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Generating evolvable, artificial metalloenzymes containing organometallic cofactors via ligand exchange

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PURPOSE OF THE ABSTRACT

In this work, ruthenium cofactors were introduced into protein scaffolds by ligand exchange between ruthenium compounds and peptidic, Lewis basic entities (Figure 1). This method ensures an intimate link between the first and second coordination-sphere, with the latter being dictated by the protein fold. By systematically adapting the electronic and steric properties of the ligands on the original ruthenium complex, the exchange process was optimised to give good yields of the desired mononuclear species rapidly using only small excess of ruthenium. The resulting modified protein was shown to be catalytically active, effectively reducing a pre-fluorescent quinolonium substrate in aqueous solution via transfer-hydrogenation from formate. [1] With a new ArM at hand, further work was started to evolve it using a high-throughput microfluidic approach. Multiple pre-fluorescent substrates were developed or adapted for use in droplets, allowing for screening of different new-to nature reactions. We further present various transition metal complexes that undergo ligand exchange in the presence of protein to yield ArMs bearing different organometallic cofactors, including iridium and ruthenium-NHC based moieties. Initial findings have shown distinct catalytic activities depending on the cofactor prior to any directed evolution, thus highlighting the potential of a combinatorial approach where informed choice of transition metal complex can install a biorthogonal reactivity into a protein that subsequently could be evolved to yield highly functional catalysts.

FIGURES

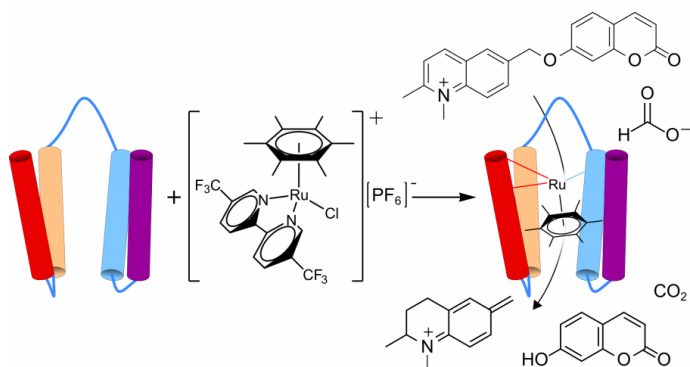


FIGURE 1

Overview

Overview of ArM formation and activity, taken from [1].

FIGURE 2

KEYWORDS

Artificial metalloenzymes | Transition metal catalysis

BIBLIOGRAPHY

[1] G. S. Biggs*, O. J. Klein*, S. L. Maslen, J. M. Skehel, T. J. Rutherford, S. M. V. Freund, F. Hollfelder, S. R. Boss, P. D. Barker, *Angew. Chem. Int. Ed.* 2021, 60, 10919.