Directed Evolution of Artificial Metalloenzymes: Genetic optimization of the catalytic activity

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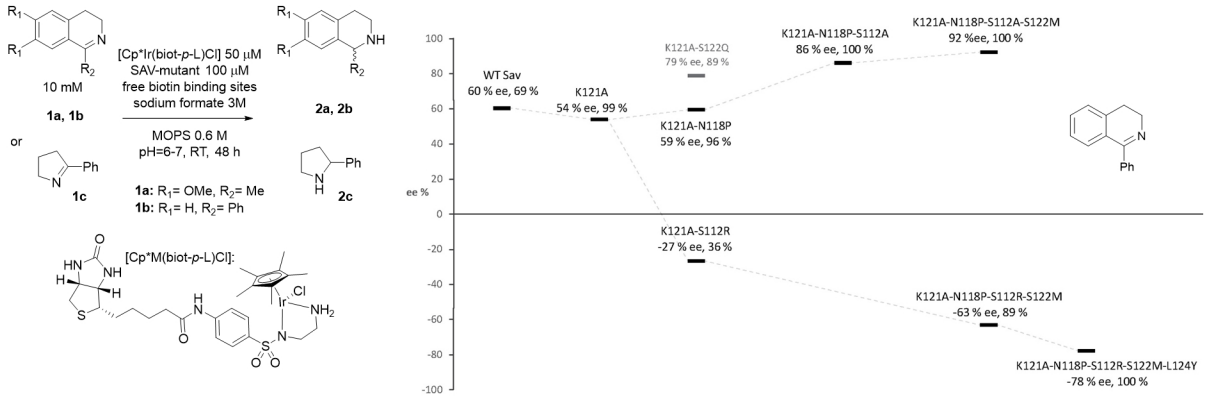
Artificial metalloenzymes (ArMs) are hybrid catalysts created by a non-covalent incorporation of an organometallic cofactor within a host protein scaffold.[1,2] This system, based on biotin-streptavidin technology, combines attractive features both of enzymatic and organometallic catalysis under near physiological conditions.[3,4] With the aim of performing catalysis *in vivo*, we have selected the transfer hydrogenation of cyclic imines as a model reaction.Herein we demonstrate the potential of directed evolution of artificial transfer hydrogenases (ATHase). Building upon a streamlined protocol[5], Sav mutants contained in *E. coli* cell free extracts were treated with diamide[6] and screened in the presence of the iridium cofactor. After identification of a successful "hit", the results were reproduced; the corresponding mutant was overexpressed, purified using an iminobiotin column and confirmed by screening.



This simplified process significantly speeds up the screening protocol and allows for the identification of improved Sav mutants for ATHase of cyclic imines. Guided by the protein crystal structure, we have performed four rounds of mutation and selection. As a result, two Sav isoforms with improved activities and yielding opposite enantiomers in the reduction of 1-phenyl-3,4-dihydroisoquinoline were obtained. X-ray analysis confirmed the presence of introduced mutations, namely K121A-N118P-S112A-S122M (96 % ee, full conversion) and K121A-N118P-S112R-S122M-L124Y (-82 % ee, full conversion). These mutants also show improved reaction rates in comparison with the use of Sav wild type.

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