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Recent Advances in the Field of Artificial Metalloenzymes: New Biocatalysts for selective oxidations and Diels-Alder Reaction

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Submitted

Abstract

Our recent research is turning towards the elaboration of artificial metalloenzymes that catalyze reactions of interest for organic chemistry under eco-compatible conditions.

First, totally artificial metalloenzymes that catalyze selective oxidations in water are described following three main lines: (i) Insertion of microperoxidase 8 into Metal Organic Frameworks leading to new artificial metalloenzymes that catalyze the selective oxidation of dyes and sulfides by H₂O₂; (ii) Design of a new polyimine polymer-based artificial reductase allowing the reductive activation of dioxygen and its use as an oxygen atom source for selective oxidations catalyzed by metal complexes including metalloporphyrins, copper complexes or Polyoxometallates and, (iii) Design of new artificial metalloenzymes that catalyze in the presence of photoactivable ruthenium complexes the photoreduction of H₂O and the concomitant oxidation of sulfides.

Second, the synthesis of new artificial metalloenzymes that catalyze the stereoselective Diels-Alder reaction is described following three strategies: (i) Covalent insertion of metal complexes into a new family of thermostable artificial proteins based on alpha-helical repeated motifs (α Reps), (ii) Substitution of the native Fe ion of a cupin-like protein, ACCO oxidase, by a copper(II) ion and (iii) Insertion of a copper(II) complex-antagonist conjugate into an adenosine receptor at the surface of living HEK cells.

Keywords: neocarzinostatin scaffold, artificial metalloenzymes, biocatalysis, copper complexes, diels-alder cyclization reaction molecular modeling

1. INTRODUCTION

Considering current economic and ecological contexts, the questions of setting up clean and eco-efficient processes as well as saving our energy resources appear to be of fundamental importance. We need to develop reactions that would address the problem of the selective transformation of chemicals under mild conditions. This urges industries to develop “green chemistry” procedures that have not only to include catalytic processes to limit waste, but also to use harmless solvents, the ideal of which would be water, with low temperatures and pressures to limit energy consumption. Enzymes, and in particular metalloenzymes, are biocatalysts that already fulfill such conditions. Because their properties are complementary to those of chemical catalysts, it is conceivable to design catalysts that would combine the robustness and wide range of reactions of chemical catalysts with the ability of enzymes to work under mild conditions in aqueous medium and with high selectivity. This can be realized by associating a metal complex with a protein, to afford a new artificial metalloenzyme or “Artzyme” that would be able to catalyze selective reactions under eco-compatible conditions.^[1] In such hybrid biocatalysts, the metal complex is responsible

for the catalytic activity and the protein by its chiral environment around the substrate induces the selectivity and also protects the metal cofactor from degradation.

In previous work, that has been extensively reviewed,^[2-4] A first generation of artificial metalloenzyme was prepared by inserting metal (Fe, Mn, Cu, Zn) complexes of various ligands including water soluble tetra-aryl-porphyrins, phenanthroline, terpyridine or tris-pyridylamine, into various proteins such as monoclonal antibodies (generation of catalytic antibodies or AbZyMes), Xylanase A, Beta-lacto-globulin or Neocarzinostatin, either non-covalently (Trojan-Horse and Host-Guest strategies) or by covalent attachment through a linker arm. The obtained hybrid biocatalysts were found to be able to stereo-selectively catalyze reactions such as the oxidation of organic compounds such as sulfides and alkenes by H₂O₂,^[2,4] the RNase like hydrolysis of oligonucleotides,^[5] the C-C bond creation through the stereoselective Diels-Alder reaction.^[6]

The present paper proposes a review of the most recent developments of our research, that are turning towards the elaboration of artificial metalloenzymes that would be able to catalyze under eco-compatible conditions reactions of interest for organic chemistry, following two main axes that are, first,

the elaboration of totally artificial metalloenzymes that would catalyze selective oxidation reactions in water and, second, the synthesis of new artificial metalloenzymes that would be able to catalyze the stereoselective Diels-Alder reaction. In the later case, the use of such biocatalysts *in vivo* for theranostic purposes will be introduced

2. RESULTS AND DISCUSSION

2.1. Selective oxidations catalyzed by artificial metalloenzymes in water

The majority of industrial chemical processes, such as oxidations, are based on harsh conditions (high temperature, pressure, corrosive oxidants and toxic solvents), leading to a harmful impact on the environment.^[7] For example, for the large scale conversion of alkanes into more valuable oxidized products such as alcohols, the major currently used pathways involve stoichiometric oxidations and severe conditions (high temperature and pressure, strong oxidants such as $S_2O_8^{2-}$, or harmful metal oxides such as permanganate, solid Molybdenum oxide, high-valent Ruthenium oxides).^[8,9] In general, the processes are long and ecologically hostile, often exhibit a low selectivity due to the harsh conditions and are only economically viable for products with a high value-added. Then, developing chemical reactions that would address the problem of transformation of hydrocarbon under mild conditions must be considered.

Nature has already solved the problem, as it uses as catalysts metal containing proteins that are able to perform oxidation reactions at room temperature, under atmospheric pressure, even the hydroxylation of hydrocarbons, in spite of the relative inertness of the C-H bond in non-activated substrates. These enzymes are known as monooxygenases and include non-heme iron enzymes such as, for example, Methane MonoOxygenase (MMO) that performs the selective oxidation of methane into methanol,^[10] and heme enzymes such as cytochrome P450 dependent monooxygenases (Cyt. P450), that catalyze the selective oxidation of endogeneous or exogeneous organic molecules by dioxygen (Fig. 1).^[11] Both kinds of enzymes thus carry out the desired oxidation reactions under conditions that fit to the current concept of “green chemistry”,^[12] as they use as oxidant, the cheapest, cleanest and naturally occurring reagent that is molecular dioxygen and

perform the catalysis at an iron center, an ecologically friendly metal, under mild conditions.

As a consequence, it was obvious that the question of clean oxidation could be solved by elaborating biomimetic systems, that would be able to perform efficiently, under mild conditions, regio- and stereoselective oxidation reactions. For more than 40 years, many teams in the world have addressed this issue by elaborating catalytic systems using complexes of synthetic heme,^[13-15] and non heme^[16] ligands with ions such as Fe(III), Mn(III), Cr(II), Ru(II) and Co(I) ions, that were found able to catalyze the oxidation of various organic compounds such as alkenes and alkanes by various oxidants such as iodosylbenzene ($PhI=O$), hydrogen peroxide (H_2O_2), alkylhydroperoxides (ROOH), oxone[®] ($KHSO_5$), sodium hypochlorite (NaOCl), etc., and, to a lesser extent, O_2 in the presence of a co-reductant.^[13-16]

With the progress in the determination of the structure and mechanism of enzymes on the one hand, and on the chemical and biochemical protein engineering on the other hand, a new strategy has emerged in the last 20 years, that rely on the design of artificial metalloproteins that are able to perform such oxidation reactions and use when possible dioxygen as oxidant. Several techniques have been used for this including the introduction of metal binding sites in the active site of a protein by site-directed mutagenesis, the design of substrate binding cavities, the chemical modification of prosthetic groups and the covalent attachment of metal cofactors.^[1-3]

In the particular case of the elaboration of artificial hemoproteins to mimic cytochrome P450s, two main questions arise. The first one is, with regard to the structure-activity relationships, which structural elements are indispensable in the biomimetic system? The second one is: which oxidant should we choose, dioxygen, like in natural systems, or other oxidants such as iodosylbenzene, organic or inorganic peroxides? To answer those questions let's have a look at structure-activity relationships in cytochromes P450 dependent monooxygenases. Cytochromes P-450 enzymes catalyze the hydroxylation of substrates according to the equation in figure 1.^[17] Their active site is a wide hydrophobic pocket that contains an heme b or iron(III)-protoporphyrin IX, as a prosthetic group, that is bound to the apoprotein through a cysteinate axial ligand of the iron,^[18,19] and a binding site for the substrate (Fig. 1).

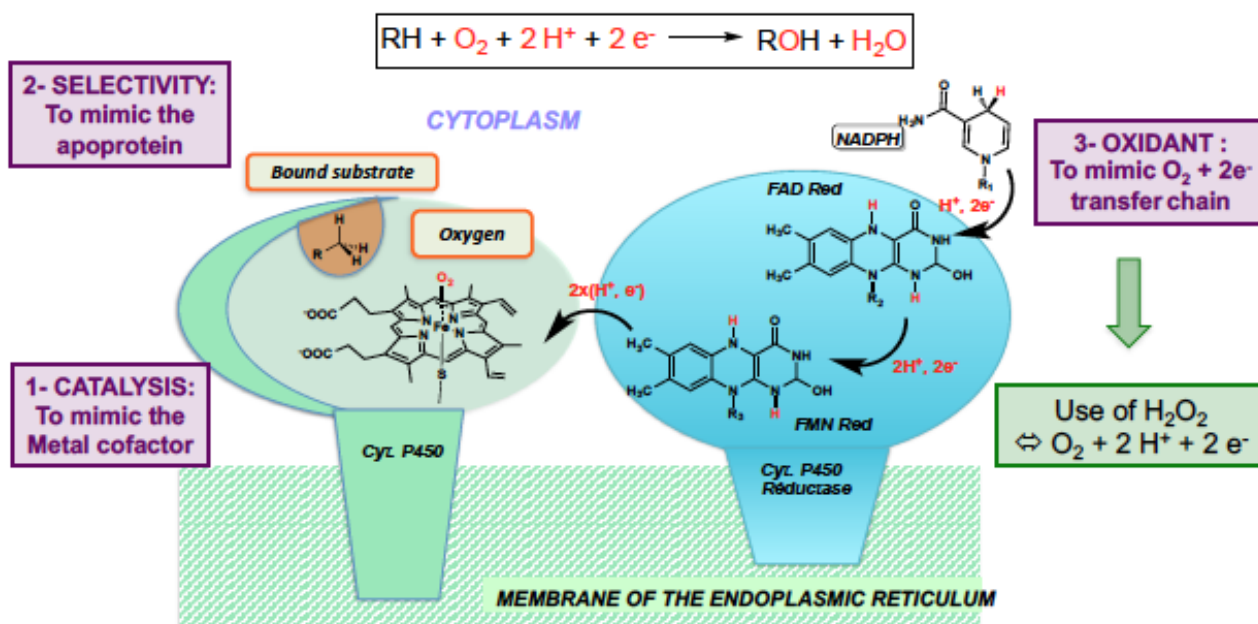


Figure 1: Reductive activation of dioxygen by the cytochrome P450-reductase complex for the oxidation of organic compounds by cyt. P450 monooxygenases and main requirements for the design of biomimetic systems.

As a consequence, at least 3 structural elements have to be mimicked to elaborate the ideal functional biomimetic system for Cyt. P450 dependent monooxygenases: (i) The heme prosthetic group, which is responsible for the oxene-transfer reactions,^[18,19] (ii) The apoprotein which binds and site-isolates heme, preventing its aggregation and its oxidative degradation, and provides an hydrophobic environment for the substrate and control its access to the heme inducing the stereoselectivity of the reaction, and (iii) The proximal axial ligand of the iron atom, a cysteinate, which has a role in controlling the heme redox potential and the reactivity of the heme iron.^[22]

Concerning the oxidant, as shown in figure 1, in the reactions catalyzed by Cyt. P450s, dioxygen is activated in the presence of 2 protons and 2 electrons that are provided by a cellular reductant NADPH, one oxygen atom being then reduced to water and the other being incorporated into the substrate. The later reaction occurs through the formation in the catalytic cycle of a high-valent iron-oxo P450 $\text{Fe}^{\text{v}}=\text{O}$ complex that is the “active oxygen species” of Cyt. P450s that is responsible for the insertion of one oxygen atom into the substrate.^[18,19] However, activation of dioxygen is a complicated process that is difficult to mimic, in which the electrons necessary for the reduction of dioxygen are not directly transferred from NADPH to the iron center, but they are delivered one by one by the cytochrome P450-reductase, a huge protein that contains two

flavin cofactors, FAD and FMN. Fortunately, it has been *in vitro*, that the high-valent iron-oxo species could directly be obtained, by the so-called “peroxide shunt”, from the reaction of iron(III)-P450 with various oxygen donating agents such as iodosylbenzene ($\text{PhI}=\text{O}$), hydrogen peroxide (H_2O_2), alkylhydroperoxides (ROOH) or inorganic oxidants such as sodium hypochlorite (NaOCl), oxone (KHSO_5).^[23] Among these, hydrogen peroxide was one of the most attractive one for 2 main reasons: first, it is an ecofriendly oxidant as it is water soluble and leads to water as a byproduct and, second, it is a perfect equivalent of dioxygen activated with 2 electrons and 2 protons (Figure 1).

Then, in the respect of the above described structural requirements, artificial hemoproteins were prepared by supramolecular insertion of synthetic water-soluble iron(III)- or manganese(III)-porphyrins into various proteins that protected the metalloporphyrin cofactor from degradative oxidations and induced some stereoselectivity in the catalyzed oxidations.^[2,3,24]

These artificial hemoproteins were obtained by several strategies including for example: (i) the non-covalent insertion of water-soluble metalloporphyrins into monoclonal anti-porphyrin antibodies (Hemoabzymes) (Fig. 2),^[25] (ii) the “Trojan Horse” strategy that involved the incorporation of steroid-metalloporphyrin conjugates into a neocarzinostatin variant,^[26] (iii) the “host-guest” strategy involved the non-covalent

incorporation of water-soluble anionic iron-porphyrins into xylanase A from *Streptomyces Lividans*.^[27] The artificial hemoproteins obtained were found able to perform efficiently the

stereoselective oxidation of organic compounds such as sulfides and alkenes by H_2O_2 and $KHSO_5$.

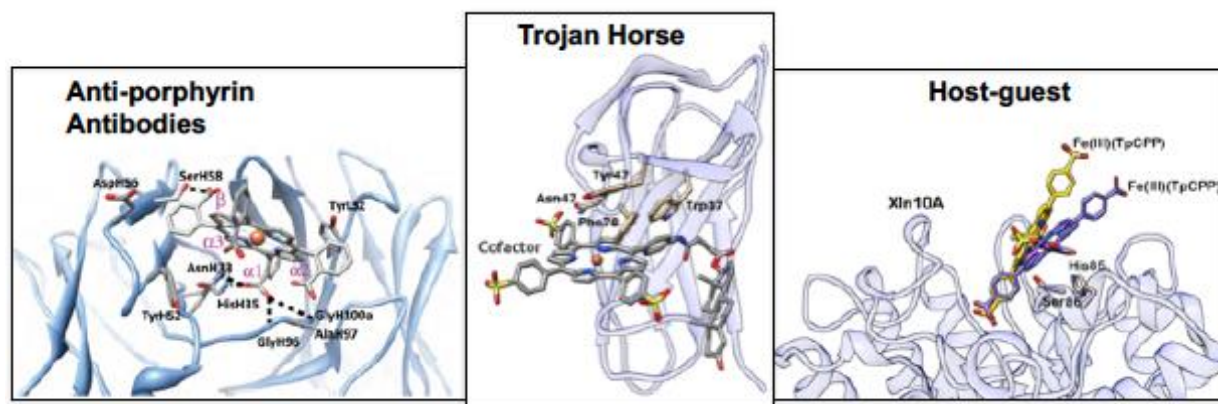


Figure 2: Artificial hemoproteins obtained by supramolecular incorporation of metalloporphyrins into proteins using various strategies: production of anti-porphyrin monoclonal antibodies, incorporation of steroid-metalloporphyrin conjugates into a neocarzinostatin variant (Trojan horse strategy), incorporation of negatively charged metalloporphyrins into xylanase A (Host-guest strategy).

The following paragraphs of this article describe the efforts that have been made in the recent years to construct new artificial hemoproteins by supramolecular insertion of a mini enzyme, microperoxidase 8 (MP8) into porous materials and the efforts that have been made to find new means for utilizing as oxygen atom sources either dioxygen in the presence of an artificial reductase or water in the presence of photoactivable ruthenium complexes.

2.2. Insertion of microperoxidase 8 (MP8) in metal organic frameworks (MOF)

The use of metal-organic frameworks (MOFs) as immobilization matrices for enzymes as a platform for emerging applications has been recently

reviewed.^[28] Enzyme-MOF biocomposites can be prepared by several strategies including surface adsorption, covalent binding, cage inclusion and in situ MOF synthesis. Several features render MOFs interesting matrices for bio-immobilization such as their robustness, high specific areas, hierarchical porosity and hybrid nature, which provides potential benefits for their use a solid-state support in the design of innovative biocomposites such as respectively enzyme protection, high loading capacities, and limitation of the leaching. Microperoxidase-8 (MP8), a small, peroxidase-type enzyme was thus immobilized into nanoparticles of the mesoporous and ultra-stable metal-organic framework (MOF) MIL-101(Cr).^[29]

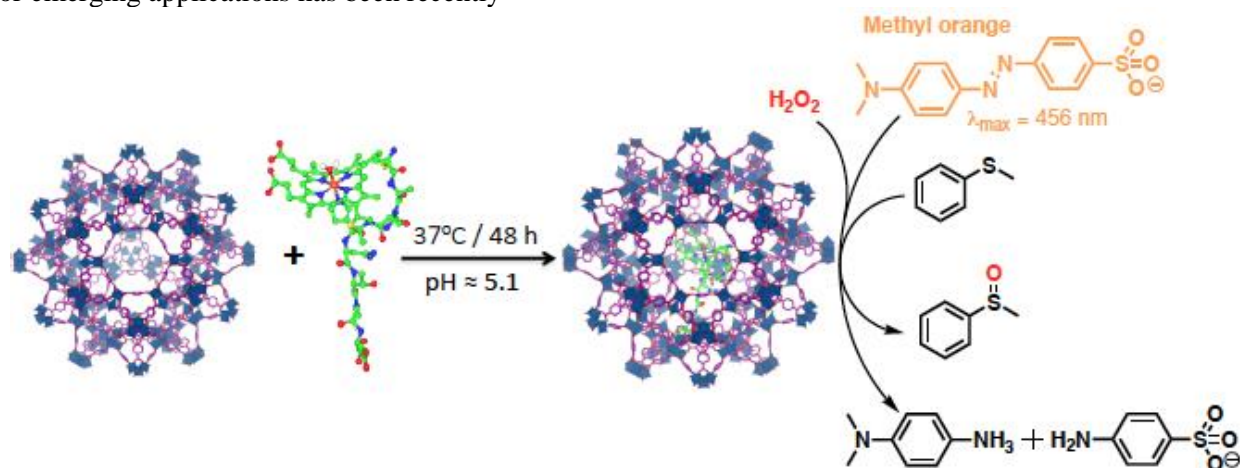


Figure 3: Encapsulation of microperoxidase 8 in mesoporous Metal-Organic Frameworks for selective biodegradation of harmful dye molecules and sulfide oxidation by H_2O_2 .

The immobilized enzyme fully retained its catalytic activity and exhibited enhanced resistance to acidic conditions. The biocatalyst was reusable and showed a long-term stability. By exploiting the properties of the MOFs framework, we demonstrated, for the first time, that the MOF matrix could act in synergy with the MP8 and enhance selectivity the oxidation reaction of dyes. The oxidation rate of the harmful negatively charged dye (methyl orange) was significantly increased after enzyme immobilization, probably as a result of the pre-concentration of the methyl orange reactant owing to a charge matching between this dye and the MOF (Fig. 3).^[29]

More recently, MIL-101(Cr), bearing different functionalized groups (-NH₂ and -SO₃H) were used for the immobilization of microperoxidase 8 (MP8), with preservation of the crystalline structure. The electrostatic interactions between the MP8 molecules and the MOF matrix were found to be a key parameter for the immobilization of higher amounts of MP8 compared to those obtained with the bare MIL-101(Cr). MP8@MIL-101(Cr) and MP8@MIL-101(Cr)-NH₂ were successfully used for the selective oxidation of thioanisole derivatives into sulfoxides (Fig. 3). MP8@MIL-101(Cr) could even be re-used to convert 4-methoxythioanisole into the corresponding sulfoxide even after 4 cycles.^[30]

In conclusion, MP8 could be entrapped and retained in mesoporous robust MOFs with minimal leaching, high catalytic activity, and long-term stability. In addition, the entrapped enzyme was protected under acidic or oxidative conditions by its confinement within the MOF matrix. The MOF matrix can even act in synergy with the enzyme by, for example selectively enhancing the oxidation

reaction of dyes, pre-concentration of reactants through charge matching between the dye and the MOF. This work expands the use of MOFs as bio-immobilization matrices beyond simple MOFs to an active component in catalytic processes.

2.3. Reductive activation of O₂ in the presence of artificial reductases

As mentioned above, the development of artificial systems, capable of delivering electrons to metal-based catalysts for the reductive activation of O₂, has been proven very difficult for decades, constituting a major scientific lock for the elaboration of environmentally friendly oxidation processes. We were recently able to demonstrate that the incorporation of a flavin mononucleotide (FMN) into a water-soluble polyimine polymer, decorated with n-octyl chains bearing a locally hydrophobic microenvironment and guanidinium chains that interacted with the phosphate groups of FMN allowed the efficient reduction of the FMN by NADH.^[31] This new artificial reductase was then able to catalyze the very fast single-electron reduction of a Mn(III)-porphyrin by splitting the electron pair issued from NADH. It then constituted a perfect mimic for natural reductases such as the cytochrome P450 reductases with kinetic parameters, which were three orders of magnitude faster compared with other artificial systems. Finally, as a proof of concept we were able to show that the reduced manganese porphyrin activated dioxygen and catalysed the oxidation of organic substrates such as sulfides in water (Fig. 4).

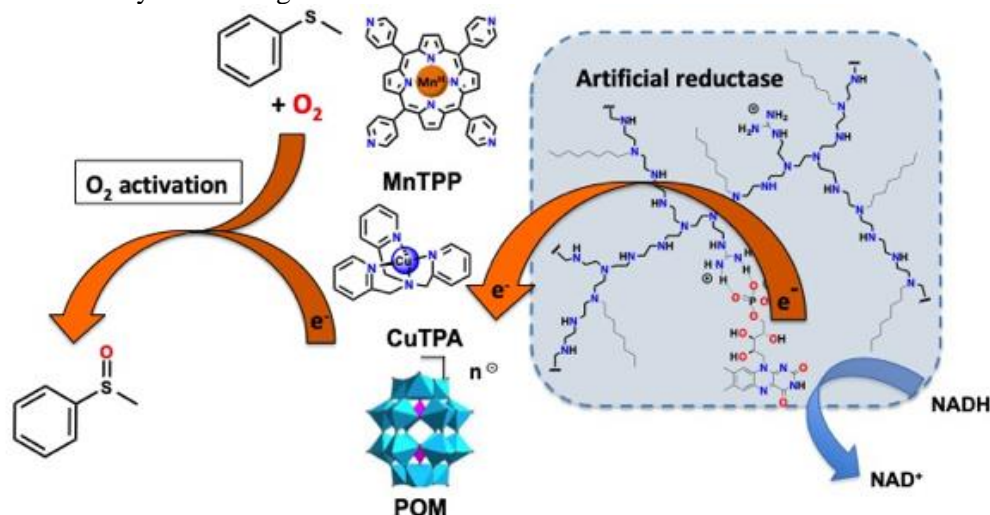


Figure 4: A new artificial polyimine polymer base flavoreductase transferring electrons from NADH to a variety of metal complexes for the reductive activation of dioxygen.

We further demonstrated that this artificial reductase, also efficiently reduced a Cu(II) TPA complex in the presence of NADH in water.^[32] Since O₂ activation at Cu(I) centers is of primary importance for the development of sustainable oxidation catalysis, this was an important finding as our artificial reductase brought a new solution for the regeneration of copper(I) centers after each catalytic cycle, which remains a major problem for multi-turn-over catalysis.

It was more recently shown that this artificial reductase was also able to reduce efficiently polyoxometallates in water which allowed them to catalyze the sulfoxidation of thioanisole derivatives by O₂.^[33] Finally we demonstrated that once reduced, this artificial flavoenzyme could directly activate molecular dioxygen under aerobic conditions and catalyzed at room temperature in water the Baeyer-Villiger reaction.^[34] This afforded a new environmentally friendly oxidation process for the direct incorporation of molecular oxygen into small organic molecules.

2.4. Photoactivation of H₂O

In the search for new eco-compatible oxidants for organic reactions, water was envisioned as an oxygen atom source, which of course required its oxidation. It was shown some years ago that this could be realized by associating metalloporphyrin or hemoproteins with photoactivable ruthenium complexes in the presence of a sacrificial oxidant. High-valent metal-oxo species, namely compound **II**

[(Por)Fe(IV)=O] and compound **I** [(Por)Fe(IV)=O]^{•+}, were then first generated from H₂O using natural peroxidases such as microperoxidase 8^[35] and horseradish peroxidase^[36] associated with a photoactivable [Ru(bpy)₃]²⁺ (bpy = 2,2'-bipyridine) complex in the presence of a [Co(NH₃)₅Cl]²⁺ sacrificial oxidant. Later on, the photooxidation of water was performed in the presence of Mn(III)-tetraarylporphyrins, with the same [Ru(III)(bpy)₃]²⁺ complex as a photosensitized electron-transfer catalyst, [Co(III)(NH₃)₅Cl]²⁺ as a low-cost and weak one-electron oxidant, in a phosphate buffer solution (pH 7.4).^[37] In this system, the Mn(III)-tetraarylporphyrins act as oxygenation catalysts, leading to the photocatalytic oxygenation of organic substrates such as sodium *p*-styrene sulfonate with nearly 100% quantum efficiency. A high-valent manganese-oxo porphyrin was proposed as an active oxidant that effects the oxygenation reactions.

This later results led us to use artificial hemoproteins in order to perform the light induced enantioselective oxidation of an organic molecule with water as the oxygen atom source demonstrated in a system where chirality is induced by the protein. We then reported the first example of the photocatalytic oxidation of water using an artificial hemoprotein built up from bovine serum albumin that was associated with a water soluble Mn-corrole, MnDSC, to afford a new conjugate that was activated with visible light using a ruthenium tris-bipyridyl chromophore (Fig. 5).^[38]

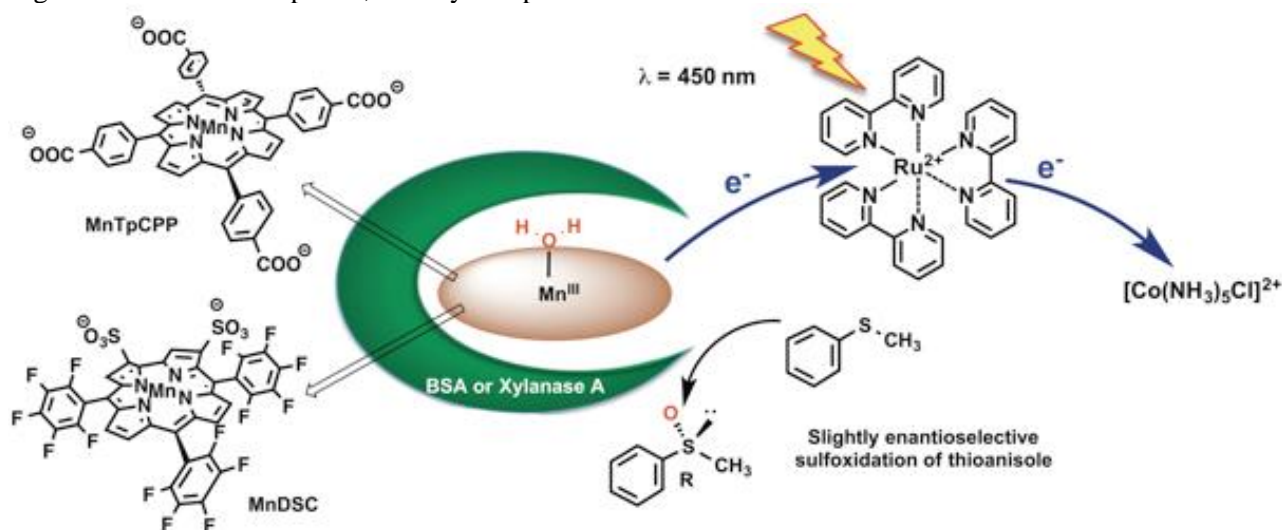


Figure 5. Photocatalytic oxygenation of thioanisole by water in the presence of Mn-corrole -BSA and Mn-tetra-carboxyphenylporphyrin-Xln 10A catalysts and of a photoactivable Ruthenium(III) complex.

Similarly, the Mn(TpCPP)-Xln10A artificial metalloenzyme, obtained by non-covalent insertion

of Mn(III)-meso-tetrakis(*p*-carboxyphenyl)porphyrin Mn(TpCPP) into xylanase 10A from *Streptomyces*

lividans (Xln10A) as a host protein, was found able to catalyze the selective photo-induced oxidation of organic substrates in the presence of $[\text{Ru}(\text{bpy})_3]^{2+}$ as a photosensitizer and $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$ as a sacrificial electron acceptor, using water as oxygen atom source. The oxidation of thioanisole was thus achieved with a slight enantiomeric excess (12-16 %) in favor of the R sulfoxide.[39]

2.5. Selective Diels Alder reaction catalyzed by artificial metalloenzymes in water

Diels-Alder cycloadditions are important reactions for chemical synthesis,[40] but unfortunately, they often take place in organic solvents and require the use of polluting catalysts. However, it has been discovered recently that a few natural enzymes were able to catalyze these reactions,[41–46] which has incited scientists to prepare artificial enzymes that can function under ecofriendly conditions.[47–49] To catalyze the reaction, these natural metal-free Diels–Alderases act as a template that position the substrates into the corresponding transition state conformation.[41–46] Artificial metal-free Diels–Alderases relying on the template effect have then

been prepared and genetically evolved to become highly efficient.[50] Catalytic antibodies that rely on this approach were also developed to catalyze Diels–Alder cycloadditions.[51–54]

However, most of the Artificial metalloenzymes catalyzing Diels–Alder cycloadditions are developed based on the coordination of dienophiles on transition metals using the benchmark substrates 2-azachalcone **1a** and cyclopentadiene **2** that provide up to four product **3** isomers (Fig. 6). Cu^{II} has appeared as the most efficient water-compatible transition metal as catalyst for this cycloaddition [55–57] and, consequently, most artificial metallo-Diels–Alderases have been prepared by incorporating Cu^{II} complexes into biomacromolecules.[58] The most efficient of these was prepared by the covalent anchoring of a 1,10-phenanthroline- Cu^{II} complex into the mutant M89C of the lactococcal multidrug resistance regulator.[59] The mechanism of the reaction has been described to proceed by the bidentate coordination of dienophile **1a** on Cu^{II} via the nitrogen and the oxygen of its amino and carbonyl groups, respectively.

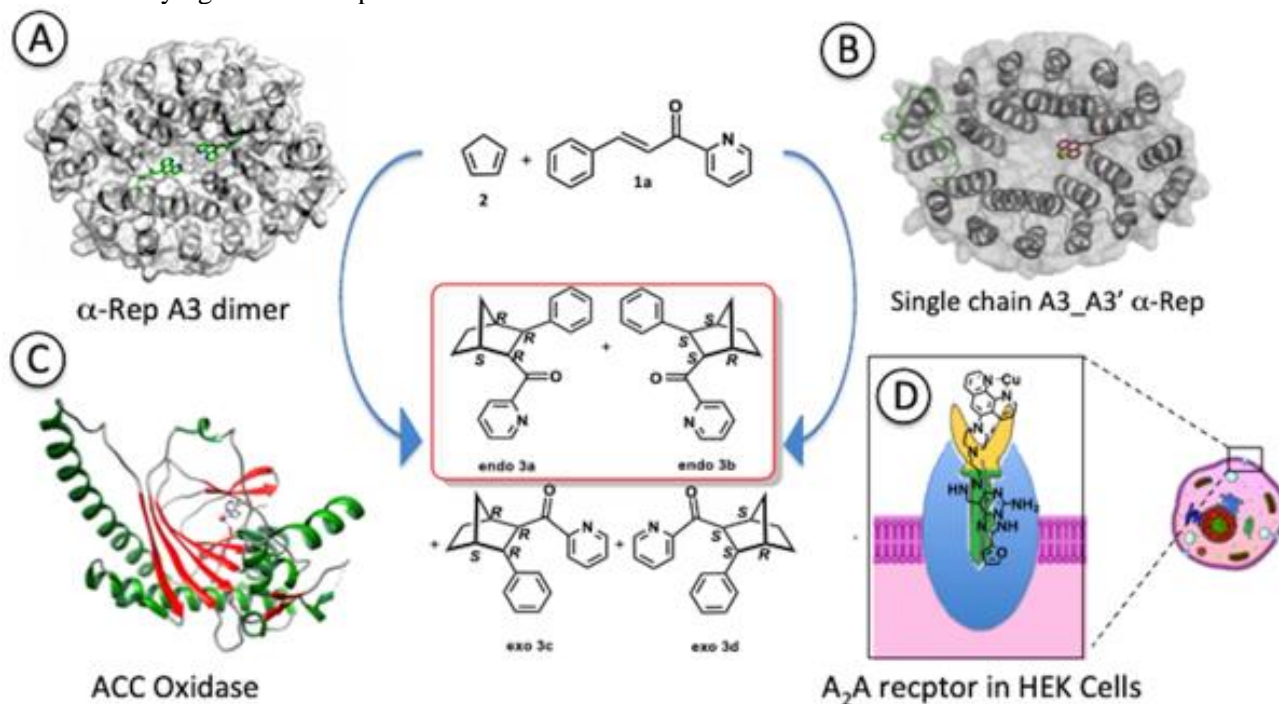


Figure 6. Diels-Alder reaction catalyzed by artificial metalloenzymes constructed by covalent and/or non-covalent insertion of copper complexes or ion into various proteins.

We ourselves prepared new artificial Diels–Alderases from alpha Rep proteins, a new family of artificial proteins based on a thermostable alpha-helical repeated motif. Indeed, one of its members, alpha Rep A3, forms a stable homo-dimer with a

wide cleft that is able to accommodate metal complexes and thus appeared as a good candidate for generating new artificial biocatalysts. Based on the crystal structure of alpha Rep A3, two positions, F119 and Y26, were independently mutated into

cysteine residues. A phenanthroline ligand was covalently attached to these cysteine residues, and Copper(II) was then added to afford the two corresponding biohybrids (Fig. 6A). One of them, the holo-biohybrid A3F119NPH-Cu(II) was found to be able to enantioselectively catalyze Diels-Alder cycloadditions with up to 62% ee. This study validated the choice of the alpha Rep A3 dimer as a protein scaffold and provided a promising new route for the design of new enantioselective biohybrids based on entirely artificial proteins obtained from a highly diverse library.^[60]

In a second time, alpha Rep A3 was converted into entirely artificial single chain bidomain metalloenzymes. A non-mutated A3 domain was covalently linked with an A3' domain bearing a unique cysteine on a chosen mutated position, F119C or Y26C. Copper(II)-phenanthroline or copper(II)-terpyridine complexes were then coupled to those cysteines to afford four new artificial metalloenzymes that were used for the catalysis of the D-A reaction (Fig. 6B). They were found to be able to catalyze the enantioselective D-A reaction of azachalcone with cyclopentadiene with up to 38% yield and 52% enantiomeric excess, which validated the proposed strategy. The data were rationalized with a computational strategy suggesting the key factors of the selectivity. These results suggest that artificial metalloenzymes based on bidomain A3_A3' proteins modified with nitrogen donor ligands may be suitable for further catalyst optimization and may constitute valuable tools toward more efficient and selective artificial biocatalysts.^[61]

We then turned to another strategy that consist in substituting the metal ion of a native metalloenzyme by another one to provide a new metalloenzyme with a new activity. We applied this strategy to 1-aminocyclopropane carboxylic acid oxidase ACCO (Fig. 6C), an iron(II) enzyme that catalyzes the oxidation of 1-aminocyclopropane carboxylic acid into ethylene, a plant growth hormone.

We thus discovered that ACCO reconstituted with Cu^{II} served as an efficient artificial Diels-Alderase. The kinetic parameters of the catalysis of the cycloaddition of cyclopentadiene and 2-azachalcone were determined ($K_M = 230 \mu\text{M}$, $k_{app} = 3 \text{ h}^{-1}$), which gave access to reaction conditions that provided the (1S,2R,3R,4R) product isomer in a quantitative yield and >99% ee. This unprecedented performance was rationalized by molecular modeling as only one docking pose of 2-azachalcone was possible in the active site of the enzyme that led to the (1S,2R,3R,4R) isomer.^[62]

We finally prepared and characterized artificial metalloenzymes based on the A2A adenosine receptor embedded in the cytoplasmic membranes of living human cells (Fig. 6D). The wild type receptor was chemically engineered into metalloenzymes by its association with strong antagonists that were covalently bound to copper(II) catalysts. The resulting cells enantioselectively catalyzed the Diels-Alder cycloaddition reaction of cyclopentadiene and azachalcone. The prospects of this strategy lie in the organ-confined in vivo preparation of receptor-based artificial metalloenzymes for the catalysis of reactions exogenous to the human metabolism. These could be used for the targeted synthesis of either drugs or deficient metabolites and for the activation of prodrugs, leading to therapeutic tools with unforeseen applications.^[63]

CONCLUSIONS

In the last few years the field of artificial metalloproteins has been expanding rapidly, following as main strategy and the incorporation of synthetic metal complexes into a chiral protein cavity.^[1-4] The present review has reported the progress we have made in this field in the last five years, to generate artificial metalloenzymes by incorporating metal (Fe, Mn, Cu) complexes of porphyrins, Tris-pyridylamine, phenanthroline, terpyridine ligands and polyoxometallates (POMs) not only into proteins, but also into several kind of envelopes including water soluble polymers and MOFs that protect the metal cofactor, induce substrate selectivity and positioning and also in some cases may participate in catalysis. This has allowed the generation of hybrid (bio)catalysts that catalyze selective oxidation reactions under eco-compatible conditions and use even O₂ as an oxidant as well as reactions not catalyzed by existing natural enzymes. However, there is still real need to optimize their performances in terms of catalytic activity (TON) and selectivity. This could possibly be realized using directed evolution techniques that have already been applied to hemoproteins such as myoglobin and cytochromes P450 to lead to a wide range of artificial hemoproteins that were found to be able to catalyze abiological reactions such as intramolecular or intermolecular carbene and nitrene transfer reactions,^[64-67] or de novo protein design that has been reported as a new attractive approach for testing and extending our understanding of metalloprotein structure and function.^[68]

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Graphic of TOC

