# Evolutionary Engineering of a Cp*Rh(III)-linked Artificial Metalloenzyme with a Chimeric β-Barrel Protein Scaffold for Isoquinoline Synthesis via C(sp$^2$)–H Bond Activation

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Artificial metalloenzymes consisting of a synthetic metal cofactor within a protein scaffold have emerged as a new type of catalyst which combines attractive features of transition metal catalysts and biocatalysts. Our group has previously reported a Cp*Rh(III)-linked artificial metalloenzyme (NB-Rh), in which a Cp*Rh(III) cofactor was covalently incorporated into the hydrophobic cavity of nitrobindin (NB).[1] NB-Rh and its engineered variants efficiently promoted cycloaddition of acetophenone oximes with alkynes to produce isoquinolines via C–H bond activation.[2] To further improve its catalytic activity, we here conducted an evolutionary engineering of the NB protein scaffold. With the aim of providing a custom-designed and confined active site for the artificial metalloenzyme, a helix-loop-helix (HLH) domain of fatty acid binding protein (FABP) were genetically recombined with the β-barrel structure of NB to generate a chimeric protein scaffold NB$^{HLH}$ (Figure 1a). After optimization of the amino acid sequence based on directed evolution methodology, a promising variant, NB$^{HLH1}$(A119/P149), with high stability was identified. Moreover, further directed evolution of NB$^{HLH1}$(A119/P149) with the Cp*Rh complex afforded an evolved artificial metalloenzyme with a 40-fold increase in the catalytic efficiency relative to original NB-Rh (Figure 1b). Herein, we will present the construction of the chimeric protein scaffold as a host for the Cp*Rh(III)-linked artificial metalloenzymes and its directed evolution for the cycloaddition reactions.

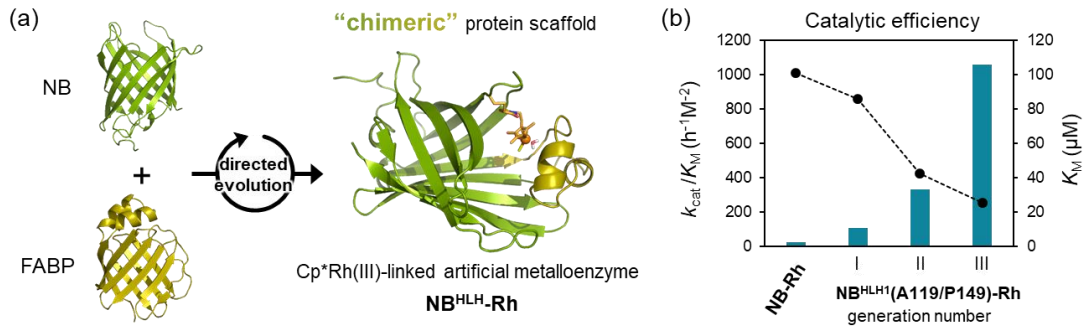


**Figure 1.** (a) Construction of a Cp*Rh(III)-linked artificial metalloenzyme with chimeric protein scaffold. (b) Michaelis-Menten parameters of the evolved NB$^{HLH}$-Rh variants for the cycloaddition reaction.

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