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Artificial Metalloenzymes for Enantioselective Catalysis: Recent Advances

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Introduction

Catalysis is the most efficient strategy for the preparation of enantiopure products.[1,2] Homogeneous and enzymatic catalyses are in many respects complementary in terms of substrate and reaction scope, operating conditions, enantioselection mechanism, etc.[3] In terms of performance optimization, directed evolution methodologies outperform combinatorial ligand libraries.[4–8]

With the aim of combining the best of both homogeneous and enzymatic worlds (e.g., reaction scope and performance optimization), the field of artificial metalloenzymes has witnessed a revival. The underlying principle of artificial metalloenzymes, relying on the "chemical mutation" of a protein to incorporate a metal-containing fragment, was developed in the late 1970s by Kaiser and Whitesides (Figure 1).[9–11]

Several recent reviews on artificial enzymes exist;[12–18] this minireview focuses on the latest developments in artificial *metallo*enzymes for enantioselective catalysis. The hybrid catalysts presented here are classified according to the nature of the metal fragment–host protein interaction: supramolecular, dative, and covalent. For simplicity (and irrespective of the nature of the metal fragment–host protein interaction), the inclusion symbol "⊂" will be used throughout the review to describe artificial metalloenzymes: metal fragment⊂host protein.[19]

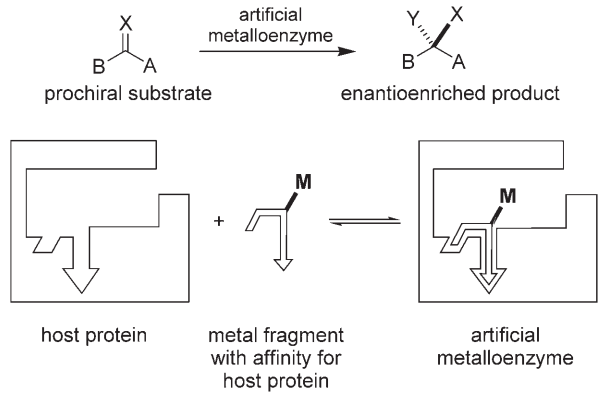
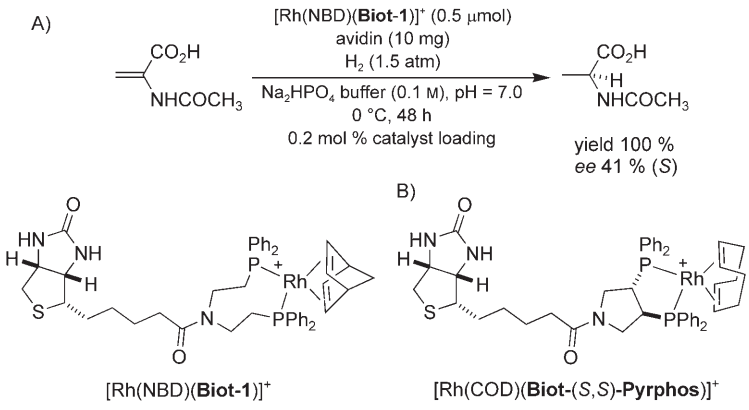


Figure 1. Artificial metalloenzymes for enantioselective catalysis based on the incorporation of a catalytically active metal fragment within a host protein. The interaction between the metal fragment and the host protein may variously be supramolecular, dative, or covalent in nature.



Scheme 1. Artificial metalloenzymes based on biotin–avidin technology for the hydrogenation of alkenes; operating conditions used by Whitesides (A) and ligand used by Chan for the reduction of itaconic acid (B).

Supramolecular Anchoring Strategies

The strongest noncovalent interaction ($K_a \approx 10^{15}\ M^{-1}$) between a protein and a small molecule is represented by the biotin–avidin couple.[20] This interaction is thus strong enough to ensure quantitative localization of a biotinylated metal fragment within a protein environment without requiring a covalent modification step that might not be quantitative. Functionalization of biotin with a rhodium diphosphine moiety affords, upon mixing with avidin, an artificial metalloenzyme for the reduction of functionalized alkenes. Wilson and Whitesides demonstrated this principle in 1978 in the reduction of *N*-acetamidoacrylate with a 0.2 mol% catalyst loading (Scheme 1), obtaining *N*-acetamidoalanine (*N*-AcAla) in 41% *ee* (*S*) and with quantitative conversion.[11] In 1999, Chan and co-workers hydrogenated itaconic acid in the presence of an enantiopure biotinylated pyrphos ligand (Scheme 1 B).[21] Depending on the operating conditions (temperature, pressure, pH) and the absolute configuration of the pyrphos ligand, the enantiopurity of the resulting methylsuccinic acid varied between 48% (*R*) and 26% (*S*).

Inspired by these findings, we reported in 2003 a biotin–streptavidin-based artificial metalloenzyme for the hydrogenation of α-acetamidoacrylic acid and subsequently of α-acetami-

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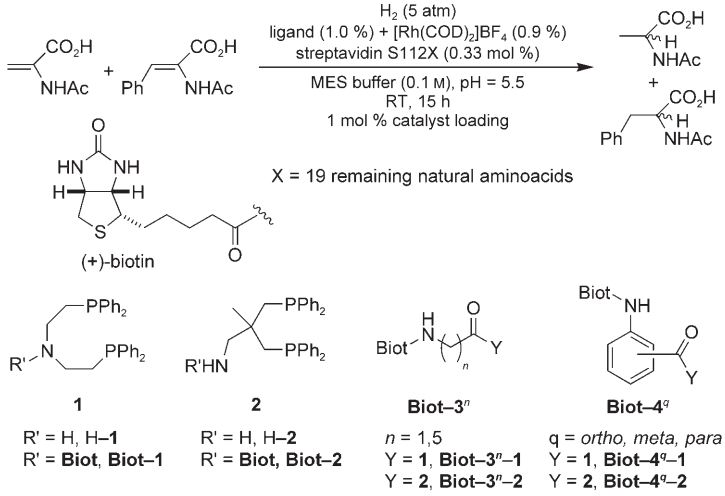
docinnamic acid.[22] Good levels of enantioselection were obtained upon substitution of avidin by streptavidin (Sav hereafter), which is known to possess a deeper binding pocket. Introduction of a spacer between the biotin anchor and the diphosphine moiety allows for chemical optimization of the performance of the artificial hydrogenases.[23–25] Genetic optimization by introduction of point mutations on the host protein allows both the activity and the selectivity of the hybrid catalysts to be fine-tuned. This chemo-genetic optimization scheme (a term initially coined by Distefano[13]) allows the rapid generation of substrate-specific and either (*R*)- or (*S*)-selective artificial metalloenzymes (Table 1).[26]

Table 1. Operating conditions and numerical summary of selected results of the chemogenetic optimization of artificial hydrogenases in the reduction of α-acetamidoacrylic and α-acetamidocinnamic acid.



	Ligand	Sav mutant	*N*-AcPhe *ee*	*N*-AcPhe Conv.	*N*-AcAla *ee*	*N*-AcAla Conv.
1	**Biot-1**	WT Sav	93 (*R*)	84	94 (*R*)	quant.
2	**Biot-1**	S112C	90 (*R*)	10	76 (*R*)	19
3	**Biot-1**	S112P	87 (*R*)	96	31 (*R*)	quant.
4	**Biot-4^{meta}-1**	S112H	81 (*S*)	88	58 (*S*)	quant.
5	**Biot-4^{meta}-1**	S112K	88 (*S*)	89	63 (*S*)	quant.
6	**Biot-4^{meta}-1**	S112P	78 (*S*)	quant.	36 (*S*)	quant.
7	**Biot-4^{meta}-1**	S112R	86 (*S*)	71	63 (*S*)	quant.
8	**Biot-2**	S112P	41 (*S*)	12	63 (*S*)	quant.
9	**Biot-3^{4}-2**	S112Q	92 (*R*)	77	87 (*R*)	quant.

N-AcPhe = α-acetamidophenylalanine. *N*-AcAla = α-acetamidoalanine. quant. = quantitative conversion.

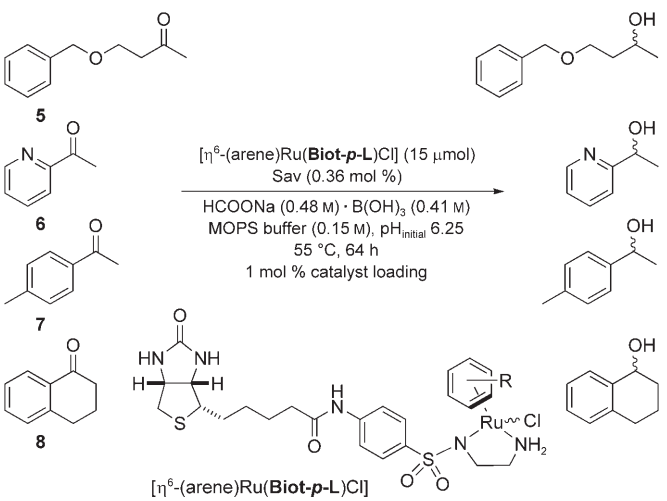
These results highlight the versatility of the chemogenetic optimization scheme. The natures of the spacer, the ligand, and the aminoacid residue at position 112, identified as a close-lying residue (Table 1, entry 2), all work in concert to afford either (*R*)- or (*S*)-selective catalysts (up to 94% *ee* (*R*), Table 1, entries 1, 3 and 9; up to 88% *ee* (*S*), Table 1, entries 4–7). Other combinations yield substrate-specific hybrid catalysts that convert the smaller substrate, leaving the larger substrate nearly untouched (Table 1, entry 8).

Motivated by these findings, we next focused on enantioselective transfer hydrogenation reactions catalyzed by d^6-piano-stool complexes. Using the same type of chemogenetic optimization procedure, we identified efficient artificial transfer hydrogenases for the enantioselective reduction of prochiral ketones (Table 2).[27,28]

Several noteworthy conclusions can be drawn from these data:

1) The initial pH plays a critical role.
2) The *para*-substituted biotinylated ligand **Biot-*p*-L** is most effective with these substrates (Table 2).
3) The nature of the capping arene plays a critical role in determining which enantiomer is produced: η^6-benzene-capped piano stools preferentially afford (*S*) products, while η^6-*p*-cymene affords (*R*) alcohols (Table 2, entries 2–4).
4) Cationic residues in position S112 favor (*S*)-reduction products (Table 2, entry 2).

Table 2. Operating conditions and numerical summary of selected results for the chemogenetic optimization of enantioselective artificial transfer hydrogenases [η^6-(arene)Ru(**Biot-*p*-L**)Cl]⊂Sav.

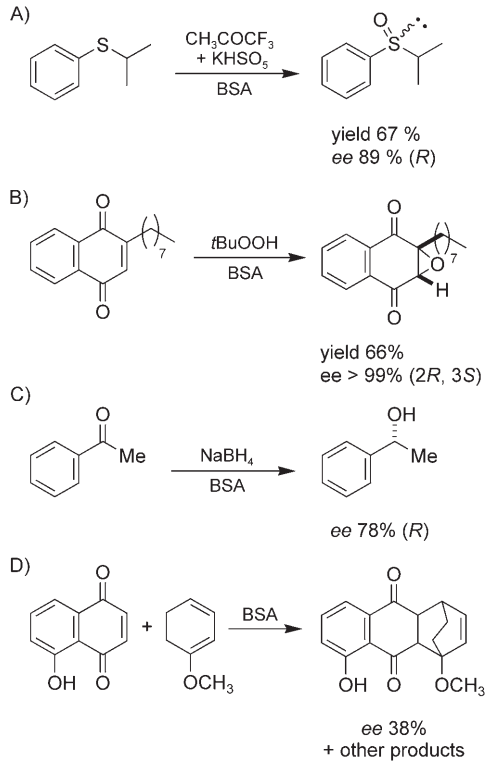


	η^6-arene	Sav mutant	Substrate	Conv. [%]	*ee* [%]
1	*p*-cymene	S112A	**5**	97	69 (*R*)
2	benzene	S112R	**6**	95	70 (*S*)
3	*p*-cymene	P64G	**7**	92	94 (*R*)
4	*p*-cymene	S112Y	**8**	79	97 (*R*)

In the context of supramolecular anchoring of metal cofactors within a host protein, albumins play a key role. Serum al-

bumins, which function as transport proteins in plasma, display a remarkable ability to bind a variety of hydrophobic guests tightly, including fatty acids, steroids, thyroxine, porphyrins, etc.

These transport proteins have been shown to catalyze a variety of enantioselective transformations, including oxidation,[29–31] reduction,[32,33] and Diels–Alder cycloaddition[34] reactions, with moderate to good enantioselectivities (Scheme 2).[35]



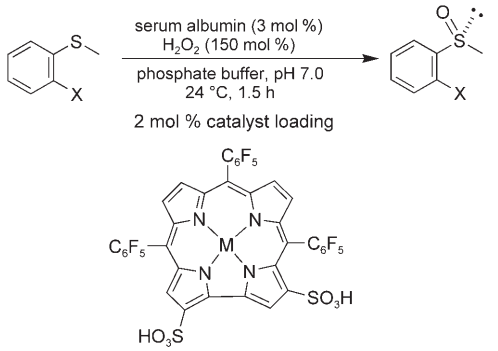
Scheme 2. Selected examples of: A) asymmetric sulfoxidation, B) epoxidation, c) reduction, and d) Diels–Alder cycloaddition catalyzed by bovine serum albumin.

The recent structural determination of a hemin⊂HSA 1:1 conjugate by Curry and co-workers[36] coincided with the development of enantioselective artificial metalloenzymes based on the noncovalent incorporation of corroles and phthalocyanines in serum albumins.[37,38]

Gross and co-workers have reported on the incorporation of amphiphilic bis-sulfonated corrole metal complexes in various serum albumins. In particular, [Mn^{III}(corrole)]⊂HSA displays a 1:1 conjugate with a K_d in the nanomolar range. The presence of an induced circular dichroism signal suggests that the metal is incorporated in a well-defined chiral environment,[37,39] consistently with the structurally characterized hemin⊂HSA conjugate.

The resulting artificial metalloenzyme catalyzes the enantioselective sulfoxidation of thioanisole derivatives (Table 3, up to 74% *ee*).[37] The Mn(corrole) derivatives are superior to Fe(corrole) in terms of activity, selectivity, and stability of the catalyst. While PhIO affords higher yields of sulfoxide, its associated enantioselectivity is smaller than that obtained with H_2O_2. Mechanistic investigations suggest the importance of a hydrogen peroxide coordinated to manganese(III) as the prime intermediate in the enantioselective sulfoxidation.

Table 3. Albumin-conjugated corrole metal complexes as artificial metalloenzymes for the enantioselective sulfoxidation of thioanisole derivatives (BSA, PSA = bovine and porcine serum albumin, respectively).



	Host protein	M	X	Conv. [%]	*ee* [%]
1	BSA	Mn	F	76	68 (*S*)
2	PSA	Mn	F	98	65 (*S*)
3	BSA	Mn	Br	16	74 (*S*)
4	BSA	Fe	H	87	38 (*S*)

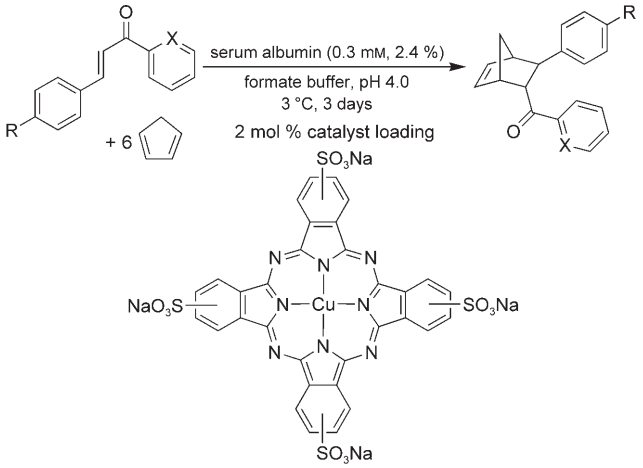
Incorporation of Cu(phthalocyanine) in a variety of serum albumins affords an efficient artificial metalloenzyme for Diels–Alder cycloadditions. Excellent *endo*-selectivities and enantioselectivities are reported for pyridine-bearing dienophiles with both BSA and HSA as host protein (up to 98% *ee* with a 10 mol% catalyst loading; Table 4, entries 1–3). Although the Cu(phthalocyanine) can formally accommodate two additional donors, the geometry imposed by the aromatic phthalocyanine virtually excludes an N–O chelation of the dienophile. As the nonchelating dienophile, devoid of an N-donor, is a very poor substrate (Table 4, entry 4), one can speculate that the pyridine functionality interacts with the host protein rather than the copper center, thus being reminiscent of enzymatic catalysis, in which the protein steers the delivery of the substrate to the coenzyme through noncovalent interactions.

Although serum albumins with no additional metal complexes have been shown to catalyze both sulfoxidation and Diels–Alder reactions (see Scheme 2), the reports by Gross and Reetz unambiguously exclude catalytic activity of the protein alone. As the hemin⊂HSA complex was shown to be anchored through an interaction between a tyrosine Y161 and the iron ion, one can speculate that both the Mn-corrole and the Cu-phthalocyanine also enter into interaction with an amino acid side chain within the albumin host protein.

In the context of hybrid catalysts for enantioselective Diels–Alder cycloadditions, Roelfes and Feringa reported on a supramolecular catalyst relying on the intercalation of a copper-based Lewis acid in salmon testes DNA (Table 5).[40,41]

Chemical optimization of the performance was achieved by replacing the intercalator-spacer-donor **9a** by a bipyridine

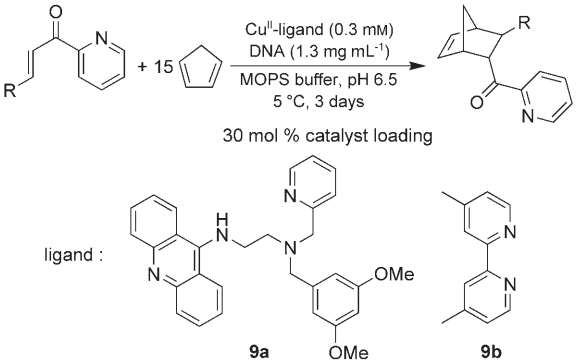
Table 4. Selected results for Diels–Alder reactions catalyzed by Cu(phthalocyanine)⊂serum albumin (BSA, HSA = bovine and human serum albumin).



	Host protein	R	X	Conv. [%]	*endo*:*exo*	*ee endo* [%]
1	BSA	H	N	80	96:4	93
2	HSA	H	N	89	91:9	85
3[a]	BSA	NO_2	N	91	91:9	98
4	BSA	H	CH	5	–	56

[a] 10 mol % catalyst loading

Table 5. Asymmetric Diels–Alder reactions catalyzed by DNA-based catalysts in the presence of aromatic bidentate ligands as DNA intercalators.



	Ligand	R	*endo*:*exo*	*ee endo* [%]	*ee exo* [%]
1[a]	**9a**	4-methoxyphenyl	91:9	−53[b]	−90[b]
2	**9b**	4-methoxyphenyl	>99:1	>99	–
3	**9b**	Ph	>99:1	99	–
4	**9b**	*t*Bu	>99:1	97	–

Conversion > 80 % for all reactions. [a] 5 equivalents of cyclopentadiene. [b] The negative sign refers to the opposite enantiomer, absolute configuration not determined.

ligand **9b**, which acts both as intercalator and as bidentate ligand. Excellent *endo*-selectivities and enantioselectivities are reported for the Diels–Alder reactions between cyclopentadiene and bidentate dienophiles (Table 5).[40,41] Although the catalyst loading remains very high at this stage, such hybrid catalysts offer an attractive alternative to artificial metalloenzymes based on proteins as hosts.

Dative Anchoring Strategies

To the best of our knowledge, Kaiser and co-workers were the first to modify the active site of an enzyme by dative modification to afford an artificial metalloenzyme with novel catalytic properties. This approach was termed chemical mutation and the first report detailed a study of the oxidase activity of a copper(II) carboxypeptidase A (CPA).[9]

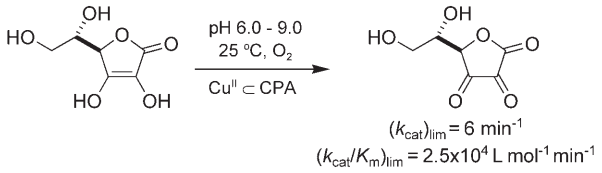
Substitution of zinc by copper in CPA affords a Cu^{II}⊂CPA conjugate that is inactive towards the hydrolysis of amides and esters. Spectroscopic studies suggest that the copper adopts a distorted N_2O_2 tetrahedral geometry and is capable of binding to typical CPA inhibitors such as β-phenylpropionate as well as to (*rac*)-α-benzylsuccinate.[9,42]

In the presence of oxygen, this artificial metalloenzyme Cu^{II}⊂CPA was shown to be an effective catalyst for the oxidation of ascorbic acid to dehydroascorbic acid. In contrast to the solvated copper(II) ion, Cu^{II}⊂CPA follows Michaelis–Menten kinetics (Scheme 3).

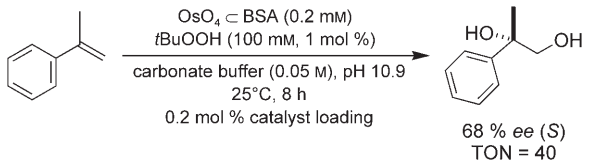
The first *enantioselective* artificial metalloenzyme based on the dative anchoring of a metal moiety was reported by Kokubo in 1983.[43] Introduction of osmium tetroxide into BSA yields a 1:1 complex that displays remarkable catalytic dihydroxylation properties in the presence of *tert*-butylhydroperoxide as oxidant (Scheme 4). The authors speculate that the OsO_4 moiety is anchored through two dative bonds to lysine residues within BSA's hydrophobic pocket. For the dihydroxylation of α-methylstyrene, enantioselectivities of up to 68 % (*S*) have been reported. This early study once again highlights the potential of albumins as host proteins for enantioselective catalysis.

A pentacoordinate vanadate ion can be regarded as a transition state analogue for phosphate hydrolysis,[44] and this property can be exploited for efficient inhibition of the hydrolytic activity of acid phosphatases[45] and phytases.[46]

Close inspection of the active site of vanadium chloroperoxidase from *C. inaequalis*[47] reveals a striking similarity to the active site of phytase from *A. ficuum* (Scheme 5),[47,48] and as a result of this observation Sheldon and co-workers investigated the sulfoxidation properties of a vanadium-incorporated phytase.[49–52] After incorporation of vanadate into

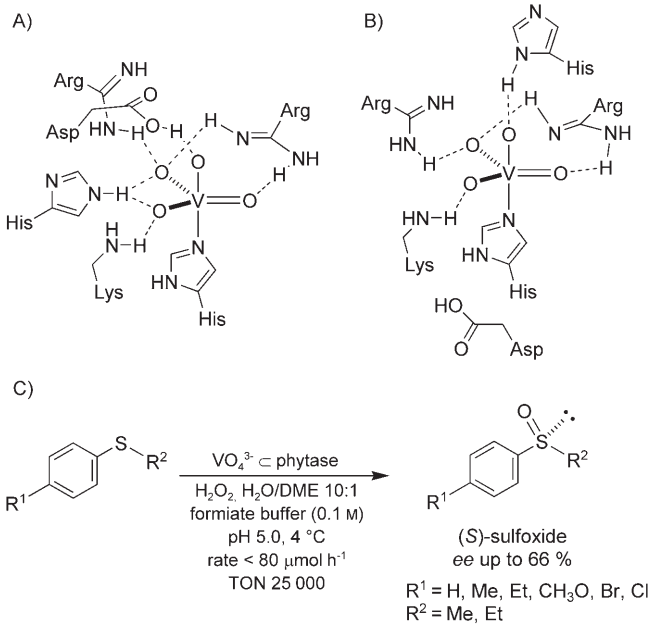


Scheme 3. Oxidase activity of copper(II) carboxypeptidase A (Cu^{II}⊂CPA), the first artificial metalloenzyme.



Scheme 4. Artificial enantioselective dihydroxylase based on the dative anchoring of OsO_4 in BSA.

phytase from *A. ficuum*, the resulting semisynthetic peroxidase displayed moderate enantioselectivity (up to 66% *ee* (*S*) at 4 °C, TOF = 11 min^{-1}) for the sulfoxidation of thioanisole derivatives in the presence of hydrogen peroxide, with very little detectable overoxidation (Scheme 5).



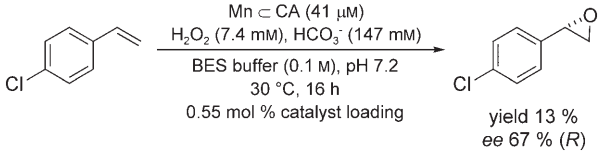
Scheme 5. Design of a semisynthetic peroxidase based on the incorporation of vanadate in phytase (A), yielding a vanadium chloroperoxidase-like active site (B). In the presence of hydrogen peroxide, the vanadium-incorporated phytase catalyzes the enantioselective oxidation of thioanisole derivatives (C).

Various other vanadium-loaded enzymes (including BSA, apo-ferritin, sulfatase, phospholipase, acid phosphatase, etc) were tested in the sulfoxidation of thioanisole: phytase from *A. ficuum* gave the highest rate and the highest enantioselectivity. The vanadate performs best of a range of oxoanions combined with phytase, in terms of both activity and selectivity. The enantioselectivity increases in the order $MoO_4^{2-} \approx ReO_4^- < WO_4^{2-} < SeO_4^{2-} < VO_4^{3-}$.[51] Similarly, substitution of Zn^{2+} from thermolysin by a tungstate yields a moderately active—but nonenantioselective—sulfoxidation artificial metalloenzyme.[53] From the restored proteolytic activity of tungstate⊂thermolysin, as well as its sulfoxidation activity towards phenylmercaptoacetophenone, the authors tentatively conclude that the anion is "correctly bound in the active site", despite its size and charge.[53]

Surprisingly, the phytase with no vanadium also catalyzes the enantioselective sulfoxidation of thioanisole (up to 66% *ee* (*S*) at 4 °C), although the sulfoxidation rate for the vanadium-free phytase-catalyzed reaction is significantly lower than that for the semisynthetic enzyme incorporating vanadium: 3.2 μmol h^{-1} vs. 124 μmol h^{-1}.[50]

Phytase, a hydrolytic enzyme, can thus be converted into a peroxidase, with no modification of the active site. Such propensity of enzymes to catalyze more than one distinct chemical transformation, known as catalytic promiscuity, has recently been reviewed by Bornscheuer and Kazlauskas.[18]

The zinc in carbonic anhydrase (CA) can be substituted by various transition metals, including manganese. Incorporation of catalytically non-innocent manganese in apo-carbonic anhydrase affords an artificial metalloenzyme Mn⊂CA for the enantioselective epoxidation of aromatic olefins: up to 67% *ee* (*R*) for *p*-chlorostyrene (Scheme 6).[54] Interestingly, percarbonate



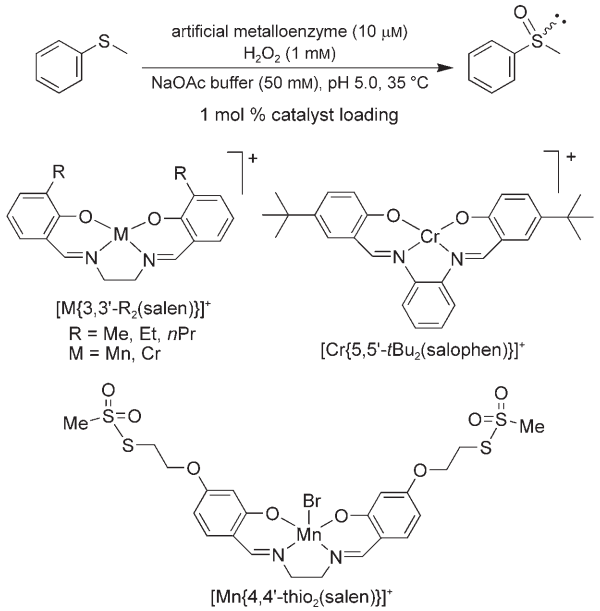
Scheme 6. Manganese-substituted carbonic anhydrase Mn⊂CA as an artificial epoxidase.

(HCO_4^- prepared from HCO_3^- and H_2O_2)[55] proved to be the best oxidizing agent: upon oxidation of the olefin, carbonate—which is the natural substrate of CA—is formed. Mass spectroscopic analysis during catalysis suggests that oxidative degradation of Mn⊂CA limits the turnover number.

Very similar results were reported nearly simultaneously by Soumillon and co-workers for the epoxidation of styrene in the presence of Mn⊂CA (up to 52% *ee* (*R*) and 9% yield with bovine carbonic anhydrase). Interestingly, they reported that a phosphate buffer affords the best selectivities as it shuts off the background epoxidation reaction of the non-protein-incorporated manganese ions.[56]

Substitution of the heme cofactor by [M(Schiff base)] moieties in myoglobin (Mb) to yield artificial metalloenzymes for enantioselective sulfoxidation was first reported by Watanabe and co-workers.[57–58] In addition to van der Waals interactions between the coordination compound and Mb, a dative bond between Histidine H93 and the metal center ensures localization of the catalyst. Selected results for the sulfoxidation of thioanisole are collected in Table 6, entries 1–4. Initial results (Table 6, entry 1)[57] could subsequently be improved by analysis of an X-ray crystal structure, which led to the rational design of site-directed Mb mutants.[58] Chemo-genetic optimization affords more active—and both (*S*)- and (*R*)-selective—hybrid catalysts (up to 33% (*S*) and 27% (*R*), entries 2–4; for entries 5 and 6, vide infra).

Table 6. Operating conditions and selected results for the sulfoxidation of thioanisole catalyzed by [M-(Schiff base)]⊂Mb mutants.



	M(R_2-Schiff base)	Mb mutant	Rate[a]	*ee* [%]
1	Cr 5,5′-*t*Bu_2(salophen)	H64D/A71G	78	13 (*S*)
2	Cr 3,3′-Me_2(salen)	H64D/A71G	210	33 (*S*)
3	Mn 3,3′-Pr_2(salen)	A71G	2724	27 (*R*)
4	Mn 3,3′-Me_2(salen)	H64D/A71G	464	32 (*S*)
5[b]	Mn 4,4′-$thio_2$(salen)[c]	Y103C	51	12 (*S*)
6[b]	Mn 4,4′-$thio_2$(salen)[d]	L72C/Y103C	390	51 (*S*)

[a] Rate unit: 10^{-3} turnover min^{-1}. [b] Reaction conditions: NH_4OAc (50 mM), pH 5.1 at 4 °C, catalyst (130 μM), thioanisole (5 mM), H_2O_2 (6.5 mM). [c] Single anchoring through a disulfide bridge. [d] Double anchoring through two disulfide bridges.

Covalent Anchoring Strategies

In the context of covalent modification of proteins to afford artificial enzymes, two papers come to mind.[10,59]

Once again, Kaiser was the first to demonstrate that novel catalytic functions can be created by covalent modification of an amino acid residue with appropriately modified coenzyme analogues. His group reported in 1977 that flavopapain can be converted into a highly effective oxidoreductase by covalent modification of the thiol group of cysteine C25 with flavins. The resulting flavopapains exhibit significant rate enhancements (up to 670-fold) over native enzymes for the oxidation of dihydronicotinamides.[60]

Hilvert developed a chemical methodology for the conversion of the serine S221, buried within subtilisin's active site, into selenocysteine.[59,61] This semisynthetic enzyme was shown to:

1) function both as an acyl transferase, and
2) mimic glutathione peroxidase.

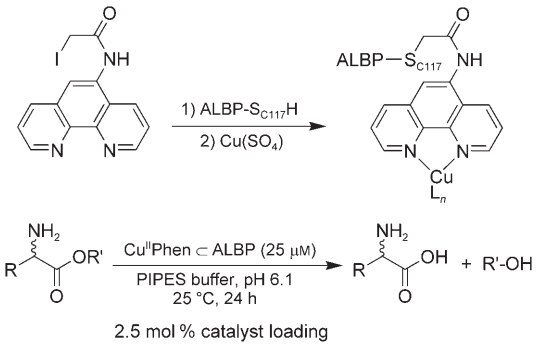
The first report on artificial *metallo*enzymes for *enantioselective* catalysis based on covalent anchoring was published by Distefano and co-workers,[13,62] who reported on the preparation of a 1,10-phenanthroline covalently linked to the adipocyte lipid-binding protein (Phen⊂ALBP). The unique cysteine residue cysteine C117, located in the interior of ALBP, reacts with iodoacetamido-1,10-phenanthroline to afford a thioether-linked artificial apo-metalloenzyme. After loading with Cu^{II}, the resulting hybrid catalyst Cu^{II}Phen⊂ALBP catalyzes the enantioselective hydrolysis of several unactivated amino acid ester substrates (Table 7).

This fascinating study reveals good kinetic resolution properties, but the turnover numbers leave room for improvement (Table 7, entries 3 and 5).

Lu and co-workers recently reported an enantioselective artificial sulfoxidation metalloenzyme based on a dual covalent anchoring strategy of a Mn(Schiff base) within Mb (Table 6).[63] The study suggests that dual anchoring to the L72C/Y103C Mb double mutant through two disulfide bridges, combined with the histidine H93 dative anchoring, affords higher selectivities than the related dative anchoring strategy pursued by Watanabe and co-workers for the sulfoxidation of thioanisole[57,58] (up to 51% *ee* (*R*), Table 6, compare entries 1–4 with entries 5 and 6).

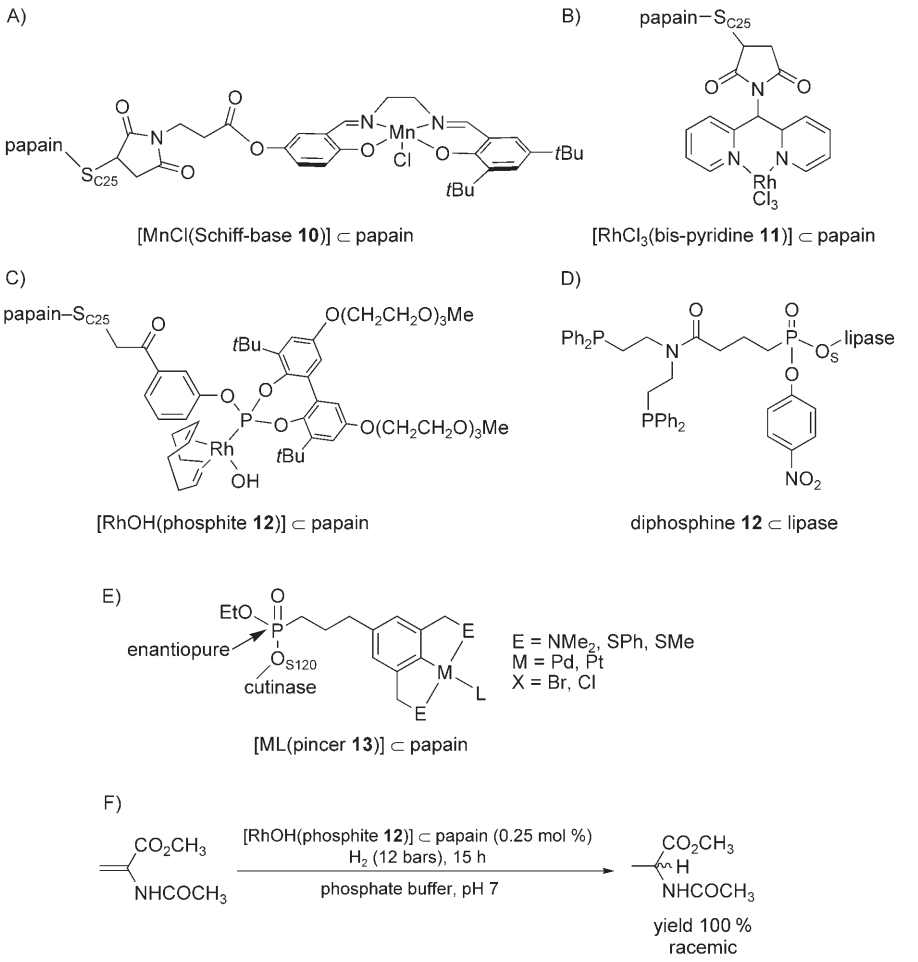
Since 2002, several groups have reported on their progress toward the covalent anchoring of achiral metal-containing catalyst precursors within host proteins to afford artificial metalloenzymes for enantioselective catalysis (Scheme 7). Reetz was the first to report covalent anchoring to papain, through Michael additions of the cysteine C25 to the maleinimide-derivatized Schiff base **10** or the bis-pyridine **11** (Scheme 7 A and B). Alternatively, a diphosphine **12**

Table 7. Preparation of Cu^{II}Phen⊂ALBP, operating conditions, and selected results for the enantioselective hydrolysis of amino acid ester derivatives.



	R	R′	TON	*ee* [%]	$k_{cat(D)}$ [$M^{-1}s^{-1}$]	$k_{cat(L)}$ [$M^{-1}s^{-1}$]
1	Me	Me	5.5	67	850	4200
2	Me	Et	3.0	40	830	1900
3	Me	*i*Pr	1.3	86	88	1200
4	$HOC_6H_4CH_2$	Me	7.6	39	2100	4900
5[a]	$HOC_6H_4CH_2$	Me	0.7	86	44	600

[a] $T = 4$ °C. TON: turnover number.



Scheme 7. Covalent anchoring of ligands and complexes to papain (A–C) and to lipases (D–E), and typical hydrogenation conditions used with [RhOH(phosphite **12**)]⊂papain (F).

was covalently anchored to a serine within a lipase through a phosphonate (Scheme 7D).[64] Catalyst localization was unambiguously established by enzyme inhibition experiments. In the case of the diphosphine **12**⊂lipase, however, esterase activity was largely restored within a day, suggesting release of the phosphonate ligand **12**.

Catalytic epoxidation and hydrogenation activity were detected for [MnCl(Schiff base **10**)]⊂papain and for [$RhCl_3$ (bis-pyridine **11**)]⊂papain. Unfortunately, the enantioselectivity turned out to be less than 10%.[64]

In a related study, de Vries and Feringa reported on the covalent anchoring of the monophosphite **12** to cysteine C25 in papain. In the presence of Rh^I, the [RhOH(phosphite **12**)]⊂papain catalyzes the reduction of methyl acetamidoacrylate. Although the artificial metalloenzyme displays good activities (quantitative conversion in the presence of 0.25 mol% within 15 h at room temperature), no enantioselectivity was detected.[65]

The reversible binding problem encountered with diphosphine **12**⊂lipase could be circumvented by using a pincer-type ligand bearing a phosphonate ester with a single nitrophenol leaving group.[66] Upon reaction with cutinase, irreversible covalent anchoring to serine S120 occurs enantioselectively (Scheme 7E). No report on the catalytic properties has thus far been reported.

Outlook

The purpose of this minireview is to present the state of the art in the area of artificial metalloenzymes for enantioselective catalysis from an historical perspective. All three anchoring strategies (i.e., supramolecular, dative, and covalent) have been exploited and, thus far, only the supramolecular approach has yielded good levels of enantioselection: >90% for hydrogenation, transfer hydrogenation, and Diels–Alder cycloadditions.

As mentioned in the Introduction, the power of directed evolution in optimizing enzymes is unrivalled. The prospects for combining both directed evolution protocols and chemical optimization are perhaps the most attractive feature of such artificial metalloenzymes. The presence of a well-defined second coordination sphere around an active catalyst might allow regio- and enantioselective derivatization of unprotected substrates containing several equally reactive functionalities. However, several technical hurdles must still be overcome:

1) Many metal-containing catalysts are incompatible with cellular extracts. This drawback requires purification of the host protein before anchoring of the metal moiety and performing of catalysis.
2) In contrast with small-molecule catalysts (e.g., organocatalysts), the molecular weights of the hybrid catalysts require very high TONs for the systems to be economically viable. Unfortunately, few water-compatible enantioselective catalytic systems to date display TONs>1000. For illustration, in the case of an artificial metalloenzyme (M_w=15 kDa) with TON=1000 for a substrate (M_w=0.15 kDa), the total mass of the catalyst would represent 10% of the mass of the substrate.
3) While efficient screening or selection protocols exist for kinetic resolutions, the number of reliable, easy to implement, and cheap assays for the enantioselectivity are unfortunately rather limited.[5,67–71]

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