTIFIED BY QUANTUM MECHANICS REVEALED TO BE THE GAME CHANGER

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A CYP450 catalyzed desaturation reaction for the synthesis of an Active Pharmaceutical Ingredient (API) via biocatalytic route that is estimated to top up the revenue of the company to 8.5 million dollars was identified in this study. Currently this route is being tested for the industrial scale production of the API. This was achieved by engineering a specific membrane bound CYP450 enzyme that was initially inactive towards the substrate. Extensive modelling studies were carried out to obtain an initial promiscuous activity (0.53/min). Here the substrate enters through the transmembrane region of the enzyme that acts as a tunnel and moves to the Heme Binding Site in the CYP450 during which it undergoes rotation of ~180°. The substrate entry was identified as the first rate limiting step in the reaction. Using a grid-based path optimization method this path was engineered to facilitate the easy entry and movement of substrate in to the active site. The second rate limiting step was observed in the formation of two transition states of the reaction which was identified using Quantum Mechanics hybridized with Molecular Mechanics (QM/MM) simulations. The enzyme was engineered using quantum polarized grid technology to reduce the energy of these transition states. The mutations introduced, improved the enzyme activity > 700 folds giving a K_{cat} value of 6.2 s^{-1} which translated to a good yield of the product in the lab scale fermenter. A greater finding in this project is the desaturation reaction mechanisms involving the hydrogen migration and double hydrogen abstracted from the same Δ^X Carbon. This was obtained using extensive QM/MM simulations studies and a high-resolution grid energy evaluation method which revealed transition and intermediate states of the reaction, not reported before in any of the desaturation mechanisms solved so far. Some important aspects of this study include, modelling yeast membrane with different lipids and varying substrate concentrations, enzyme behavior at high substrate concentrations and process optimization. In conclusion, this is a cost effective route with a conversion rate that is ~10 times more than the chemical route. This process can be applied to engineer CYP450 for any chemical routes that involve a desaturation reaction.

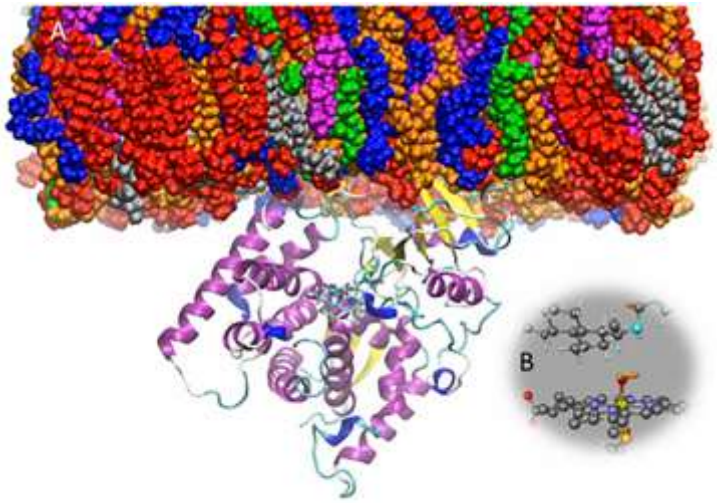

Figure 1A. – Cyp450 complexed with heme and substrate integrated in the yeast hypo membrane B. Shows the rate limiting transitions state of the enzymatic reaction

Variants	Number of mutations	Activation energy of TS1 and TS2 [kcal mol^{-1}]	*invitro* Activity K_{cat} Sec^{-1}
Wild	-	48.3 and 43.6	0.008
V1	3	35.8 and 30.3	6.2±0.8
V2	3	35.6 and 33.2	4.8±1.2
V3	2	32.7 and 33.8	3.5±0.5

Table 1 – Energies of the crucial transition states and the experimental K_{cat} values