Artificial Metalloenzymes: Towards Olefin Metathesis in Cells

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Enzymes are very attractive in the field of catalysis because of their exquisite performance. They are evolved through the process of natural evolution to have high reaction speed, turnover numbers, and selectivity, as well as mild reaction conditions in aqueous media. Unfortunately, their reactions scope is limited to those that have emerged in nature[1]. On the other hand, transition metal catalysts provide a broad range of chemical transformations, many of which have no precedent in nature, that offer a great toolbox for the synthetic chemist. However, these small-molecule catalysts are usually not compatible with biological systems; they are often toxic to the cells or are inactivated by cellular components. By combining these two different paradigms, Artificial Metalloenzymes are an attempt to make catalysts which catalyze new-to-nature reactions, and are genetically encoded and evolvable. Artificial Metalloenzymes are made by the incorporation of a small-molecule transition metal catalyst into a protein scaffold. There are different strategies to construct Artificial Metalloenzymes (Figure 1)[2].

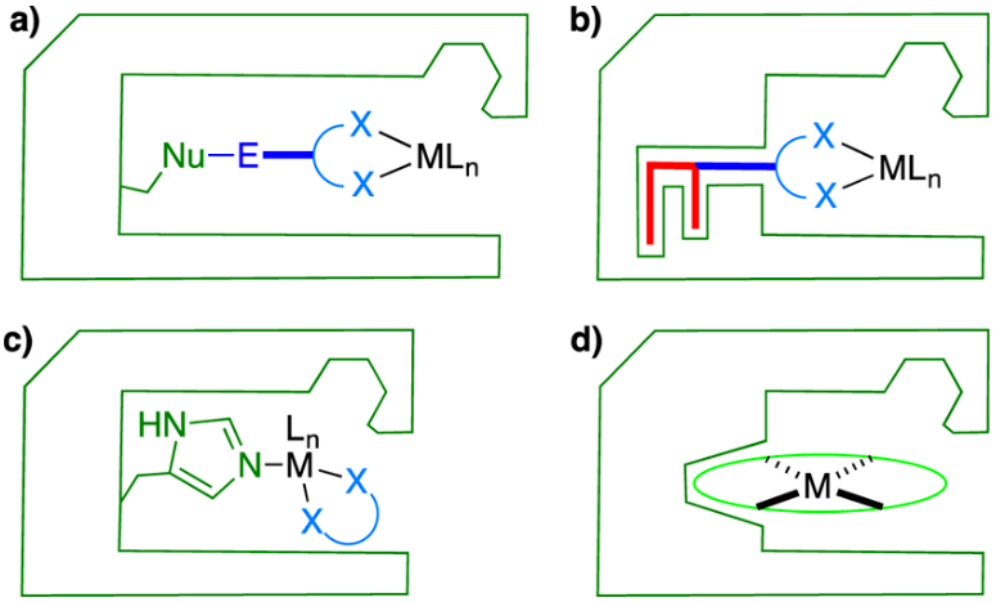

Figure 1. Different anchoring strategies to make Artificial Metalloenzymes: (a) covalent, (b) supra- molecular, (c) dative, and (d) metal substitution.

To this day, Artificial Metalloenzymes can catalyze a wide variety of natural and abiotic reactions. Reactions such as sulfoxidation, alcohol oxidation, epoxidation, dihydroxylation, C-H activation, hydrolytic cleavage, and C=C hydration are among those that have natural counterparts[2]. Furthermore, Artificial Metalloenzymes can catalyze new-to-nature reactions including hydrogenation, hydroformylation, Suzuki and Heck reactions, click reaction, and olefin metathesis[2]. This presentation will focus on the development of Artificial Metalloenzymes capable of catalyzing olefin metathesis, or "*metathases*".

The first two reports of metathases appeared in the same issue of Chemical Communications in 2011[3,4]. Both papers used the same metallocofactor, the Grubbs-Hoveyda catalyst, but used different anchoring strategies. Hilvert and co-workers, generated their metathase via covalent modification of a small heat-shock protein[3]. Ward and co-workers, on the other hand utilized a supramolecular anchoring strategy based on (strept)avidin-biotin technology[4]. Both works functioned as proof-of-principle and did not show stellar catalytic performance. Since then, several works have attempted to improve the performance of artificial metathases by trying different anchoring chemistries and/or different protein scaffolds[5,6,7]. In addition, Okuda and Schwaneberg reported the first artificial metathase for ring-opening metathesis polymerization (ROMP)[8,9].

A major breakthrough in the field was achieved by the groups of Ward and Panke[10]. Based on the streptavidin-biotin couple, they successfully assembled an artificial metathase in the periplasm of *Escherichia coli* (Figure 2).

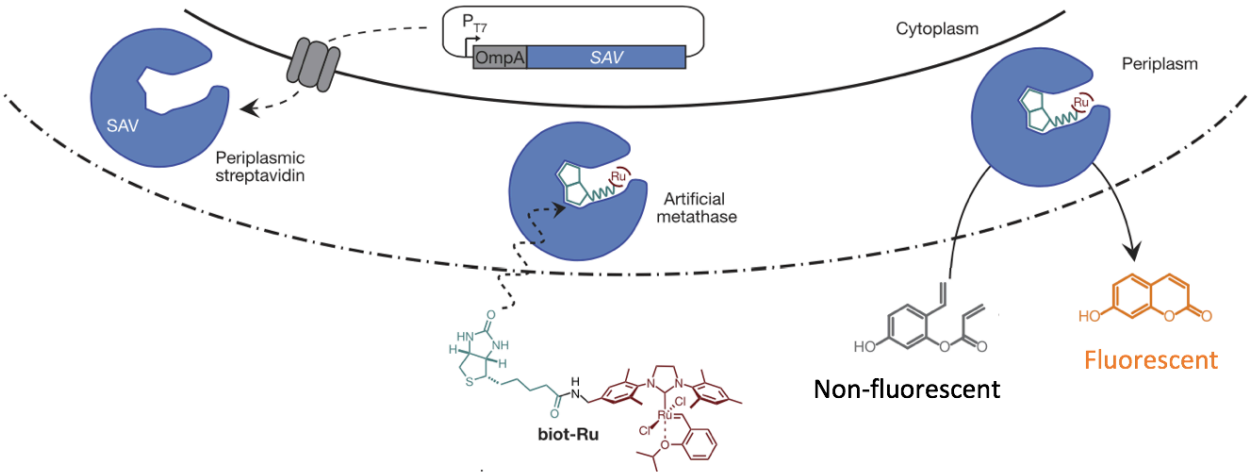

Figure 2. Construction of an Artificial Metalloenzyme for metathesis in the periplasm of *E. coli*.

Being able to conduct metathesis reactions *in vivo* let the authors to speed up genetic optimization of the artificial metathases through the process of directed evolution[11]. A library of random mutations was generated in 20 amino acid positions in the protein scaffold and over 3000 clones were screened. After five rounds of directed evolution, a quintuple mutant (47A-49K-114Q-119G-121R) was identified with a cell-specific activity 5.4 times higher than the wild-type metathase. The catalytic performance of the evolved enzyme was then tested *in vitro* on two water-soluble substrates. The enzyme showed significant improvement over the free catalyst as well as two benchmark, commercially available metathesis catalysts for the neutral substrate. For the positively charged substrate, however, the evolved enzyme showed decreased activity with respect to the wild-type metathase. Another round of directed evolution exchanged the positively charged arginine residue for the neutral leucine (R121L) and improved the catalytic performance for the positively charged substrate both *in vivo* and *in vitro*.

In conclusion, within the past five years, the efficiency of metathases has significantly improved. Additionally, all common applications of metathesis including ring-closing metathesis (RCM) and ring-opening metathesis polymerization (ROMP) have been achieved by Artificial Metalloenzymes. It has also been shown that such metathases, under specific conditions, can work inside cells. This opens a window of opportunity for further improving the efficiency and selectivity of such catalysts as well as future *in vivo* applications such as non-natural metabolic pathways.

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