

IN SILICO SCREENING OF TRANSAMINASE USING SEMI-EMPIRICAL QM/MM APPROACH

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Transaminases (TAs) are being widely used to produce chiral amines, and the demand of engineered TA variants to meet specific production processes is increasing. However, the engineering of TA by directed evolution methodology is still heavily relying on wet lab screening, which is often costly and time consuming. In this study we developed a reliable high throughput *in silico* (pre-)screening method for TA engineering. We established an *in silico* model of the catalytic reactions of wild-type (WT) TA from *Aspergillus fumigatus* Af293 (AfTA), identified the rate limiting step of TA reaction, and performed *in silico* screening of a large number of AfTA variants using a new framework for computer-aided directed evolution of enzymes (CADEE)¹ with semi-empirical QM/MM approach in combination with DFT studies at B3LYP/6-31G-level for calibration. Firstly, we used quantum mechanical/molecular mechanical (QM/MM) calculations to investigate the detailed catalytic reaction mechanism and the rate limiting step of PLP-dependent transaminase reaction for several chiral amines such as (R)- α -phenylethylamine (R-PEA) and (R)-1-naphthylethylamine (R-NEA). By calculating the proton at different positions on the amines (shown in figure 1 inset), we proposed a mechanism that differs from the one reported for a different TA in literature².

After validation of the energy profile of the reaction (shown in Figure 1) with WT AfTA, we performed *in silico* screening of AfTA variants with CADEE calculations and were able to identify activity-enhancing mutations in the active-site for R-PEA vs. R-NEA in good agreement with the experimental results of AfTA variants. In conclusion, our study demonstrated that CADEE with semi-empirical QM/MM approach can be used as a fast and accurate *in silico* (pre-)screening method for TA engineering.

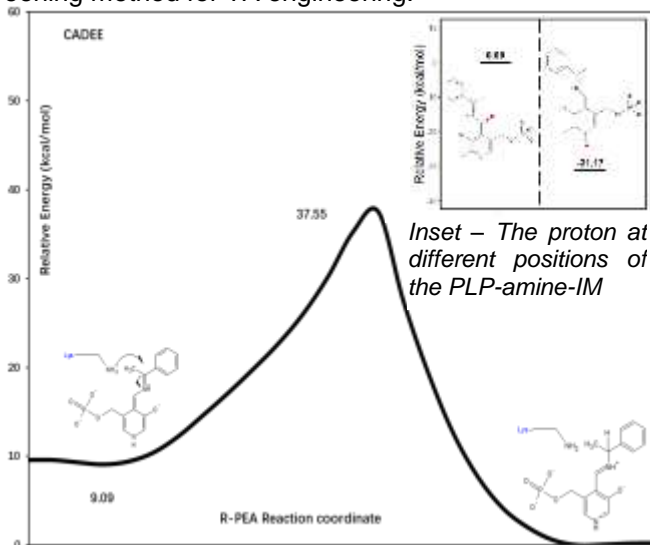


Figure 1 – Rate limiting step of transamination reaction for R-PEA.

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