

EXPANDING SUBSTRATE SCOPE AND ALTERING STEREOPREFERENCE OF ENZYMES THROUGH ADVANCED PROTEIN ENGINEERING

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Key Words: Baeyer-Villiger Monooxygenases, Biocatalysis, Transaminases, Protein Engineering

This lecture will highlight principle strategies and current challenges in enzyme discovery and protein engineering [1]. These will be exemplified for amine transaminases (ATA) and Baeyer-Villiger monooxygenases (BVMO). We took advantage of the vast number of protein sequences available from databases to facilitate the discovery of novel enzymes and guide the design of 'small, but smart' mutant libraries. For the synthesis of chiral amines, we performed an in silico analysis and identified a toolbox of novel (R)-selective ATAs [2] as well as (S)-selective enzymes from a structure-guided search [3]. We also performed an in-depth bioinformatic analysis of a PLP-dependent superfamily resulting in the identification of distinct motifs for 21 types of PLP-dependent enzymes [4]. More recently, we could engineer (S)-selective ATA for the acceptance of bulky ketones in the asymmetric synthesis of chiral amines [5, 6].

For BVMOs, we could recently engineer these enzymes to efficiently accept the cofactor NADH instead of NADPH [7]. Furthermore, we demonstrated how we could invert their stereopreference [8, unpublished].

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