Expanding Nature's Catalytic Repertoire – Directed Evolution of Artificial Metathases

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Artificial metalloenzymes offer a promising means to expand the natural enzymatic tool box with novel reaction mechanisms and combine attractive features of organometallic catalysis with the superior properties of enzymes. Relying on the streptavidin-biotin interaction we have recently developed a screening platform for the directed evolution of these hybrid biocatalysts in the periplasm of *Escherichia coli*[1, 2].
This periplasmic compartmentalization strategy allowed us to assemble a functional, ruthenium-based enzyme for olefin metathesis (i.e. a metathase) in the bacterial cell envelope endowing the corresponding strain with the capability to perform an archetypal transition metal reaction with no known equivalent in Nature[1]. Using a fluorescent surrogate reaction we were able to evolve the artificial metathase yielding a set of mutants with significantly improved catalytic behaviour both *in vivo* and *in vitro*, which may be traced back to an increased active site flexibility in the evolved variant.
The novel metathase shows activity for other commonly used metathesis substrates and compares favourably with commercial catalyst for metathesis in water. Moreover, we were able to use the periplasmic screening strategy to further improve activity for an alternative, non-fluorescent substrate indicating that the introduced technology is broadly applicable and can be used to evolve the novel biocatalysts into different directions.
We believe that our proposed system represents a promising strategy to generate new-to-nature enzymatic activities for various potential applications in green chemistry and synthetic biology.

[1] M. Jeschek, R. Reuter, T. Heinisch, C. Trindler, J. Klehr, S. Panke, T.R. Ward, *Nature*, **2016**, in press.
[2] M. Jeschek, S. Panke, T.R. Ward, *Methods in Enzymology*, **2016**, in press.