ARTIFICIAL METALLOENZYMES FOR SYNTHETIC BIOLOGY APPLICATIONS: CHALLENGES AND OPPORTUNITIES

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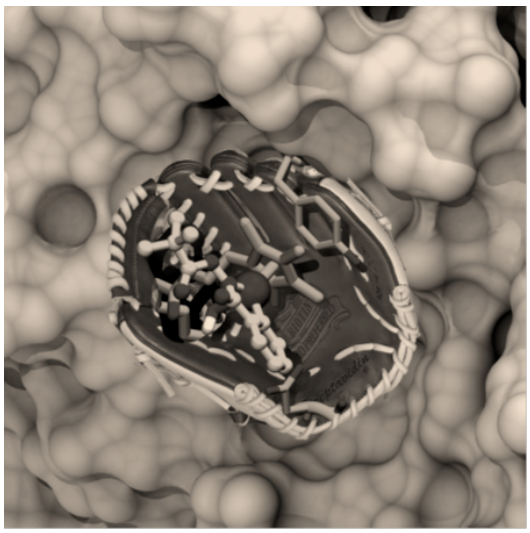
Artificial metalloenzymes result from incorporation of a catalytically competent organometallic moiety within a host protein. We and others have been exploiting the potential of the biotin-(strept)avidin technology for the creation of artificial metalloenzymes, Figure. Thanks to the remarkable supramolecular affinity of biotin for either avidin or streptavidin ($K_D > 10^{-13}$ M), linking of a biotin anchor to a catalyst precursor ensures that, upon stoichiometric addition of (strept)avidin, the metal moiety is quantitatively incorporated within the host protein.

Such artificial metalloenzymes are optimized either by chemical (variation of the biotin-spacer-ligand moiety) or genetic- (mutation of (strept)avidin) means. These chemogenetic schemes were applied to optimize the performance for eight different catalyzed transformations as well reaction cascades in the presence of natural enzymes. [1-4]

More recently, we have been investigating the potential of artificial metalloenzymes for *in vivo* catalysis to complement metabolic pathways. In this context, *E coli*'s periplasm has proven particularly versatile.



Reactions implemented thus far:
Hydrogenation (up to 96 % ee)
Transfer Hydrogenation of
- ketones (up to 98 % ee)
- imines (up to 96 % ee)
- enones (up to 1000 TONs)

Allylic Alkylation (up to 95% ee)
C-H Activation (up to 86 % ee)
Olefin Metathesis (up to 140 TONs)
Alcohol Oxidation (up to 250 TONs)
Sulfoxidation (up to 93 % ee)
Dihydroxylation (up to 98 % ee)
NAD^+ regeneration (up to 20'000 TONs)

Figure Artificial metalloenzymes obtained upon supramolecular incorporation of a biotinylated organometallic catalyst within streptavidin

[1] Ward, T. R. Acc Chem Res 44, 47 (2011).
[2] Köhler, V. et al. Nature Chem 5, 93 (2013).
[3] Hyster, T. K., Knorr, L., Ward, T. R. & Rovis, T. Science 338, 500 (2012).
[4] Wilson, Y. et al. J. Am. Chem. Soc.,136, 8928 (2014).