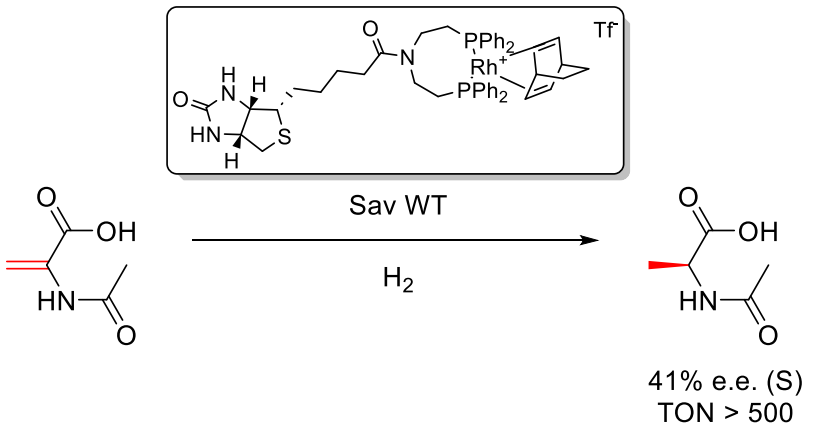
C—H Bond Activation with Artificial Metalloenzymes

David Freytag Literature Seminar 11/28/23

The functionalization of the C—H bond is of great import due to the ubiquity of this bond in organic chemistry and the prospect of remediation of pollutants like methane to potential fuels like methanol. C—H activation is also attractive in that it allows one to bypass traditional functional group interconversion schema in favor of a more direct synthetic logic, enabling more environmental and cost-effective methodologies.[1] One promising approach for the development of facile and thermodynamically feasible C—H functionalization methods involves the use of artificial metalloenzymes (ArMs).

ArMs are metalloproteins that are prepared in a laboratory, instead of occurring naturally. These types of enzymes occupy a unique niche in bioinorganic chemistry due to their biological characteristics, yet ability to catalyze reactions never seen amongst the naturally occurring enzymatic repertoire, such as Suzuki coupling,[2] olefin metathesis,[3] and indeed C—H bond activation.[4] The first ever reported ArM was prepared by Wilson and Whitesides by situating a biotin-derived rhodium(I) cofactor within streptavidin via the well-known, strong complexation between the two, which was then used to perform enantioselective hydrogenation (**Scheme 1**).[5]



Scheme 1: ArM-catalyzed enantioselective homogenous hydrogenation of 2-acetamidoacrylic acid.

Since this seminal contribution, several other methodologies have been developed for the preparation of ArMs, including covalent immobilization, dative coordination with an unsaturated metal complex, and substitution of a metal in a naturally occurring cofactor (**Figure 1**).[6] Several recent approaches to the preparation of effective ArMs involving some of these types, in addition to a mechanistic study, will be the focus of this seminar.

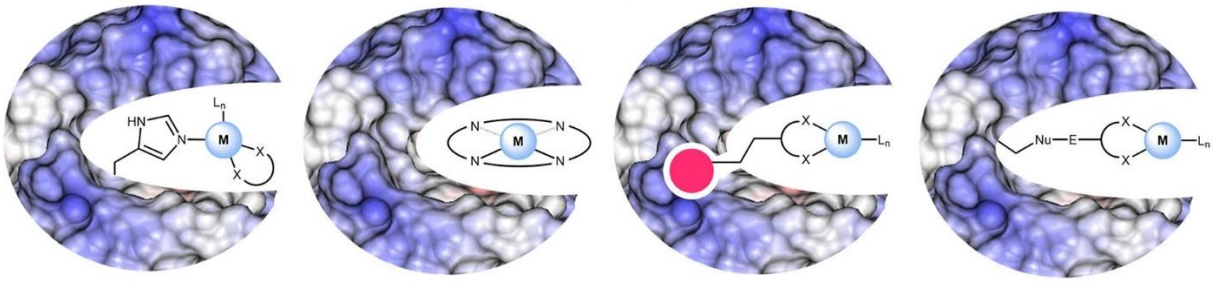
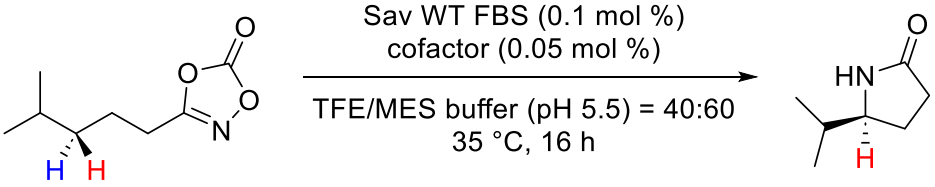


Figure 1: Dative coordination with an unsaturated metal complex, substitution of a natural cofactor, supramolecular coordination using a high-affinity anchor, and covalent immobilization as methods of ArM formation (shown left to right).

The Ward group recently reported a similar cofactor incorporation strategy as Whitesides demonstrated 45 years prior, utilizing an iridium Cp cofactor covalently linked to biotin to effect a tight linkage between the cofactor and streptavidin.[7] Chemical optimization of the cofactor was achieved by conjugating eight different Cp*Ir cofactors to wild-type streptavidin and testing the performance of the holoenzyme on an alkyl dioxazolone as a model substrate (**Scheme 2**). Genetic optimization was additionally carried out by performing site directed mutagenesis at the two closest lying residues to the cofactor, as determined from a crystal structure. The best mutant streptavidin in conjugation with the best Cp*Ir cofactor was able to catalyze a range of substrates with turnover numbers (TON) up to 768 (±25) and enantiomeric excesses of up to 92% (±1.4%). Quantum mechanical modelling additionally revealed a preferential *S* conformation of the pseudo-chiral center on iridium, which was hypothesized as due to sundry secondary sphere interactions that stabilized the transition state relative to other possible arrangements, leading to *S* enantioenriched γ-lactam.



Scheme 2: ArM catalyzed preparation of alkyl γ-lactam via alkyl dioxazolone model substrate.

A different strategy was employed by Hartwig and coworkers in 2022, where the native porphyrin IX cofactor of CYP199 was substituted for a methyl iridium variant.[8] A special mutant strain of *E. coli* (Nissile 1917) featuring a genetically encoded heme transporter was utilized to import the cofactor and assemble the CYP119 ArM *in vivo*, bypassing the need for *in vitro* reconstitution steps and supplying a pathway toward efficient directed evolution. This method

additionally avoids common pitfalls of *in vivo* assembly systems such as inhibition by cellular components, inefficient cofactor uptake, and specific assembly within the cytoplasm. Hartwig and coworkers demonstrated proper iridium uptake by Nissile 1917 via inductively coupled plasma-mass spectrometry (ICP-MS), followed by confirmation of catalytic activity via carbene insertion into phthalane. Evaluation of this reaction's site specificity was performed with a variety of 4-substituted phthalanes, resulting in different para/meta substitution ratios depending on the substrate and enzyme mutant. A directed evolution platform was developed wherein an NNK-generated combinatorial library targeting 10 residues around the active site was screened for catalysis of (+)-nootkatone cyclopropanation. Three rounds of mutation produced an enzyme yielding 75% diastereoselectivity and a 28% increase in reaction yield relative to the starting species.

While the above two studies successfully demonstrated preparation of effective ArMs in novel and useful ways, they did not investigate in any detail the mechanism of the associated catalytic cycles or probe the dynamics of enzyme-cofactor interactions. The Hartwig group reported just this in early 2023, with the study of an artificial cytochrome P450 containing an iridium porphyrin.[9] Following structural characterization via single-crystal X-ray diffraction, the kinetics of carvone cyclopropanation using ethyl diazoacetate (EDA) was probed, leading to discovery that the kinetics of the reaction suggested a ping-pong mechanism. The existence of an induction period for enzyme activation in concert with kinetic data further suggested this period was the result of reversible cofactor dissociation and reassociation in an upside-down geometry, allowing access of substrate reagents to the active site pocket (**Figure 2**). Finally, quantum mechanical computations and molecular modeling further confirmed the hypothesized mechanistic details, predicting the major cyclopropane diastereomer and supplying additional information on relevant structural features of active site residues.

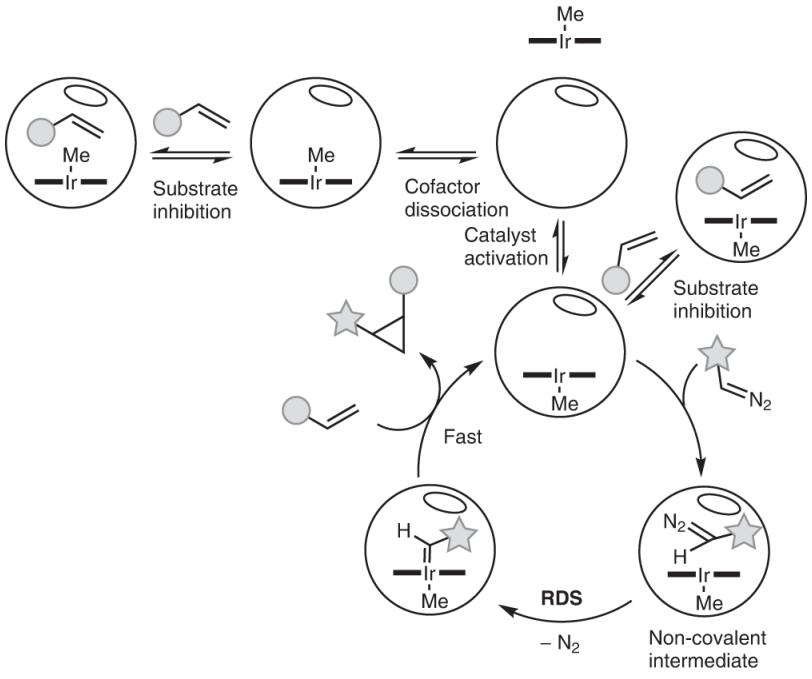


Figure 2: Proposed catalytic mechanism for CYP119 ArM featuring a methyliridium mesoporphyrin IX cofactor.

In summary, the studies discussed demonstrate the preparation of effective artificial metalloenzymes for C—H bond activation, utilizing diverse strategies such as streptavidin-biotin conjugation incorporation and *in vivo* assembly. While highlighting the advantages of ArMs as versatile tools for accessing new chemistry, the studies by Ward and Hartwig also emphasize the need for mechanistic exploration. The Hartwig group's mechanistic investigation into an artificial cytochrome P450 provides insightful details into the catalytic cycle, unveiling the dynamics of enzyme-cofactor interactions in the context of a model cyclopropanation reaction. These findings collectively advance the scientific understanding of ArMs, contributing to the development of sustainable and efficient methodologies in C—H bond activation.

References

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