In vivo metathesis of artificial metalloenzymes: progress towards non-natural metabolism

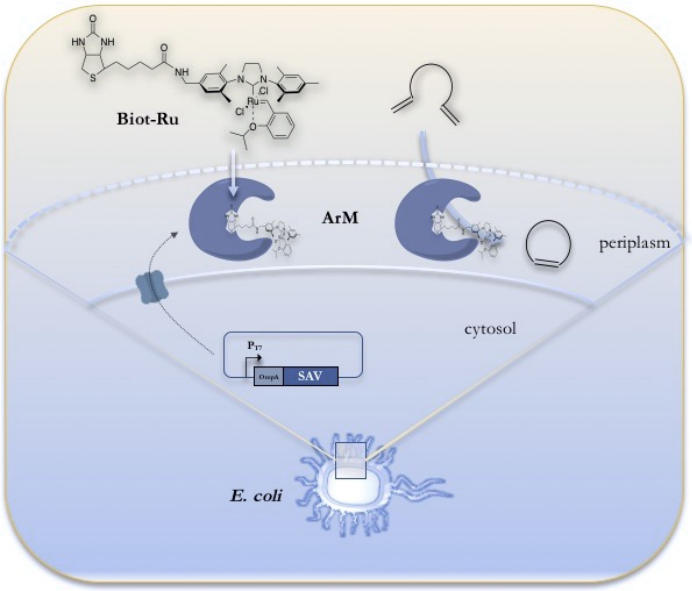
V. Sabatino[1], T. R. Ward[1]*

[1]University of Basel, Switzerland

Biocatalysis is a continuously evolving field with applications in the pharmaceutical and agrochemical industries. Genetic engineering tools allow to enhance biological systems by engineering new-to-nature reactions. To perform such reactions, artificial metalloenzymes (ArM) represent a versatile tool resulting from the incorporation of organometallic moieties within a protein scaffold. Artificial metalloenzymes based on the biotin-streptavidin technology have been shown to enable bioorthogonal reactions and their chemogenetic optimisation.

A recent report in our group led to the development of a ruthenium-based artificial metalloenzyme evolved by directed evolution that catalyzes ring closing metathesis in the periplasm of *Escherichia Coli* [1]. This suggests the possibility to perform *in vivo* non-natural reactions, paving the way for innovative applications in metabolic engineering.

Herein, we describe a "metathase-dependent" system based on a biotinylated Hoveyda-Grubbs catalyst, aiming to introduce non-natural substrates which can be readily converted in metabolic precursors upon ring closing metathesis. Metathesis reactions have no equivalent in nature, therefore allowing to bypass biochemical pathways and allow cell survival in engineered *E. coli* cells lacking specific metabolic routes.



[1] M. Jeschek, et al. *Nature,* **2016**, 537, 661-665