Stereoselective Inverse Electron Demand Hetero-Diels-Alder Reactions Catalyzed by Non-Heme Metalloenzymes

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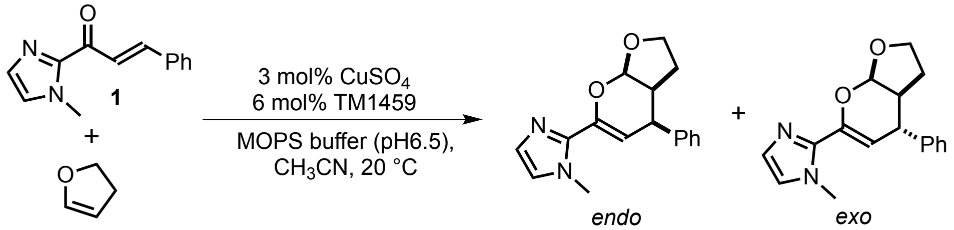
Recently, a variety of approach toward developing artificial metalloenzymes (ArMs) have emerged all over the world. ArMs are defined as highly regio- and/or enantioselective catalysts consisting of a protein matrix and a synthetic metal complex. Therefore, ArMs can harness excellent reactivity derived from the metal complexes as well as enzymatic ability such as exquisite chemical micro-environment to accelerate even difficult and desirable chemical reactions.[1]

Our group developed artificial metalloenzymes by using a cupin-type protein (TM1459) obtained from hyperthermophile, *Thermotoga Maritima*, where well-defined amino acid residues are disposed around the metal center. This metal binding site consists of 4-histidine residues in a same geometry to that of the tris(2-pyridylmethyl)amine (TPA) ligand system. By using this protein as a metal-ligands, we have recently developed the artificial non-heme metalloenzyme with high stereoselectivity by mutating 4-histidine tetrad at the metal binding site for some asymmetric reactions.[2]

In this study, we screened thus obtained mini-library of mutants for the inverse electron-demand hetero Diels-Alder reaction. As a result, Cu-H52A mutant with 3-his triad, showed *endo*-selectivity, but low enantioselectivity and yield as well. Therefore, the pose of substrate docked into cavity by *in silico* simulation suggested that there are some steric repulsion between the substrate and surrounded amino acids. Finally based on this notion, we constructed the Cu-H52G/I108D mutant which showed enhanced selectivity (94 % ee) and yield (92 %). In addition, further substrate scope was investigated and the series of substrate also showed good stereoselectivity.



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