SWITCHING THE COFACTOR SPECIFICITY OF AN IMINE REDUCTASE

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Chiral amines have proven to be powerful building blocks for defining new pharmaceutical and agrochemicals due to their high density of structural information. In this light, the reduction of prochiral C=N double bonds is a well-established route in synthetic chemistry due to the easy accessibility of imines from their ketone precursors with the asymmetric addition of hydrogen or a hydride as the key stereo-differentiating step. Recently, we have witnessed remarkable advances in the enzyme-catalyzed asymmetric reduction of imines by NADPH-dependent imine reductases (IREDs).[1,2] Imine reductases were presented that catalyze the asymmetric reduction of various imines and the chemo- and stereoselective reductive amination as a useful method for the preparation of amines derived from aldehydes and ketones.[3,4]

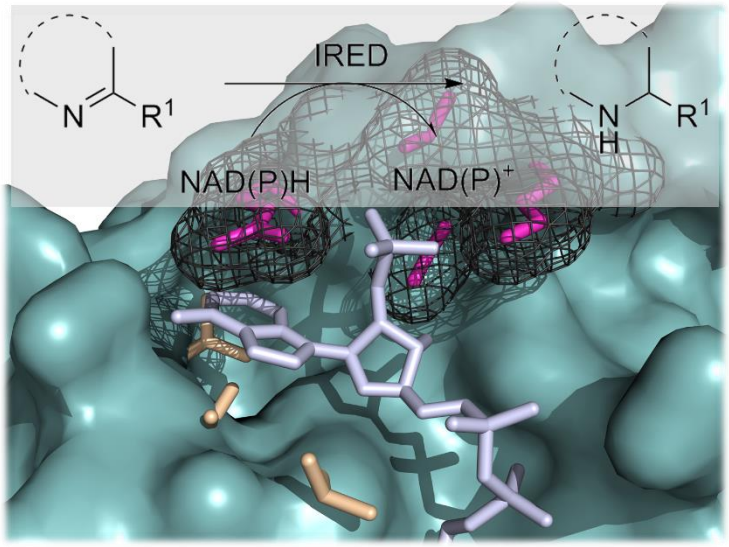

Figure 1 – Switching the NADPH cofactor specificity of an imine reductase

Most of reported cases involving the relaxing of switching of cofactor specificity have utilized sequence alignments and structural information, typically from either X-ray crystallography or homology modeling, in identifying amino acid residues likely to affect the specificity. The utilization of the 'Cofactor Specificity Reversal–Structural Analysis and Library Design' (CSR-SALAD)[5] tool and further structure analysis enabled us to substantially alter the cofactor specificity of the imine reductase from *Myxococcus stipitatus* from NADPH to NADH. A 96-well plate screen was developed and variants were screened based on the spectral signature of reduced nicotinamide. Replacement of four residues in the nicotinamide cofactor phosphate-binding site in combination resulted in imine reductase variants with reversed cofactor specificity. Furthermore, we have constructed imine reductase variants that recovered the catalytic efficiency. The kinetic parameters of WT and best variants were determined to further validate the influence of mutations.

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