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Directed Evolution of De Novo Designed Artificial Metatheases

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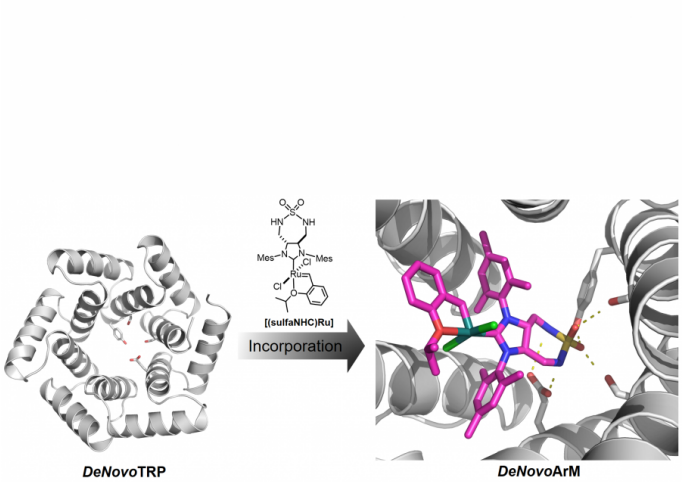
PURPOSE OF THE ABSTRACT
Artificial metalloenzymes are metalloproteins made in the laboratory which aim to catalyze highly selective and efficient abiotic chemical reactions. These biohybrid catalysts are often designed by tethering a catalytic metal cofactor to a preexisting (or repurposed) protein scaffold. Owning to the advances in computational biology, de novo protein design is emerging as a powerful methodology to generate proteins with user-defined functions. Developing artificial metalloenzymes using de novo designed protein scaffolds is of high interest. Here we report the designing and evolution of novel artificial metalloenzymes for highly efficient and selective olefin metathesis, one of the most widely used reactions in chemical synthesis, using modular and robust de novo designed proteins as scaffolds. Taking advantage of protein engineering methodologies, especially directed evolution campaigns, we optimized the supramolecular interactions toward the metal cofactor and improved the activity of the de novo designed artificial metathases (DeNovoArMs). We envision that this strategy will also evolve the regio-/enantio-selectivity of the DeNovoArMs in a wide range of olefin substrates.

FIGURES



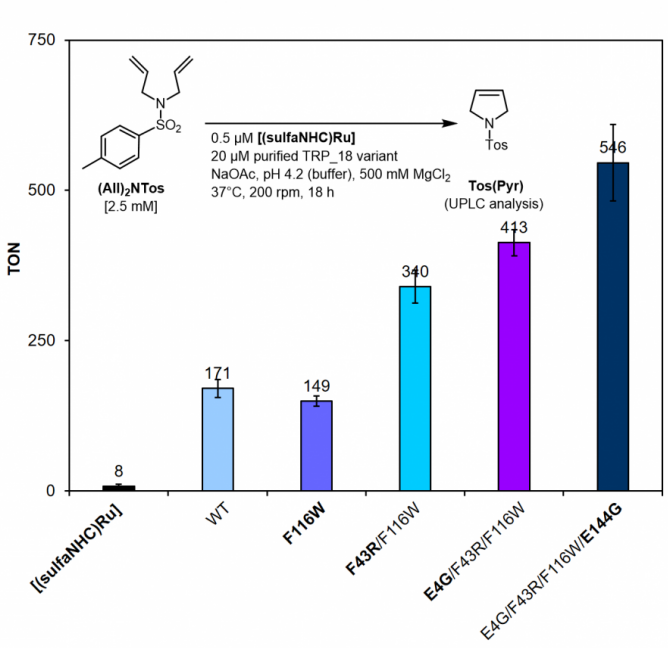


FIGURE 1

Figure 1

Assembling of the de novo designed artificial metathase (DeNovoArM) via supramolecular interactions of DeNovoTRP host (left, marked in grey) and [(sulfaNHC)Ru] cofactor (right, marked in magenta).

FIGURE 2

Figure 2

Directed evolution of the DeNovoArM using an (All)2NTos as the substrate. Screening of three iterative rounds of site-saturation mutagenesis libraries has generated a tetra-variant (E4G/F43R/F116W/E144G) which gains 3.7-fold in TON.

KEYWORDS

Artificial Metalloenzyme | De Novo Protein Design | Directed Evolution | Metathesis

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