EXPLORING TRANSAMINASE STABILITY FOR BIOCATALYSIS

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In recent years, sustainable development has driven the evolution of biocatalysis as a green technology for the efficient synthesis of different amine-containing compounds. Amine transaminases (ATAs), the pyridoxal 5'-phosphate (PLP) dependent enzymes, which under mild conditions mediate the highly stereoselective transfer of an amino group from an amino donor to a carbonyl group, have attracted tremendous attention as a powerful biocatalyst for production of enantioenriched chiral amines [1]. We are developing enzymatic cascades in our lab involving ATAs to transform renewable compounds, such as substituted furan derivatives, to refined products. A high operational stability and performance of the enzymes in such applications are crucial for product formation and therefore understanding and improving enzyme stability is the key to success. To this end, we have characterized several (S)-selective ATAs with respect to their thermodynamic and operational stability.

We have identified four different ATAs that can catalyze the reductive amination of 5-(hydroxymethyl)furfural and 2,5-diformylfuran. These ATAs were further immobilized using glutaraldehyde-functionalized amine beads and site-selective binding and then applied in continuous flow for the amination of HMF. ATA from *Silicibacter pomeroyi* achieved high conversion rates after 12 days with alanine and isopropylamine.[2]

Our parallel strategies for exploring ATA stability include both the role and stability of the cofactor [3] and the thermodynamic and kinetic stability of the protein [4]. In the talk, I will discuss the impact on stability explored by (i) light-induced deactivation of the cofactor, (ii) consensus mutations, (iii) ancestral sequence reconstruction, (iv) B-factor guided proline engineering, and (v) immobilization. We achieved some stability improvements through enzyme engineering based on the previously discovered inactivation mechanism of (*S*)-selective ATAs [5].

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