QM/MM STUDIES OF THE PHENYLALANINE AMMONIA-LYASE VARIANTS HELPED TO UNDERSTAND THE MECHANISTIC ROLE OF THE MUTATIONS

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Pegvaliase, the only FDA-approved drug for treating classical phenylketonuria (PKU), is a PEGylated Phenylalanine ammonia-lyase (PAL). PAL from *Anabaena variabilis*, which known for the conversion of l-phenylalanine to *trans*-cinnamic acid and has widespread application in chemoenzymatic synthesis, agriculture, and medicine. Deep mutational scanning (DMS) has recently emerged as a powerful method to study protein sequence–function relationships which used for identifying hotspots and mutations to improve reaction kinetics in cell-free and cellular contexts. Behavioural studies of enzyme after the incorporation of the mutations were thoroughly studied using quantum mechanics/molecular mechanics (QM/MM) and molecular dynamics (MD) studies. QM/MM studies were conducted on 4 chains of the enzyme simultaneously which helped to understand the mechanistic role of the mutations including stabilizing transition and intermediate states. Molecular Dynamics Based Metadynamics studies with unique collective Variable (CV) helped us to understand changes in substrate binding affinity among mutations and umbrella sampling was used to understand the association and dissociation path of the substrate which helped to reduce product inhibition.

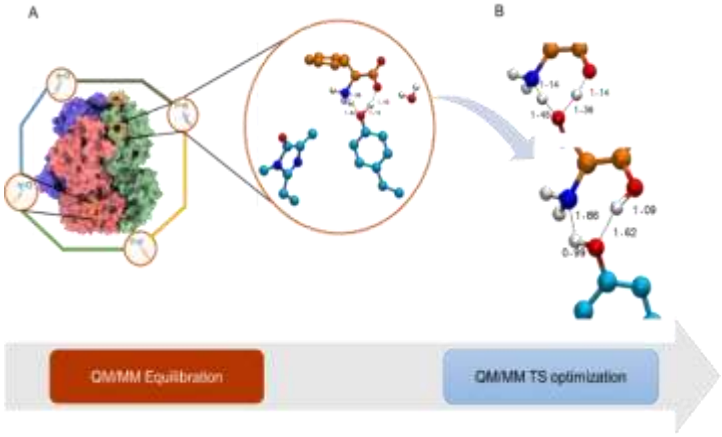

Figure 1. QM/MM implemented in this study (A) 1 µs MCV equilibrated E-S conformation was extended with QMMM-PM7 simulation using NAMD 2.13 to derive the attack conformation. This reduced the distance between Y-OH and NH3-Sub from ~3.5 Å to 1.5 Å in all the 4 chains. (B) Post this, TS level TS optimizations were adapted to obtain transition state and the final state

References:

1. In-Depth Sequence–Function Characterization Reveals Multiple Pathways to Enhance Enzymatic Activity Vikas D. Trivedi, Todd C. Chappell, Naveen B. Krishna, Anuj Shetty, Gladstone G. Sigamani, Karishma Mohan, Athreya Ramesh, Pravin Kumar R, and Nikhil U. Nair. ACS Catalysis 2022 12 (4), 2381-2396, DOI: 10.1021/acscatal.1c05508