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Artificial Metalloenzymes

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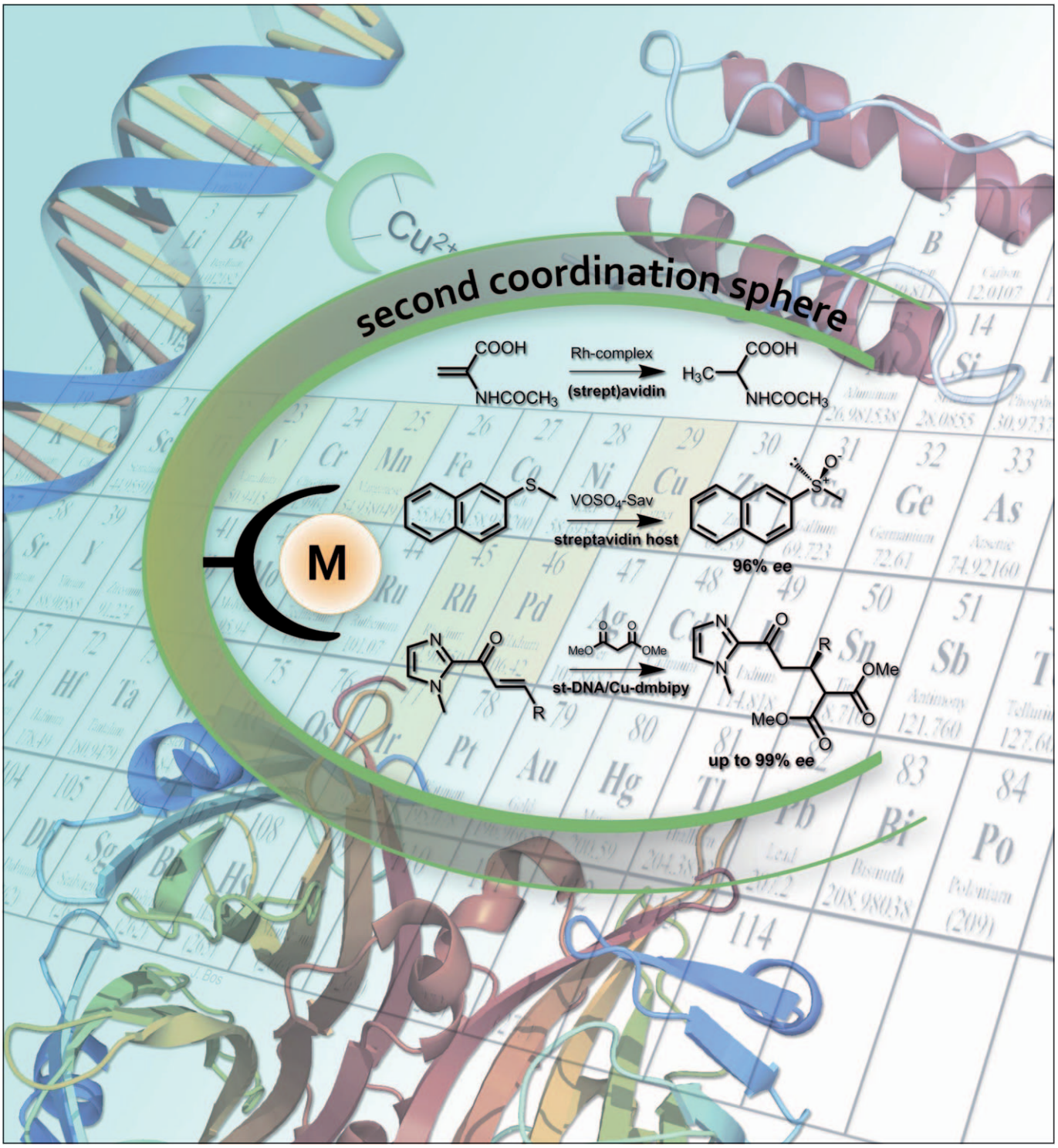

Artificial metalloenzymes have emerged as a promising approach to merge the attractive properties of homogeneous catalysis and biocatalysis. The activity and selectivity, including enantioselectivity, of natural metalloenzymes are due to the second coordination sphere interactions provided by the protein. Artificial metalloenzymes aim at harnessing second coordination sphere interactions to create transition metal complexes that display enzyme-like activities and selectivities. In this Review, the various approaches that can be followed for the design and optimization of an artificial metalloenzyme are discussed. An overview of the synthetic transformations that have been achieved using artificial metalloenzymes is provided, with a particular focus on recent developments. Finally, the role that the second coordination sphere plays in artificial metalloenzymes and their potential for synthetic applications are evaluated.

Introduction

The dominant catalytic approaches towards obtaining enantiomerically pure compounds are homogeneous catalysis, which includes transition metal catalysis,[1] organocatalysis,[2] and biocatalysis.[3] Until recently, these approaches to catalysis have developed in parallel as separate fields. Over the last decade, however, significant efforts have been made to bridge the gap between these fields, resulting in artificial metalloenzymes.[4,5] These are hybrid catalysts in which a catalytically active transition metal complex is incorporated into a host biomacromolecule, typically a protein[6] or DNA.[7,8] The incentives for the creation of artificial metalloenzymes are both practical and fundamental. It is envisioned that artificial metalloenzymes can combine the best of both worlds, that is, broad catalytic scope, a hallmark of homogeneous catalysis, and high activity and selectivity under mild conditions, which typically characterize enzymatic catalysis. The creation of active and selective artificial metalloenzymes is fundamentally important as it holds important lessons for catalyst design and catalytic reaction mechanisms in general.

The catalytic activity and selectivity of conventional transition metal catalysts are almost exclusively controlled by the first coordination sphere provided by the chelating ligand. The dominant strategy towards achieving enantioselectivity involves forcing an incoming reagent to approach selectively from one prochiral side of a substrate, by sterically blocking the other side.

In contrast, in enzymatic catalysis the second coordination sphere, which is the term used for the combination of interactions provided by the biomolecular scaffold, such as hydrogen bonding and hydrophobic interactions, is an important contributor to the catalytic activity and selectivity. It is usually the second coordination sphere that is responsible for chiral discrimination; it can induce selectivity in catalyzed reactions by structurally complementing the transition state towards one enantiomer of the product and/or directing a chemical reactant to one prochiral face of a bound substrate. Artificial metalloenzymes aim at harnessing second coordination sphere interactions to create transition metal complexes that display enzyme-like activities and selectivities.

In the present Review, the various approaches that can be followed for the design and optimization of an artificial metalloenzyme are described. An overview of the synthetic transformations that have been achieved using artificial metalloenzymes is provided, with a particular focus on recent developments. Finally, the lessons learned from artificial metalloenzymes, in particular about the role of the second coordination sphere, and their potential for synthetic applications are discussed.

1. Design of Artificial Metalloenzymes

Ideally, an artificial metalloenzyme would be designed de novo from the 20 natural amino acids. The de novo design of a metalloprotein is based on the construction of a polypeptide sequence which is not directly correlated to any natural protein and that can fold in a well-defined three-dimensional structure capable of binding metal ions. This would allow all the structural features required to achieve highly active and enantioselective catalysis to be included, from the start. Examples of this approach include the de novo design of α helical bundles, a common scaffold for several artificial heme proteins.[9] Significant advances have been made in the computational methods that are required for this purpose and recently some successful examples of de novo designed enzymes have been reported.[10,11] However, our understanding of protein folding is still far from sufficient to allow the routine design of novel enzymes for any desired synthetic transformation.

For this reason, the design of artificial metalloenzymes has focused on the creation of active sites in existing, native, biomolecular scaffolds, such as proteins and DNA. The design of active sites into native scaffolds offers more choices and it is, in principle, simpler than de novo design considering that most native scaffolds already have sufficient thermodynamic stability and tolerance for mutations. Furthermore, structural information for most of the scaffolds used to date is available, facilitating the design and optimization process considerably.

The three key parameters in the design of an artificial metalloenzyme are the transition metal catalyst, the biomolecular scaffold, and the mode of attachment of the transition metal catalyst to the scaffold. The choice of the transition metal catalyst is principally guided by the catalytic activity that is desired.

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An important consideration is that the reactivity of the transition metal catalyst needs to be orthogonal to the biomolecular scaffold, that is, it should be inert to the chemical functionalities presented by the biomolecule. Furthermore, the catalyst should be tolerant to water, as the use of artificial metalloenzymes implies working in aqueous solutions. The factors involved in the selection of the biomolecular scaffold and the mode of attachment of the catalyst are discussed below.

1.1. Selection of biomolecular scaffolds

Important considerations in the selection of biomolecular scaffolds are their chemical properties, such as the overall charge, pH and temperature stability, and tolerance to organic solvents. Another important choice is whether to use a protein or nucleotide scaffold. Both have been used with considerable success and the catalytic scope of protein and DNA-based artificial metalloenzymes reported to date is mostly complementary, as discussed later. The choice for protein or DNA has consequences for the transition metal catalyst and the catalytic chemistry that can be used. For example, whereas catalytic oxidation is one of the most explored reaction types with protein-based artificial metalloenzymes, complications can be expected when using a DNA scaffold, due to its susceptibility to undergo oxidative DNA strand scission.[12]

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Gerard Roelfes obtained his MSc from the University of Groningen, where he also completed his PhD in 2000, under the guidance of Prof. Ben L. Feringa, in a collaborative project with Prof. L. Que, Jr, University of Minnesota. He then went to do a postdoc with Prof. Donald Hilvert at the ETH Zürich. In 2003, he returned to the University of Groningen as a junior project leader. Currently, he is an Assistant Professor of biomolecular chemistry. The central theme in his research is developing new concepts in catalysis, inspired by nature. The main research topics in the Roelfes group are DNA-based asymmetric catalyis, modular assembly of novel DNA-based (catalytic) systems and semisynthetic proteins, with a particular focus on artificial metalloenzymes.

In addition to the chemical properties of the scaffold, fundamental choices about the design approach need to be made. An artificial metalloenzyme can be based on a scaffold that comprises an existing active site and/or binding pocket, which can be reengineered, or alternatively, a new active site can be created in a native biomolecular scaffold.

Using an existing active site or binding pocket is attractive because the second coordination sphere, in principle, is already setup and after the initial design, it can be re-engineered to optimize the performance of the catalyst. This approach was followed in the seminal report of an artificial metalloenzyme by Wilson and Whitesides.[13] The protein avidin was used as the biomolecular scaffold. This protein, which has an exceptionally strong affinity for biotin, contains a very large and deep binding pocket that can accommodate both the catalyst and the substrates. The choice of biomolecular scaffolds that can be used, however, is limited since the binding pocket has to be sufficiently large enough to accommodate both the transition metal catalyst and the substrates. The scaffolds that have been used up to date to achieve enantioselective catalysis with considerable success are proteins such as avidin (Av),[13] streptavidin (Sav),[14] bovine serum albumin (BSA),[15] and apo-myoglobin (apo-Mb).[16] Examples of other scaffolds that have been investigated or proposed include papain,[17,18] cutinase,[19] carbonic anhydrase,[20] and tHisf.[21]

The alternative is to create a new active site in an existing biomolecular scaffold that does not yet possess a binding pocket. The advantage of this approach is that it greatly expands the number of scaffolds that can be used. However, a priori, it is not clear how incorporation of a novel active site will affect the structure and stability of the biomolecular scaffold, as it may result in disruption of some important intra- and intermolecular interactions.

A key example of the second approach is the DNA-based asymmetric catalysis concept, introduced by Roelfes and Feringa.[22] In this approach, an active site is created in or near the DNA groove by binding of a transition metal complex to DNA. The second chiral coordination sphere provided by the DNA directs the reaction towards one of the enantiomers of the product, resulting in an enantiomeric excess. Another scaffold that has been used for this approach is the peptide hormone bovine pancreatic polypeptide (bPP).[23]

A special category consists of the proteins that present a large vacant space, such as the iron storage protein ferritin.[24] In addition to binding of transition metal complexes, the large space available in these protein cages also allows for incorporation of metallic nanoparticles. These protein encapsulated nanoparticles have mainly been investigated for their material properties.[24,25] However, catalytic applications have been explored as well.[24] In these approaches, the role of the biomolecular scaffold is different from the artificial metalloenzymes above and not necessarily intended to achieve enantiomeric excess. Rather, the scaffold acts as a container and/or reactor to grow nanoparticles of a certain size and shape, which may be important for catalysis. Moreover, the pores of the protein cage can be used to discriminate substrates based on their shape and size.[26]

1.2. Anchoring strategies

To ensure the localization of the metal containing moiety into the host, two common strategies are used: noncovalent, which include anchoring through supramolecular and dative interactions, and covalent anchoring (Figure 1).[6]

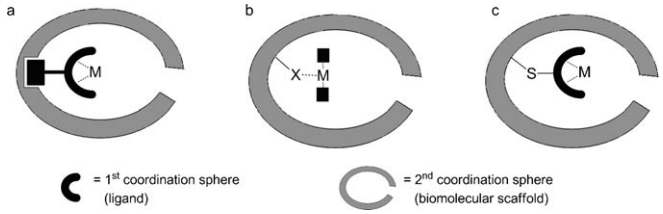


Figure 1. Representation of the concept of artificial metalloenzymes and the various anchoring strategies: a) supramolecular, b) dative, and c) covalent. M denotes the catalytically active transition metal.

The supramolecular approach makes use of the strong and highly specific covalent interactions between biopolymers and small molecules, such as ligands/inhibitors to generate artificial enzymes. The above mentioned examples of artificial metalloenzymes based on the biotin/(strept)avidin interaction and DNA-based asymmetric catalysis involve supramolecular anchoring. The success of this approach is related to the ease of self-assembly of these artificial metalloenzymes, which allows for rapid optimization. A potential complication is that there may be some ambiguity about where the catalyst might bind, depending on the strength and selectivity of the supramolecular interactions. As a result, the structure of the active site may be less well-defined. As the example of DNA-based asymmetric catalysis shows, such an approach can also give rise to a very heterogeneous catalytic system in which every catalytic site is in a different microenvironment, and hence, displays different activity and selectivity.[27] In contrast, the biotin/streptavidin interaction is so strong that it will give rise to a single species, which can be characterized structurally.[28]

A closely related anchoring approach involves dative bonds, such as coordinative bonds between the catalytic metal ion and functionalities presented by the biomolecular scaffold. Examples include artificial metalloenzymes assembled from Mn–salen complexes in myoglobin and Fe and Mn corroles in serum albumins, which have been used in catalytic enantioselective sulfoxidation reactions.[16,29] In addition to the dative bond, other supramolecular interactions are likely to be involved as well. An added advantage of this approach is that the dative bond allows for precise positioning of the transition metal catalyst in the biomolecular scaffold.

The covalent anchoring approach builds on the seminal work of Kaiser and involves the covalent incorporation of a transition metal complex via the ligand to a predetermined position in a biomolecular scaffold.[30] In case of proteins, often a cysteine residue is used as the anchoring site. This allows for highly regio- and site-selective anchoring of the catalytic moiety, with control over the structure and geometry of the catalytic site. A considerable drawback is that the optimization and redesign is not straightforward and is time-consuming, involving chemical modification and non-trivial purification steps. For this reason, highly efficient and chemoselective conjugation reactions are desirable.

2. Tools for Optimization

An attractive feature of artificial metalloenzymes is that the chemical catalyst and the biomolecular scaffold can be optimized independently, followed by screening of all combinations in a matrix format, if desired. This has been referred to as the chemogenetic approach.[31] Combinatorial approaches in combination with high-throughput screening are desirable.

The chemical catalyst is generally optimized by rational design, preferably based on structural information for the artificial metalloenzyme as a whole. Compared to traditional transition metal catalysis, the advantage is that the ligand in most instances is not chiral, which reduces the synthetic complexity of the optimization process.

Optimization of the scaffold can be achieved by rational design and design in combination with evolutionary approaches, such as designed and directed evolution. The different possible approaches to rationally (re)design a metal-binding site in a protein have been recently summarized by Lu et al: 1) the empirical approach. This includes the design by inspection, homology, and by replacement of modular units; 2) the theoretical approach that uses computer search algorithms that make predictions and help design a new active site into a native scaffold by finding the optimal locations for ligands; 3) semitheoretical approach that combines visual inspection of proteins to identify optimal locations for ligands to create a new metal-binding site and computer program evaluation for the energetics of positioning appropriate amino acid residues.[32]

Impressive demonstrations of rational redesign of metalloenzymes were recently reported by the group of Lu. In the first example, the redox potential of a single curpredoxin was tuned by judicious replacement of amino acid residues in the second coordination sphere.[33] These residues were selected based on structural information of the enzyme. In this manner the hydrophobicity and hydrogen bonding of the second coordination sphere were modulated such that the entire range of redox potentials that is known for the family of cupredoxins could be accessed by a single cupredoxin, without significant structural change in the first coordination sphere. Moreover, it was shown that the effect of mutations was additive, which means that the redox potential can be tuned in a predictable manner.

In another contribution, myoglobin (Mb) was re-designed into a functional nitric oxide reductase, by creation of a novel non-heme iron binding site in the distal pocket of Mb.[34] The design was based on the X-ray structure of Fe-Mb in combination with a minimized computer model. The X-ray structure of the rationally designed enzyme overlaid closely with the minimized computer model, confirming the accuracy of the predicted structure.

In evolutionary approaches, several parameters are varied randomly to generate a library of variants that are then screened for the desired catalytic activity. Designed evolution is guided by structural information of the artificial metalloenzyme or the apo-scaffold and involves identification of critical residues, which are then subjected to saturation mutagenesis, either consecutively or simultaneously, to generate a small sized library that is then screened for the desired catalytic activity.[35]

In directed evolution, in principle, structural information is not required. By introducing random mutations, for example by error prone PCR, a library of artificial metalloenzymes can be created.[4,36] Screening and selection of the most active or selective variants is followed by more rounds of randomization and selection, until the desired activity and selectivity have been achieved.

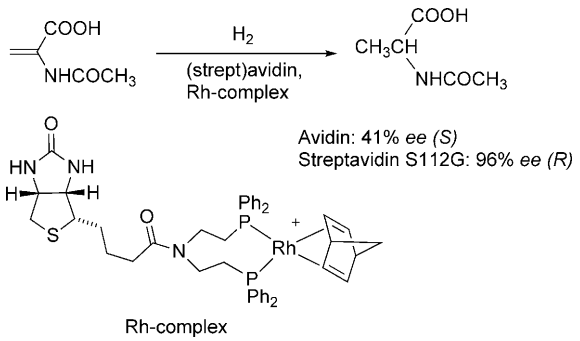
The advantage, in principle, is that also positions remote from the active site are probed. It has been shown on more than one occasion that subtle changes remote from the catalytic site had a dramatic effect on catalyst activity. Challenges that need to be addressed include that with increasing library size, the project becomes increasingly dependent on high throughput methodologies. Moreover, directed evolution approaches also require highly efficient and chemoselective anchoring of the transition metal complexes, since intermediate purification steps should be avoided. In this regard, supramolecular anchoring approaches are particularly attractive. Finally, the quality of the library, that is, the degree of catalyst improvement and the number of hits, can be disappointing. Therefore a variety of other more focused library techniques have been introduced, most notably the combinatorial active-site saturation test (CAST), which is directed at the amino acids directly associated with the active site. Reetz et al. demonstrated the potential of the CAST methodology for optimization of an artificial metalloenzyme based on a biotin linked rhodium diphosphine complex that was anchored to streptavidin. In three rounds, the enantioselectivity was increased from 23 to 65% *ee*.[37]

3. Catalytic Scope of Artificial Metalloenzymes

The catalytic scope of artificial metalloenzymes to date is already quite broad. An overview of the main classes of reactions catalyzed by artificial metalloenzyme is provided. This Review focuses on the key examples for every reaction class, supplemented with the most recent developments. For a comprehensive overview of all the reactions catalyzed by artificial metalloenzymes up until 2008, a number of reviews are available.[6,24,31,38,39]

3.1. Reductions

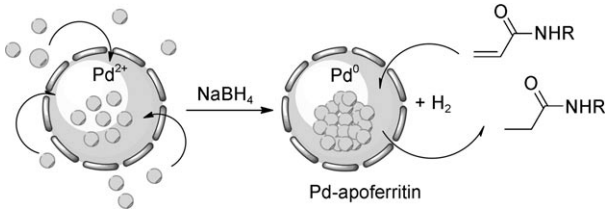
Artificial metalloenzymes for catalytic enantioselective hydrogenations constitute some of the early successes for this field. This is the class of reactions that was studied in the seminal report of an artificial metalloenzyme by Wilson and Whitesides.[13] A rhodium phosphine complex was incorporated into the binding pocket of avidin by a biotin moiety that was attached covalently to the ligand. The resulting artificial rhodium enzyme gave rise to a modest enantioselectivity of 41% *ee* in catalytic asymmetric hydrogenation (Scheme 1).



Scheme 1. Catalytic enantioselective hydrogenation by artificial rhodium enzymes based on avidin and streptavidin.

The design was later matured by Ward and co-workers.[14,40] It was found that the best results were obtained by switching to streptavidin. This improvement was attributed to streptavidin possessing a deeper binding pocket compared to avidin. By using a chemogenetic approach, the design was further improved. Firstly, the design of the ligand was optimized by changing the spacer between the ligand for Rh and the biotin. Then mutation of various residues close to the binding site by glycine, resulted in the discovery of a mutant, S112G, which in combination with the Rh complex gave rise to 96% *ee* in the hydrogenation of acetamidoacrylic acid. Further chemogenetic optimization, which included saturation mutagenesis at position S112 of streptavidin, afforded a second generation of artificial metalloenzymes, in which $>95\%$ *ee* of both enantiomers could be obtained. Catalytic hydrogenation has also been achieved with biomolecular scaffolds such as papain[17] and antibodies.[41]

Encapsulation of Pd nanoparticles in an apo-ferritin cage provided an artificial metalloenzyme capable of catalyzing hydrogenation of acrylamide substrates (Scheme 2).[26] No enantioselectivity was observed, but the observed turnover numbers did depend strongly on the size of the substrate, with larger substrates reacting less efficiently. This was related to the dimensions of the pores of the protein cage; larger substrates diffuse less efficiently through the pores to the inside of the cage, where the catalyst resides. As a result, a lower

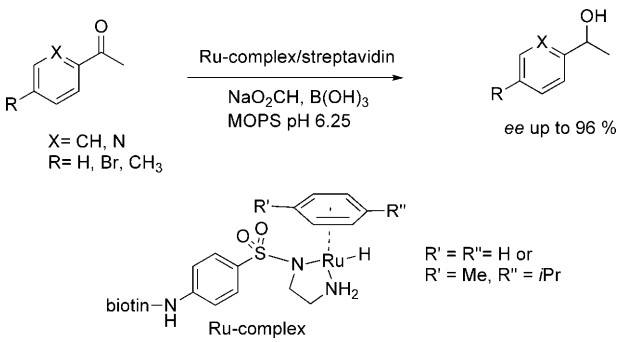


Scheme 2. Hydrogenation catalyzed by Pd nanoparticles encapsulated in ferritin.

reactivity is detected. By encapsulation of Au/Pd core-shell nanoparticles in the protein cage, the reactivity of the artificial metalloenzyme in catalytic hydrogenation of acrylamide was further increased up to fourfold.[42]

Recently, stereoselective hydrogenation of olefins by Rh substituted carbonic anyhydrases was reported.[43] Replacement of the Zn^{2+} from the active site in bovine carbonic anhydrase isoenzyme II by Rh, using $[Rh(cod)_2)]BF_4$, resulted in an artificial enzyme capable of catalytic hydrogenation. This artificial metalloenzyme hydrogenated *Z*-stilbene 20 times more efficiently than *E*-stilbene. In contrast, in the absence of the biomolecular scaffold, *E* and *Z* stilbene were hydrogenated by the Rh complex almost equally efficiently. A preliminary modeling study suggested that this stereoselectivity was due to the better compatibility of *Z*-stilbene with the structure of the active site.

Artificial ruthenium enzymes for catalytic enantioselective transfer hydrogenation of ketones have been developed using the biotin/(strept)avidin concept (Scheme 3). [η^6-*p*-cymene)Ru(ligand)Cl] complexes, in which the ligand is a biotinylated aminosulfonamide ligand, bound to streptavidin, were used to catalyze the transfer hydrogenation of acetophenone and derivatives with formate as the hydrogen source, giving rise to enantiomerically enriched 1-phenylethanols.[44] The artificial metalloenzyme was optimized by saturation mutagenesis at position S112. The highest enantioselectivities were achieved by replacement of S112 by an aromatic amino acid, such as Phe or Tyr; up to 97% *ee* of the *R* enantiomer was obtained. It was proposed that attractive CH/π interactions between the *p*-cymene ligand and the aromatic ketone substrate are important for the enantioselectivity. Interestingly, mutants containing a cationic residue at position 112, such as S112K or S112R produced the *S* enantiomer in up to 70% *ee*.[45]



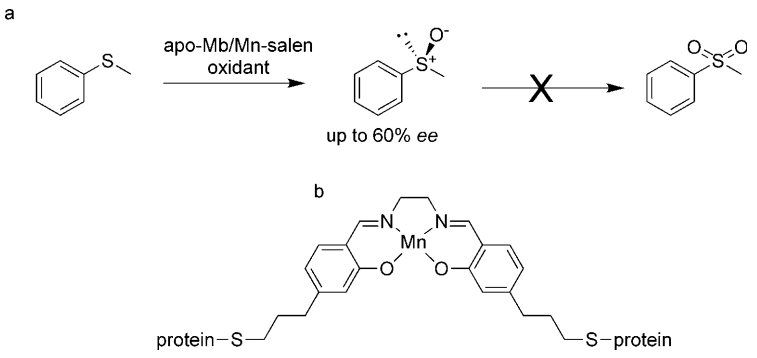
Scheme 3. Enantioselective transfer hydrogenations by an artificial ruthenium enzyme based on streptavidin.

Based on the crystal structure of the [η^6-benzene)Ru(biotinylated ligand)]/Sav S112 artificial metalloenzyme, positions K121 and L124 were selected for further mutagenesis studies. A screening protocol involving immobilization of the artificial metalloenzymes on biotin sepharose was used to facilitate the optimization study. Using either the S112A K121N or the S112A K121T mutant, excellent enantioselectivities were obtained for a broad range of methyl alkyl and methyl aryl ketones. Surprisingly, it was found that the η^6-coordinated arene ligand is important in determining which mirror image of the product is obtained in excess, with benzene and *p*-cymene giving rise to the formation of opposite enantiomers.[28]

3.2. Oxidations

Mn–salen complexes are some of the most powerful catalysts for oxidation reactions.[46] By incorporation of Mn–salen complexes into apo-Myoglobin, artificial metalloenzymes capable of moderately enantioselective sulfoxidation using H_2O_2 as terminal oxidant, have been reported. Using noncovalent anchoring of the metal complex, up to 32% *ee* was obtained for the *S* enantiomer.[16,47] Using the related Cr complex, 33% was achieved. Alternative designs of the salen ligand have been investigated, which resulted in an increase of the stability of the artificial protein.[48] However, no catalysis was reported with these variants. Covalent anchoring of the Mn–salen cofactor, using a dual point attachment strategy, provided higher enantioselectivities; up to 51% *ee* was obtained.[49] Interestingly, the *R* sulfoxide product was now obtained as the major enantiomer. By changing one of the attachment sites in the protein, the *ee* value could be increased to 60% (Scheme 4).[50] Moreover, this artificial metalloenzyme displayed complete chemoselectivity towards the sulfoxide product; no formation of sulfone was detected. This was attributed to the relatively apolar nature of the active site, which does not favor binding of the sulfoxide. As a result, overoxidation does not occur.

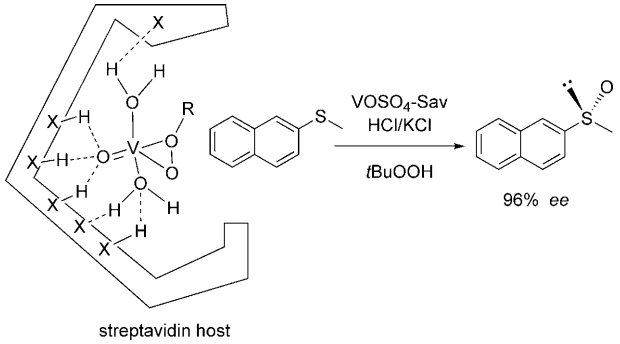
In a related design involving supramolecular anchoring of the Mn–salen complex, the selectivity was also found to be pH dependent.[51] At a pH of 6.4, an *ee* value of 67% was obtained. At higher pH, the rate of sulfoxidation increased significantly, albeit accompanied by a decrease in *ee*. Moreover, at this pH, the artificial metalloenzyme is also capable of oxidizing 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), something it does not do at lower pH values, which suggests the involvement of a different oxidizing intermediate. A crucial role was identified for the distal hystidine ligand, which is involved in the binding, orientation, and activation of the metal bound H_2O_2 by hydrogen bonding interactions.



Scheme 4. a) Enantioselective and chemoselective sulfoxidations catalyzed by apo-Mb/Mn–salen; b) Schematic representation of the protein bound Mn–salen complex.

Serum albumins have emerged as one of the preferred scaffolds for the design of artificial metallo-oxygenases. In an early report, bovine serum albumin conjugated with OsO_4 gave rise to an intriguing 68% *ee* in the dihydroxylation of alkenes, using *t*BuOOH as terminal oxidant.[15] Fe and Mn corroles bound noncovalently to serum albumins from various sources have been used in enantioselective sulfoxidation reactions.[29] Mn-corroles proved to be particularly effective; up to 74% *ee* was obtained in the sulfoxidation of thioanisole derivatives with H_2O_2. Mn–salen complexes have also been anchored noncovalently to human serum albumin (HSA). Using NaOCl as the terminal oxidant, chemoselective oxidation to the sulfone was achieved, whereas in the absence of HSA the sulfone was the major product. As with the Mn–salen/myoglobin system described, the chemoselectivity was proposed to be related to the unfavorable binding of the polar sulfoxide into the relatively apolar binding pocket.[52]

Inspired by the vanadium haloperoxidases, which are capable of enantioselective sulfoxidation reactions using H_2O_2,[53] artificial vanadium enzymes have been prepared by anchoring of vanadate $(VO_4)^{3-}$ to phytase, giving rise to the chiral sulfoxide product with up to 66% of the *S* enantiomer.[54] Recently, a streptavidin-based artificial vanadium enzyme was created by addition of $VOSO_4$. It is believed that the pentahydrated vanadyl ion $[VO(H_2O)_5]^{2+}$ is bound in the binding pocket using weak second coordination sphere contacts (Scheme 5). Excellent *ee* values of up to 93% were obtained in the sulfoxidation of broad range thioanisole derivatives and dialkyl sulfides.[55]



Scheme 5. Representation of the $VOSO_4$/streptavidin artificial metalloenzyme and enantioselective sulfoxidation.

Streptavidin has also been used in combination with biotin linked Mn–salen complexes.[21] However, to date, only low enantioselectivities in catalytic sulfoxidations were found using *t*BuOOH.[56]

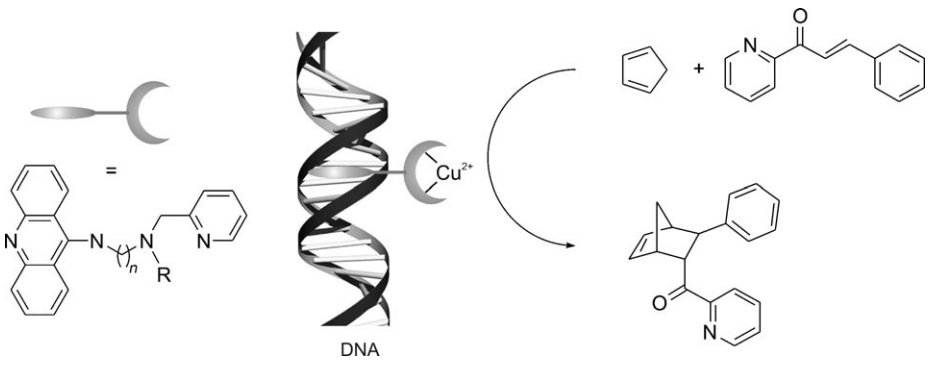
By substitution of the active site Zn of carbonic anhydrase with Mn^{2+}, an artificial peroxidase enzyme was created.[20] Moderate enantioselectivities were obtained in the epoxidation of various alkenes. An important role for the amino alcohol buffer, for example tris(hydroxymethyl)aminomethane (Tris), was found; this buffer can bind in the active site, thus reducing the space and limiting the way in which a substrate such as styrene can bind. A mechanistic proposal involving the formation of peroxycarbonates was put forward. In a similar approach, moderate *ee* values were obtained for a variety of alkene substrates.[57] It was shown that a mutation of an active site (threonine to alanine), significantly increased epoxidation yields, albeit that this mutation did not give rise to improved enantioselectivity.

Artificial Fe hemoenzymes have been created by anchoring an iron porphyrin to an anti-estradiol antibody, via a porphyrin-bound estradiol moiety,[58] or by supramolecular binding to the enzyme xylanase A.[59] Only with the latter system were significant enantioselectivities, up to 40%, obtained in the sulfoxidation of thioanisole.

3.3. Lewis acid-catalyzed C–C bond forming reactions

The concept of DNA-based asymmetric catalysis has mainly found applications in enantioselective Lewis acid-catalyzed C–C bond forming reactions. In this concept, a new active site is created on a salmon testes DNA (st-DNA) scaffold by noncovalent anchoring of an achiral Cu^{II} complex to the DNA double helix by intercalation and/or groove binding. The proximity of the catalytic center to the chiral DNA structure allows for transfer of the chirality to the catalyzed reaction (Scheme 6). Proof of principle for this concept was demonstrated in the Cu^{II}-catalyzed Diels–Alder reaction of azachalcone with cyclopentadiene in water.[22,60] Using a ligand comprising a 9-aminoacridine moiety, a strong DNA intercalator, that is connected by a spacer to an aminomethyl pyridine Cu^{II} binding moiety, moderate *ee* values in the Diels–Alder product were obtained.

In an improved design, ligands that integrate the DNA and metal binding moiety were employed, which eliminates the spacer and allows for placing the active Cu^{II} center closer to the DNA. The highest enantioselectivities were obtained with 2,2′-bipyridine ligands and in particular 4,4′-dimethyl-2,2′-bipyridine (dmbipy): >99% *ee* of the *endo* isomer was obtained.[61] Interestingly, the Cu–dmbipy complex has only a moderate



Scheme 6. Representation of the DNA-based asymmetric catalysis concept.

binding affinity for DNA, which means that under catalytic concentrations not all the Cu–dmbipy is bound to the DNA. Moreover, no apparent sequence selectivity for binding to DNA was detected. This means that the DNA-based catalyst is actually a heterogeneous mixture of Cu complexes that all reside in a different microenvironment. Yet, despite this, $>99\%$ *ee* could be achieved for multiple substrates.

In a kinetic study, the reaction in the presence of DNA proved to be 58-fold faster than the reaction of Cu–dmbipy alone.[27] It was also observed that the reaction was strongly DNA-sequence dependent, with the best *ee* values obtained for sequences containing tracts of three guanines. Importantly, the DNA sequences that gave rise to the highest *ee* values, also caused the largest rate accelerations. Combined, these results explain why DNA-based asymmetric catalysis works with a heterogeneous scaffold such as st-DNA: the DNA accelerates the reaction significantly, which means that unbound Cu–dmbipy does not contribute significantly to the catalysis, and the Cu–dmbipy complexes that are bound to DNA sequences that cause the highest *ee* values, also give rise to the largest rate accelerations and, hence, dominate the outcome of the catalytic reaction. In other words, the enantioselectivities induced by st-DNA are the weighted average of all contributing DNA sequences.

In Cu^{II}-catalyzed reactions, usually bidentate binding of the substrate is required.[62] However, from a synthetic perspective, aza-chalcones are not particularly interesting substrates. The introduction of α,β-unsaturated 2-acyl imidazoles as an alternative class of substrates,[63] allowed a significant expansion of the scope of the DNA-based catalysis concept (Scheme 7). Initially, these substrates were evaluated in the enantioselective Diels–Alder reaction, where similar *ee* values were found compared to the reactions with aza-chalcone.[64] The substrate scope of these reactions was quite broad and after the reaction, the *N*-methyl imidazole moiety was displaced readily, which allowed for determination of the absolute configuration of the product.

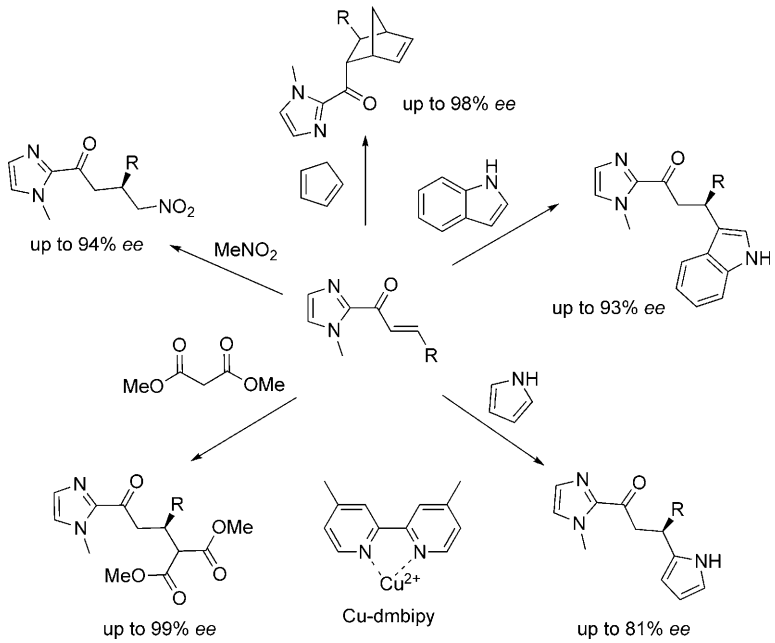
These substrates also proved to be suitable electrophiles in conjugate addition reactions catalyzed by st-DNA/Cu–dmbipy. Using dimethyl malonate or nitromethane as the Michael donors, the corresponding Michael addition products were obtained with excellent enantioselectivities.[65] Again, the scope of enone substrates accepted proved to be very broad. Using neutral π-nucleophiles, such as indoles or pyrrole, the conjugate addition products were obtained in up to 93% *ee*.[66] These reactions represent the first examples of catalytic enantioselective vinologous Friedel–Crafts alkylations in water, reactions that are typically associated with strictly anhydrous conditions. In addition, in this case a significant DNA-acceleration of up to 30-fold was observed. Interestingly, in all st-DNA/Cu–dmbipy-catalyzed C–C bond forming reactions, the stereochemistry was predictable; in every case the enantiomer resulting from attack of the diene or nucleophile from the same π-face was found in excess.

The Cu^{II}-catalyzed Diels–Alder reaction of aza-chalcone with cyclopentadiene has also been used as the benchmark reaction for a DNA-based catalyst containing a covalently attached copper complex. Using a synthetic DNA that incorporates a polyaza crown ether capable of binding Cu^{II} as a nucleotide substitute, a low enantioselectivity was obtained.[67]

A modular assembly strategy towards covalently anchored DNA-based catalysts has been introduced recently.[68] In this approach a Cu-(2,2′-bipyridine) complex was linked covalently to the 3′ or 5′ terminus of an oligonucleotide containing a terminal amino linker. These modified oligonucleotides are readily available from commercial sources. Combination of the bipy-linked oligonucleotide with another oligonucleotide and a template strand that is complementary to both allowed for assembly of a duplex that contains the bipyridine ligand internally at the interface between the two oligonucleotides. The corresponding Cu^{II} complex was evaluated in the Diels–Alder reaction of aza-chalcone with cyclopentadiene. Depending of the nucleotides flanking the covalently bound Cu^{II} complex, up to 93% *ee* was obtained for the Diels–Alder product.

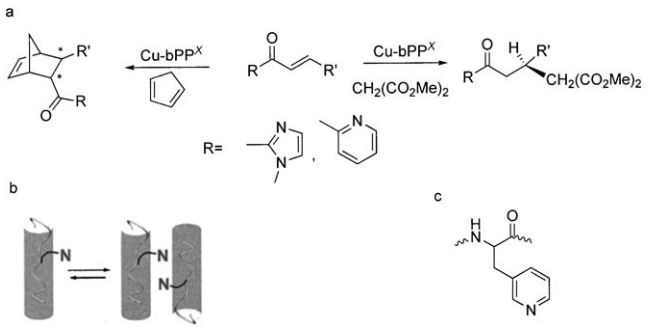
Enantioselective Lewis acid-catalyzed reactions are not limited to DNA scaffolds. BSA has been used as source of chirality in Diels–Alder reactions in the absence of transition metal catalysts, resulting in *ee* values of up to 38%.[69] By anchoring a copper phthalocyanine complex to BSA through a combination of dative and supramolecular interactions, Reetz et al. created a highly enantioselective artificial metalloenzyme that gave rise to *ee* values up to 98% in the Cu^{II}-catalyzed Diels–Alder reaction of aza-chalcone with cyclopentadiene.[70]

Incorporation of a non-proteinogenic Cu^{II} binding amino acid into the peptide hormone bPP provided an artificial copper enzyme capable of enantioselec-



Scheme 7. Scope of the C–C bond forming reactions catalyzed by st-DNA/Cu–dmbipy.

tive Diels–Alder and Michael additions (Scheme 8).[23] Native bPP exists as a dimer in solution and Tyr7, which is located at the dimer interface, was replaced by 3-pyridylalanine, using

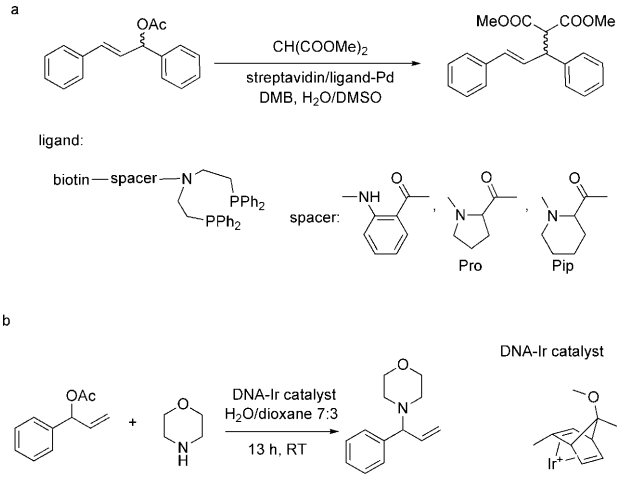


Scheme 8. a) Enantioselective Diels–Alder and Michael addition reactions catalyzed by Cu-bPP^x; b) representation of the monomer/dimer equilibrium of bPP^x; c) a 3-pyridylalanine residue.

solid phase peptide synthesis. In the absence and presence of Cu^{II}, the resulting bPP variant (bPP^x) proved to be a mixture of monomer and dimer, with the monomer being favored. It was found that this artificial metalloenzyme displayed a high substrate specificity. In case of the Diels–Alder reaction of aza-chalcones or α,β-unsaturated 2-acyl imidazoles with cyclopentadiene, the highest *ee* values, that is, up to 83%, were found for R′ = phenyl. With larger substituents, there was a significant loss of activity and selectivity, whereas with a smaller substituent R′, such as R′ = CH_3, the activity was restored, but the reaction was no longer enantioselective. In contrast, in the Michael addition of these substrates with dimethyl malonate, a good enantioselectivity of 86% was found for this substrate, whereas with R′ = phenyl hardly any activity and no enantioselectivity was obtained. It was proposed that the active site provided by this artificial metalloenzyme was structurally compatible with certain substrate/reactant combinations only. Incompatibility with the structure of the active site resulted in loss of activity and/or enantioselectivity.

3.4. Allylic alkylations and aminations

Artificial metalloenzymes catalyzing organometallic C–C and C–N bond forming reactions have been discovered recently. Catalytic asymmetric allylic alkylation has been achieved using the biotin/streptavidin system.[71] A Pd^{II} complex of a biotinylated ligand was anchored to (strept)avidin; the resulting artificial palladium enzyme was evaluated in the allylic alkylation of 1,3-diphenylallylacetate, using dimethyl malonate as the nucleophile. Initial results showed only low reactivity towards the desired product; instead hydrolysis of the 1,3-diphenylallylacetate occurred. Upon addition of a surfactant, that is, didodecyldimethylammonium bromide (DMB), the yield of the allylic alkylation product increased significantly. Starting from this initial design, using a chemogenetic optimization procedure, excellent *ee* values and conversions could be achieved. A key aspect in the design proved to be the nature of the amino acid spacer between the diphosphine ligand and the biotin moiety, with the highest enantioselectivities obtained in case of an *o*-aminobenzoate spacer: up to 93% *ee* was achieved using an S112A streptavidin mutant. The 31% *ee* of the other enantiomer of the product was obtained when a streptavidin variant containing a S112Q mutation was used. Using a constrained amino acid spacer such as (*S*)-proline (Pro) or (*S*)-Pip also gave rise to good *ee* values (Scheme 9). By using the *R* enantiomer of these spacers, the opposite enantiomer of the product could be obtained. In the absence of streptavidin, only low to moderate *ee* values were obtained, underlining that, even though the ligand is chiral, the presence of the scaffold is important.



Scheme 9. a) Enantioselective allylic alkylation by an artificial palladium enzyme based on streptavidin; b) DNA–Ir-catalyzed enantioselective allylic amination.

The group of Jäschke reported the first DNA-based organometallic catalyst.[72] A diene ligand was anchored covalently to DNA. Hybridization with the complementary oligonucleotide and coordination of an Ir^{I} salt gave rise to a DNA-based system capable of catalyzing the allylic amination of phenyl allyl acetate with morpholine (Scheme 9b). The enantioselectivities were not high and were most likely related to the chiral diene ligand. However, the enantiomeric outcome of the reaction, that is, which enantiomer is obtained in excess, depended on the nature and structure of the polynucleotide scaffold.

3.5. Miscellaneous

Two other enantioselective transformations have been reported using DNA as the biomolecular scaffold. Enantioselective electrophilic fluorination of indanone-based β-ketoesters using selectfluor has been achieved using a st-DNA/Cu–dmbipy catalyst. Depending on the structure of the β-ketoester substrate, up to 74% was obtained.[73]

In addition, using st-DNA/Cu–dmbipy, the hydrolytic kinetic resolution of pyridyloxiranes was reported. A low selectivity factor ($S = 2.7$) was found in case of trans-β-phenyl pyridyloxirane. The selectivity proved to be structure dependent; no

significant selectivity was observed in case of *cis*-β-phenyl pyridyloxirane.[74]

4. The Role of the Second Coordination Sphere

The field of artificial metalloenzymes was built on the promise that the second coordination sphere provided by the biomolecular scaffold would confer enzyme-like properties to transition metal catalysis. This would then result in a new branch of catalysis in which the attractive properties of chemo- and biocatalysis could be merged, resulting in catalysis that combines the best of both worlds. With the field maturing rapidly, it is a good time to take stock and assess whether the field has lived up to its expectations, to date. Herein, we provide an overview of the role that the second coordination sphere of the biomolecular scaffold plays in catalysis. Evaluation of the data available reveals a number of important parameters in a catalyzed reaction that are affected by the biomolecular scaffold:

1) Reaction rate. The transition metal catalyst is principally responsible for the catalysis and rate acceleration. But the rate of the reaction is also affected by the presence of the biomolecular scaffold. In some cases, the catalyzed reaction becomes slower.[26,41,70] However, there are now many examples of artificial metalloenzymes in which the second coordination sphere affects the rate positively, which is evident from the increased conversions that were found for the artificial metalloenzyme compared to the transition metal catalysts alone, and the kinetic data.[27,49,54,55,65,66,71,75] The rate accelerations reported range from a modest twofold increase, to up to two orders of magnitude. Often these rate accelerations are substrate dependent, which is consistent with an enzyme active site that prefers binding of some substrates over others or that is compatible with certain activated complexes only. Obviously, rate acceleration induced by the biomolecular scaffold is an attractive phenomenon from a catalytic perspective. However, in particular when using supramolecular anchoring approaches, it is also highly desirable since at low concentrations, the position of the equilibrium between bound and unbound catalyst can become unfavorable. This results in the presence of significant amounts of unbound catalyst, which gives rise to the formation of a racemic product, thus causing a lower overall *ee*. As the case with DNA-based asymmetric catalysis,[27] this is not a problem when the DNA-bound metal complex is significantly faster than the unbound catalyst.

2) Chemoselectivity. The biomolecular scaffold determines the chemical properties, such as the polarity of the microenvironment around the incorporated transition metal complex and, hence, can effect the chemoselectivity of a catalyzed reaction. For example, in the sulfoxidation of thioanisole to the corresponding sulfoxide by a artificial metalloenzymes, such as Mn–salen/apo-Mb[50] or Mn–salen/HAS,[52] the sulfoxide product was obtained selectively; whereas in the absence of the biomolecular scaffold, significant amounts of sulfone also were found. It was demonstrated that the relative apolar nature and the proton donating capabilities of the environment surrounding the Mn–salen inside the protein disfavors binding of the polar sulfoxide, and therefore, prevents overoxidation to the sulfone.

3) Enantioselectivity. The chiral structure provided by the biomolecular scaffold is used to generate reaction products in an enantiomerically enriched form. As discussed earlier, many examples of enantioselective catalysis with artificial metalloenzymes have been reported. In particular, the biotin/(strept)avidin and the DNA-based catalysts have been effective in multiple reaction classes with enantioselectivities of greater than 90%. For many of these reactions, these enantioselectivities represent the highest that have been obtained for reactions in aqueous solutions to date.

4) Size and shape selectivity. Many of the artificial metalloenzymes have a broad substrate scope, which is a typical characteristic of chemocatalysis. In some cases, however, the biomolecular scaffold imposes restrictions on which substrates or substrate/reactant combinations will be reactive. In case of the Pd nanoparticles encapsulated in the protein cage ferritin, it was shown that the size and structure of the pore allows passage into the cage of certain substrates only.[26] Larger substrates cannot reach the catalytic site and, as a result, are not converted into product. In the case of Cu-bPP, which was active in both the Diels–Alder reaction and the Michael addition reaction, it was found that this artificial metalloenzyme displayed a high substrate specificity.[23] This was attributed to the structure of the active site provided by this artificial metalloenzyme being compatible with certain substrate/reactant combinations only. Incompatibility with the structure of the active site results in loss of activity and/or enantioselectivity. In this sense, this system resembles a true enzymatic catalyst, which generally also has active sites that are compatible with certain substrates only.

Often, several of these parameters are affected simultaneously by the biomolecular scaffold. For example, in many of the reactions catalyzed by artificial metalloenzymes based on the biotin/(strept)avidin system or the DNA-based asymmetric catalysis concept, high enantioselectivities are often accompanied by significant rate accelerations induced by the biomolecular scaffold. These observations defy the conventional wisdom in asymmetric homogeneous catalysis, which says that enantioselectivity is usually achieved at the expense of activity.

5. Potential Application in Synthesis and Outlook

To date, the field of artificial metalloenzymes has mainly focused on the fundamental aspects of the design and the catalysis. Only in a limited number of cases have artificial metalloenzymes been applied in reactions on a preparative scale. The DNA-based catalytic enantioselective Diels–Alder, Michael addition, and Friedel–Crafts alkylation have all been carried out on a preparative scale, that is, a mmol scale of substrates, which corresponds to multiple hundreds of milligrams. In all cases, good isolated yields of the products were obtained with excellent enantioselectivities. It was found that after extraction of the product from the aqueous phase, which still contains the DNA-based catalyst, fresh reagents could be

added and the reaction proceeded as efficient as before. Thus, the catalyst was recycled multiple times without loss in isolated yields and enantioselectivity. Recently, it was reported that significant amounts of organic cosolvents were tolerated by the catalyst,[76] which allowed for the use of higher substrate concentrations and lower temperatures. In this manner, the Michael addition and the Friedel–Crafts alkylation reactions were carried out at a gram scale at 4 and $-18\,^{\circ}C$, respectively, giving rise to excellent isolated yields and enantioselectivities.

In general, for artificial metalloenzymes to become attractive for applications in synthesis and be competitive with conventional catalysis approaches, a number of important conditions are identified:

1) The biomolecular scaffold should be readily available in large quantities at reasonable costs.

2) The artificial metalloenzyme should be straightforward to assemble and easy to handle. In this respect, the supramolecular anchoring approaches are preferred since they rely on self-assembly; covalent anchoring usually requires an intermediate purification step, which is undesirable.

3) Scalability requires that low catalyst loadings can be used in combination with high substrate concentrations to keep the total reaction volume acceptable. Since the solubility of many organic substrates in water is limited, the use of organic cosolvents will be required in many cases, which should be tolerated by the biomolecular scaffold.

Even if all the above criteria are met, most synthetic chemists will most likely still be hesitant to use an artificial metalloenzyme for their reactions. One important reason is that for all enantioselective transformations reported to date, good alternatives using conventional approaches, such as homogeneous or biocatalysis, are available. The main advantage that artificial metalloenzymes offer from a synthetic perspective is that they allow for asymmetric catalysis in water as the reaction medium. Indeed, many of the highly enantioselective reactions reported, that is reactions giving $>90\%$ *ee*, represent the best results that have been reported for asymmetric catalysis in water, to date.

For the long term, however, it is imperative that the field of artificial metalloenzymes starts to address some of the challenges in enantioselective catalysis, for which there are no solutions available in conventional asymmetric catalysis, thereby making full use of the clear advantages that the second coordination sphere has to offer. In view of the impressive progress that has been achieved in this field in the last years, it is expected that the field of artificial metalloenzymes will live up to its promise and demonstrate that the best of both worlds really can be achieved.

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