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Artificial Metalloenzymes and Metallopeptide Catalysts for Organic Synthesis

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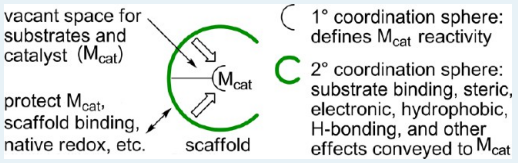
ABSTRACT: Transition metal catalysts and enzymes possess unique and often complementary properties that have made them important tools for chemical synthesis. The potential practical benefits of catalysts that combine these properties and a desire to understand how the structure and reactivity of metal and peptide components affect each other have driven researchers to create hybrid metal−peptide catalysts since the 1970s. The hybrid catalysts developed to date possess unique compositions of matter at the inorganic/biological interface that often pose significant challenges from design, synthesis, and characterization perspectives. Despite these obstacles, researchers have developed systems in which secondary coordination sphere effects impart selectivity to metal catalysts, accelerate chemical reactions, and are systematically optimized via directed evolution. This perspective outlines fundamental principles, key developments, and future prospects for the design, preparation, and application of peptide- and protein-based hybrid catalysts for organic transformations.



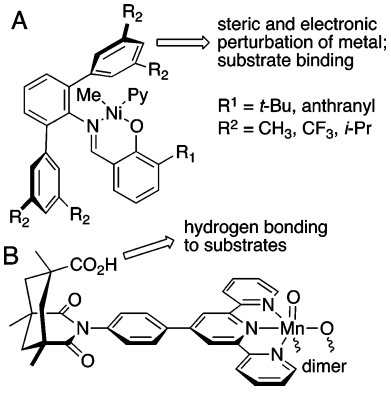
KEYWORDS: *artificial metalloenzymes, metallopeptide, molecular recognition, evolution, catalysis*

1. INTRODUCTION

Catalytic chemistry plays an essential role in the production and functionalization of an enormous range of chemicals, biomolecules, and materials.[1] The resulting products have a direct impact on human health, quality of life, and the global economy. Given this scope and importance, a range of different systems, including heterogeneous solids, homogeneous small molecules and metal complexes, and enzymes, have been developed as catalysts for chemical synthesis.[2] Each of these catalyst classes possesses a number of defining characteristics that differentiate their potential utility for problems in organic synthesis.

Homogeneous Transition Metal Catalysis. Homogeneous transition metal complexes catalyze a broad range of challenging chemical transformations, including cross-coupling reactions,[3] C−H bond functionalization,[4] and olefin polymerization[5] and metathesis.[6] In general, such catalysts consist of a metal ion bound to some number of ligands that comprise the primary coordination sphere of the metal.[7] These ligands can be readily and rationally modulated to alter the reactivity of the metal center and to generate chemoselective catalysts that react with a particular functional group, ideally, regardless of its molecular context. Such catalysts are said to have broad substrate scope. Ligand substituents proximal to the metal center can be used to modulate the interaction of the metal with substrates to impart stereo- and site selectivity. Importantly, ligand substituents distal to metal centers, those comprising the secondary coordination sphere of the metal, are increasingly appreciated to impact catalyst reactivity and selectivity.[8−11] Hydrogen bonding, hydrophobic, electrostatic, and steric interactions have been invoked in this regard,[12] as exemplified by two classes of catalysts. The first includes a number of Ni−salicylaldiminato complexes that exploit bulky substituted terphenyl groups on the salicylaldiminato core to control polyethylene molecular weight, branching, and microstructure through both steric and electrostatic (C−F···H−C hydrogen bonding) effects (Scheme 1A).[13,14] The second involves a carboxylic acid-substituted Mn−terpyridine complex that uses hydrogen bonding to position suitable carboxylic acid-substituted substrates for selective oxo-insertion into C−H and C═C bonds (Scheme 1B).[15,16] Altering such structures to fine-

Scheme 1. Structures of Substituted (A) Ni−Salicylaldiminato[13,14] and (B) Mn−Terpyridine[15,16] Catalysts Whose Activity Depends on Secondary Coordination Sphere Effects



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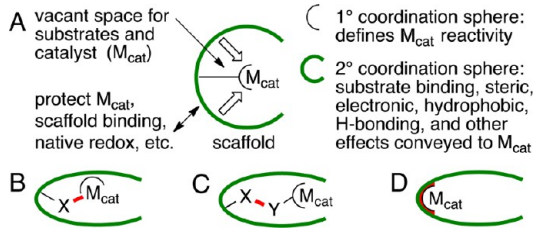
tune catalyst−substrate interactions can be synthetically challenging, and small molecule ligands offer limited control over the orientations of distal substituents in both a static and dynamic sense. The effects of these interactions also tend to be quite substrate-dependent,[8,9] which compromises catalyst generality. Given such limitations, the need persists for transition metal catalysts with well-defined secondary coordination environments that can be readily and extensively fine-tuned for particular applications.

Enzyme Catalysis. Enzyme catalysis is typically lauded for the extreme levels of rate acceleration and exquisite selectivity exhibited by many natural enzymes[17] on their native substrates.[18] Unlike small molecule catalysts, enzymes can differentially bind substrates and their associated activated complexes in three dimensionally defined yet dynamically fluxional[19] active sites to reduce barriers to chemical reactions.[20,21] Amino acid residues and cofactors are precisely oriented by the *entire* enzyme structure to modulate reaction energy surfaces through a combination of coordinated hydrogen bonding, hydrophobic, electrostatic, steric, and other interactions. Of course, this control arose through evolutionary processes (natural selection, neutral drift, etc.),[22] which can be mimicked in the laboratory using iterative rounds of catalyst diversification and functional screening or selection, to engineer enzymes with improved efficiency.[23,24] Thus, although a given enzyme may not catalyze a particular reaction or do so with sufficient selectivity or substrate scope, it can be evolved to increasingly extreme extent[25] to do so.[26] Furthermore, these catalysts can be genetically incorporated into living organisms to enable direct synthesis of complex molecules without the need for isolation and purification of intermediates.[27] However, whereas laboratory evolution has been used to create enzymes from noncatalytic scaffolds,[28,29] it is most effective at optimizing activities that are already present,[23] and despite exciting progress toward the design of enzymes for new reactions,[30−32] many reactions, particularly those catalyzed by nonbiological metals, are likely not possible without the introduction of such species.

2. HYBRID METAL−PEPTIDE CATALYSTS

As can be appreciated from the discussion above, transition metal catalysts and enzymes possess unique and often complementary[33] properties that have made them important tools for chemical synthesis. The potential practical benefits of catalysts that combine these properties and a desire to understand how the structure and reactivity of metal and peptide components affect each other have driven researchers to create hybrid metal−peptide catalysts since the 1970s (Scheme 2A).[34−37] Central to these efforts are robust methods to incorporate transition metals into peptides or proteins, hereafter collectively referred to as scaffolds. These can be broadly classified as involving coordination of metal atoms by scaffold residues (scaffold−M, where M is a free ion or complexed with other nonprotein ligands), covalent scaffold modification using functionalized catalysts (scaffold−cat), or noncovalent catalyst binding, either to the catalyst itself or to a catalyst substituent (cat⊂scaffold) (Scheme 2B−D).[34] Combinations of these classes are possible; the latter two can also involve the introduction of ligands that can be subsequently metalated, and each has advantages and disadvantages that make it more or less suitable for particular applications. The hybrid catalysts developed to date using these methods possess unique compositions of matter at the inorganic/biological

Scheme 2. (A) General Hybrid Catalyst Structure and Structures of Hybrid Catalysts Formed via (B) Coordination of Metal Ions or Catalysts, (C) Covalent Scaffold Modification, and (D) Noncovalent Scaffold Modification[a]



[a]Scaffold linkage sites for each method are highlighted in red.

interface that often pose significant challenges from design, synthesis, and characterization perspectives. Despite these obstacles, researchers have developed systems in which secondary coordination sphere effects[38] impart selectivity to metal catalysts,[39,40] accelerate chemical reactions,[41] and are systematically optimized via directed evolution.[39,40] Although much work will be required to enable practical application of hybrid catalysts, these capabilities have the potential to impact chemical synthesis in ways not readily achieved using small molecule catalysts.

This perspective outlines a number of fundamental principles and key developments in the design, preparation, and application of peptide-based hybrid catalysts for organic transformations. Catalysts derived from both peptide and protein scaffolds are covered and will be referred to as metallopeptide catalysts and artificial metalloenzymes (ArMs), respectively. A practical division between these two scaffold classes of ∼50 residues in a single peptide chain, the upper range available directly via solid phase synthesis,[42] was selected. Related catalysts and materials based on DNA[43] and PNA[44] or describing only metal binding,[45−48] probe bioconjugation,[38] or inorganic redox chemistry[49] are generally not covered, despite the importance of these advances. The many interesting examples in which natural metalloenzymes have been used to catalyze unnatural reactions or modified via mutagenesis using natural amino acids to do so are not broadly discussed because of the different challenges encountered in such efforts relative to incorporating nonnative metal catalysts into scaffolds.[50−53] Even with these limitations, a large body of work has emerged regarding peptide-based hybrid catalysts for chemical synthesis. These will be discussed in terms of the classifications noted above, but as will be evident, these are not always absolute and serve only as a means of organization.

3. ARTIFICIAL METALLOENZYMES

3.1. Binding Metal Ions and Complexes (Scaffold−M).

Preparing a homogeneous transition metal catalyst typically involves combining a metal catalyst precursor (M) with one or more small molecule ligands (L) to form a complex via one or more metal−ligand bonds (L−M).[7] The ligands employed can range from simple, low-molecular-weight, monodentate species (e.g., halides, carboxylic acids, amines, phosphines, etc.) to complex, polydentate, high-molecular-weight structures, such as the substituted salicylaldiminato and terpyridine ligands noted above.[13−15] Proteins, which contain a range of metal-binding N, O, and S functional groups within well-defined, three-dimensional, chiral structures, can be viewed as yet another step along this ligand complexity continuum and have therefore

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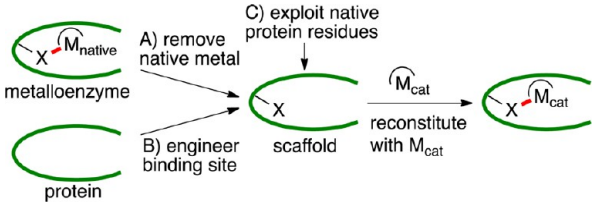
attracted attention as scaffolds for ArM formation via metal coordination (Scheme 2B).[49,54]

Of course, binding sites for catalytically active metal ions and cofactors have evolved in naturally occurring metalloenzymes,[55,56] and these have been investigated as ligands for nonnative metal ions commonly used to catalyze organic transformations (Scheme 3A).[54] This approach requires that the desired protein can be expressed without the native metal ion (as an apoprotein) or that an efficient method, such as dialysis against a metal chelator, can be used to remove the native metal ion. Furthermore, the apoprotein must be stable, and efficient reconstitution of this protein with the nonnative metal ion, often via dialysis against this species, must be possible. This approach was first used by Kaiser and co-workers in 1976 at the University of Chicago to convert carboxypeptidase A (CPA), which naturally contains a Zn(II) ion, to a Cu(II) CPA oxidase.[57] Spectroscopic studies of the resulting ArM indicated that the coordination environment about Cu(II) was significantly perturbed from that observed with Zn(II). The ArM completely lacked the peptidase and esterase activities of the native enzyme but gained the ability to oxidize ascorbic acid to dehydroascorbic acid following Michaelis–Menten kinetics. These results showed that although natural enzymes offer a means to create ArMs, care must be taken in generalizing the structure (and thus activity) of the resulting ArM on the basis of the coordination environment of the original enzyme. In analogy to this work, the tris-histidine-ligated active site Zn(II) of human carbonic anhydrase (hCA) or carbonic anhydrase II (hCAII) was removed using dialysis with 2,6-pyridinedicarboxylate and reconstituted with both Mn(II) and Rh(I). The resulting ArMs catalyze enantioselective alkene epoxidation (up to 66.5% ee, 12.5% conversion, Scheme 4A),[54,58,59] chemoselective alkene hydrogenation (Scheme 4B),[60] and regioselective alkene hydroformylation (up to 8:1 L/B, Scheme 4C),[61] demonstrating a clear impact of the scaffold on catalyst selectivity in each case. Crystal structures of hCAII substituted with Co(II), Cu(II), Ni(II), and Mn(II) have been solved,[62] and as described for metal-substituted CPA, the primary coordination sphere of each of these is uniquely perturbed (e.g., residue distances, bond angles, and water/sulfate ligands) relative to the native Zn(II) CA,[63] although the overall metal binding motif remains intact (Figure 1).

Scheme 3. Different Approaches to ArM Formation via Scaffold Metallation[a]



[a](A) Reconstituting metalloenzymes, (B) engineering metal binding sites into proteins, and (C) exploiting fortuitous metal binding by native residues in proteins.

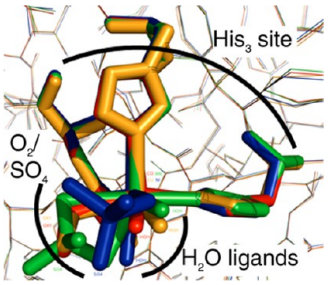
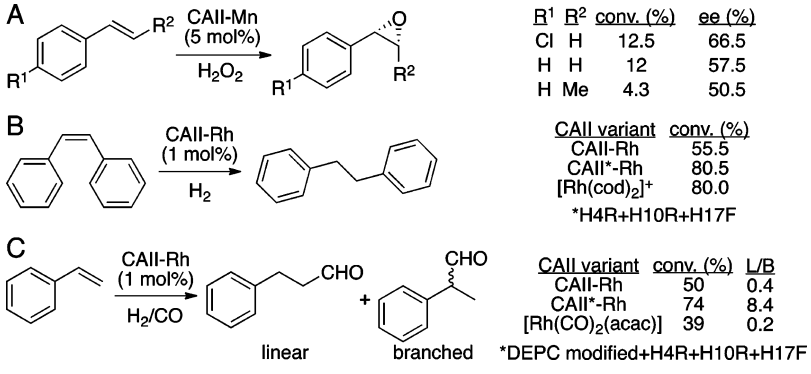


Figure 1. Overlay of structures of CA substituted with Co (red), Cu (yellow), Mn (green), and Ni (blue) showing different primary coordination sphere orientations of water, O_2 (Co and Cu), and sulfate (Mn and Ni) ligands. Arcs indicate the general positions of the ligands indicated.[62]

Ferritin, a spherical iron storage protein assembly consisting of 24 subunits, can be recombinantly expressed in the apo form and reconstituted with a range of metal ions and complexes.[64−67] Watanabe has reported that apo-ferritin (Figure 2A) reacts selectively with $[PdCl(\eta^3\text{-}C_3H_5)]_2$ to form dinuclear $Pd(\eta^3\text{-}C_3H_5)$ adducts at two unique Pd-binding sites (Figure 2B) on each subunit (4 Pd atoms per subunit), leading to a total of 96 Pd atoms on the ferritin interior.[64] Site-directed mutagenesis was used to replace different histidine residues involved in the two Pd-binding sites with alanine, which led to alternate Pd coordination environments and stoichiometry, as confirmed by X-ray crystallography (Figure 2C). Each of these species catalyzed a Suzuki coupling between 4-iodoaniline and phenylboronic acid with efficiencies that roughly correlated with the ArM Pd loading. Potential effects of the different Pd binding sites on catalysis and on substrate entry into the ArM

Scheme 4. Representative Examples of Reactions Catalyzed by Metal-Substituted CA Variants[a]



R1	R2	conv. (%)	ee (%)
Cl	H	12.5	66.5
H	H	12	57.5
H	Me	4.3	50.5

CAII variant	conv. (%)
CAII-Rh	55.5
CAII*-Rh	80.5
$[Rh(cod)_2]^+$	80.0

*H4R+H10R+H17F

CAII variant	conv. (%)	L/B
CAII-Rh	50	0.4
CAII*-Rh	74	8.4
$[Rh(CO)_2(acac)]$	39	0.2

*DEPC modified+H4R+H10R+H17F

[a](A) Enantioselective olefin epoxidation.[54,58,59] (B) Olefin hydrogenation: variable ratios of olefin hydrogenation and isomerization were observed (data not shown).[60] (C) Olefin hydroformylation: significantly improved B/L ratio observed for ArM-catalyzed process.[61].

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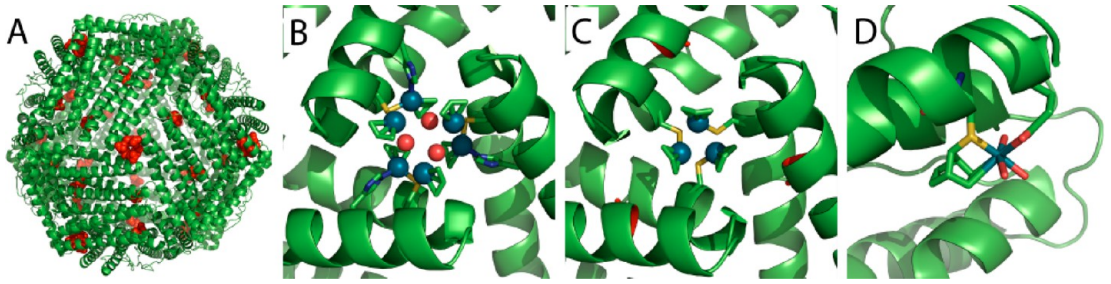
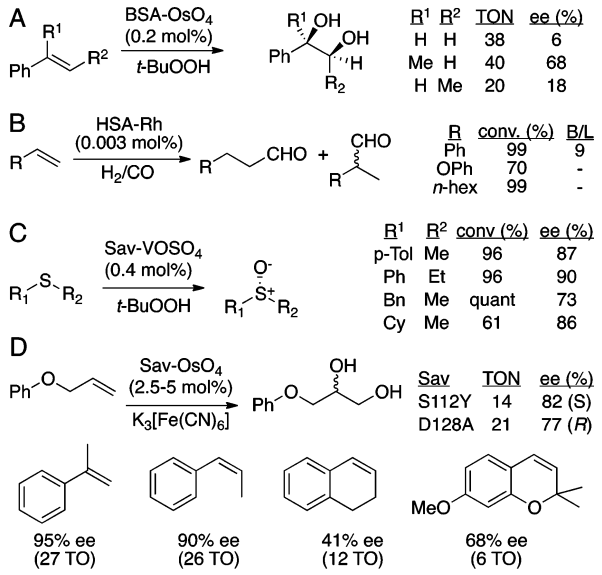


Figure 2. (A) Structure of apo-ferritin–Pd with Pd(η^3-C_3H_5) fragments shown as red spheres.[64] (B) One of the two Pd binding sites in apo-ferritin–Pd. Pd and water are shown as turquoise and red spheres, respectively. (C) H114A (red) causes a change in Pd binding and stoichiometry at the binding site shown in B. (D) Covalent norbornyl adduct formed at a Rh binding site in apo-ferritin–Rh generated upon addition of $[Rh(NBD)Cl]_2$ to apo-ferritin.[65]

were proposed, but no mention was made of possible catalysis by dissociated Pd.[68] Watanabe also showed that selective reaction of apo-ferritin with $[Rh(NBD)Cl_2]_2$ was possible, and crystal structure analysis revealed three unique Rh-binding sites per subunit, one of which involved covalent attachment of NBD to a cysteine thiol (Figure 2D).[65] Notably, the ferritin–Rh complex catalyzed phenylacetylene polymerization, and differences in the properties of polymer produced by this ArM versus $[Rh(NBD)Cl]_2$, including increased polymer solubility, narrowed polydispersity, and a maximum polymer molecular weight that did not increase with extended reaction time or excess monomer, led the authors to conclude that catalysis occurred within the ArM interior in this case.

Although naturally occurring metal binding sites can facilitate ArM formation, they also limit the range of primary coordination environments, metal ions, and catalytic activities that can be used. Researchers have therefore explored the metal-binding capabilities of proteins that do not naturally bind metals (Scheme 3C). For example, serum albumins, which naturally bind and transport hydrophobic substrates in blood serum, can also bind catalytically active metal ions and complexes. In an early example exploiting this capability, researchers generated a complex between bovine serum albumin (BSA) and OsO_4.[69] On the basis of comparison of the absorption spectra for this complex and related small-molecule osmate complexes, a primary amine (i.e., lysine) linkage was inferred, but no further structural details were provided. The BSA–OsO_4 ArM catalyzed enantioselective cis-dihydroxylation of alkenes using *t*-butyl hydroperoxide (up to 68% ee, Scheme 5A). A human serum albumin (HSA) complex with $Rh(CO)_2(acac)$ provided up to 500 000 turnovers for hydroformylation of styrene (99% conversion, 9:1 B/L, Scheme 5B), and this efficiency was attributed to exclusion of O_2 and other poisons from the Rh center by the ArM scaffold.[70] Maximal activities were observed using >5:1 Rh/HSA, suggesting that multiple nonspecific binding sites existed (no enantioselectivity data were presented), but similar adducts with papain and egg albumin led to variable B/L ratios on 1-octene, 45:55 and 0:100, respectively, indicating that scaffold control of catalyst selectivity was possible. Albumins and several other proteins have also been used to bind vanadate ions to generate ArMs for enantioselective sulfoxidation.[71] Notably, vanadate-loaded streptavidin (Sav) catalyzes enantioselective thioether sulfoxidation with up to 90% ee at 96% conversion (Scheme 5C),[72] and this same scaffold binds OsO_4 to generate ArMs that catalyze enantioselective olefin cis-dihydroxylation (>90% ee at ~20 TON, Scheme 5D).[73] In this later case, ArMs selective for either product enantiomer were identified, and

Scheme 5. Selective Catalysis Using ArMs Generated from Coordination of Metal Complexes to Scaffold Proteins Whose Native Function Is Not Primarily Metal Binding[a]



[a]Enantioselectivity was observed in A,[69] C,[72] and D,[73] but only regioselectivity was reported for B.[70].

although X-ray crystallography revealed three Os-binding sites in Sav, the actual catalytic site(s) could not be confirmed.

Although novel metal binding capabilities of proteins can certainly be exploited for ArM formation and catalysis, different metal species can bind to proteins with varying degrees of stoichiometry and selectivity, as illustrated in the Sav–OsO_4 and ferritin–Pd and –Rh examples outlined above, which can lead to catalyst mixtures with varying selectivity. To expand the range of coordination environments that can be used to *selectively* bind metals, researchers have turned to engineering metal binding sites into proteins (Scheme 3B). Whereas metal binding sites have been designed, selected, or evolved in a number of proteins,[36,49,74] few of these were intended for catalysis. Of the examples for which catalysis was a goal, reactions of oxygen[75−77] or ROS[78] but no organic substrate are the most common. More relevant to organic synthesis, Reetz and co-workers engineered a copper-binding site (His/His/Asp, HHD) near the top rim of the α/β-barrel protein tHisF, the thermostable synthase subunit of the glutaminase synthase enzyme complex from *Thermotoga maritima*.[79] Following removal of a native cysteine residue, the resulting protein was metalated with $CuSO_4$, and the resulting ArM (tHisF-HHD–

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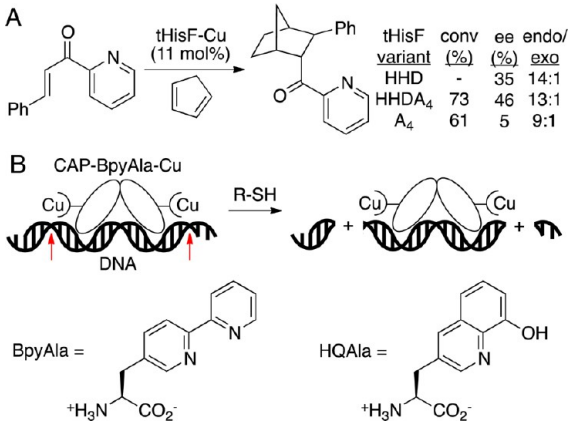
Cu) catalyzed enantioselective Diels–Alder reaction between an azachalcone and cyclopentadiene with 35% enantioselectivity (Scheme 6A). Notably, mutating to alanine (A_4) four

Scheme 6. (A) Enantioselective Catalysis Using an ArM Generated from a Designed Metal Binding Site[79] and (B) Cartoon Representation of Hybrid Catalyst Nuclease Generated from Incorporation of the Metal Binding Amino Acid BpyAla into the Transcription Factor CAP[83]

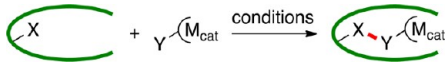


histidine residues that could also bind Cu to generate nonselective catalysts increased reaction selectivity (up to 46% ee, 13:1 endo/exo). To eliminate the design challenges associated with engineering metal-binding sites into proteins, Schultz used unnatural amino acid (UAA) mutagenesis[80] to install specific metal-binding amino acids into proteins. Initially, (2,2′-bipyridin-5-yl)alanine (BpyAla) was incorporated into T4 lysozyme, which was then selectively metalated using Cu(II) to generate the corresponding Cu(II)–BpyAla metalloprotein.[81] Cu(II)Bpy and other Cu(II) and Fe(II) complexes react with O_2 in the presence of sulfide reducing agents to generate ROS (e.g., hydroxyl radical) that randomly cleaves nucleic acids and proteins. Several groups have developed hybrid catalysts exploiting this reactivity for selective nuclease and protease activity (vide infra).[82] Indeed, Schultz found that incorporating BpyAla into the *Escherichia coli* catabolite activator protein (CAP) at a site proximal to the CAP–DNA interface led to the formation, after metalation with Cu(II), of a site-specific ArM nuclease (Scheme 6B).[83] A hydroxyquinone-based UAA (HQAla) was also incorporated into proteins and metalated with Zn(II), but no catalysis has been described to date.[84]

3.2. Covalent Linkage of Metal Catalysts and Ligands.

Coordinating metals with proteins can enable ArM formation in much the same way that metals and ligands are mixed together to form small molecule catalysts; however, this approach is generally limited to coordination by the 20 natural amino acids, and many reactions are catalyzed by metals with ligands not found in nature (e.g., carbenes, phosphines, etc.). Although UAA mutagenesis can be used to expand this scope, this process itself requires extensive engineering for each desired amino acid and is limited to complexes that can be formed in the presence of a protein. To more readily expand the range of catalysts that can be incorporated into proteins, researchers have developed methods to covalently link synthetic, catalytically active transition metal cofactors to proteins (Scheme 2C).[85,86] At a minimum, covalent ArM formation (bioconjugation) requires a scaffold protein containing a uniquely reactive residue (typically a nucleophile) and a cofactor substituted with the corresponding reaction partner (typically an electrophile), both of which present unique synthetic challenges (Scheme 7).

Scheme 7. Representative Covalent Attachment Methods



Of the many bioconjugation reactions developed to date,[87] cysteine alkylation has found the most application in ArM formation (Table 1, entries 1–6). Cysteine mutations can be readily introduced into proteins, but any additional reactive cysteine residues must also be removed, which is time-consuming and can be problematic if these residues are structurally important. The native activity of some enzymes can simplify the bioconjugation process. For example, many hydrolase enzymes contain uniquely nucleophilic serine or cysteine residues in their active sites that can react selectively with suitable electrophiles (Table 1, entry 8). This eliminates the need for scaffold engineering (e.g., installing or removing cysteine residues) but limits the approach to hydrolase enzymes. Several other bioconjugation reactions have been used to form ArMs, and these will be discussed below (Table 1). In addition to facilitating bioconjugation, a scaffold's native function can be exploited for ArM catalysis (Scheme 2A). For example, substrate binding by an antibody or nuclease can be used to convey selectivity to ArMs generated from these scaffolds, and native electron transport proteins can be used to reduce synthetic cofactors to enable new chemistry in an ArM.[88] Regardless of their native function, scaffolds must be sufficiently large to contain both the cofactor linker and metal complex. This has led to the exploration of enzymes and complexes that contain significant amounts of "vacant space,"[37] including α/β-barrel proteins, apo-heme proteins, and protein dimer interfaces as ArM scaffolds. Because vacant space also provides room for different linker conformations and cofactor positioning within the ArM, a balance must be struck between providing room for cofactor introduction and restricting cofactor movement for selective catalysis.

Althuogh the range of potential bioconjugation reactions available provides some flexibility for scaffold preparation, cofactor synthesis is often complicated by the need to incorporate a reactive metal center for catalysis and a reactive functional group for bioconjugation in the same molecule (Table 1). The number of potential catalyst/functional group combinations makes any generalization of cofactor synthesis impossible. In some cases, it may be possible to metalate an otherwise complete cofactor, but in other cases, it may be preferable to install a reactive functional group on a substituted metal catalyst. If neither of these is possible, apo-cofactors can be covalently linked to proteins and subsequently metalated in analogy to the metal coordination approaches outlined above.

Kaiser provided seminal examples of cofactor bioconjugation to generate artificial enzymes in his work on flavopapain oxidoreductases.[89] In these systems, the active site cysteine of papain, a cysteine protease, was alkylated by bromomethyl- and α-bromoacetyl-substituted flavins (Table 1, entries 1 and 2).[90] The resulting flavopapains catalyzed oxidation of dihydronicotinamides with up to 50-fold rate acceleration over the uncatalyzed air oxidation reactions and exhibited k_{cat}/K_m values similar to those of natural flavin oxidases (e.g., glucose oxidase). Although no metals were involved in these examples, they

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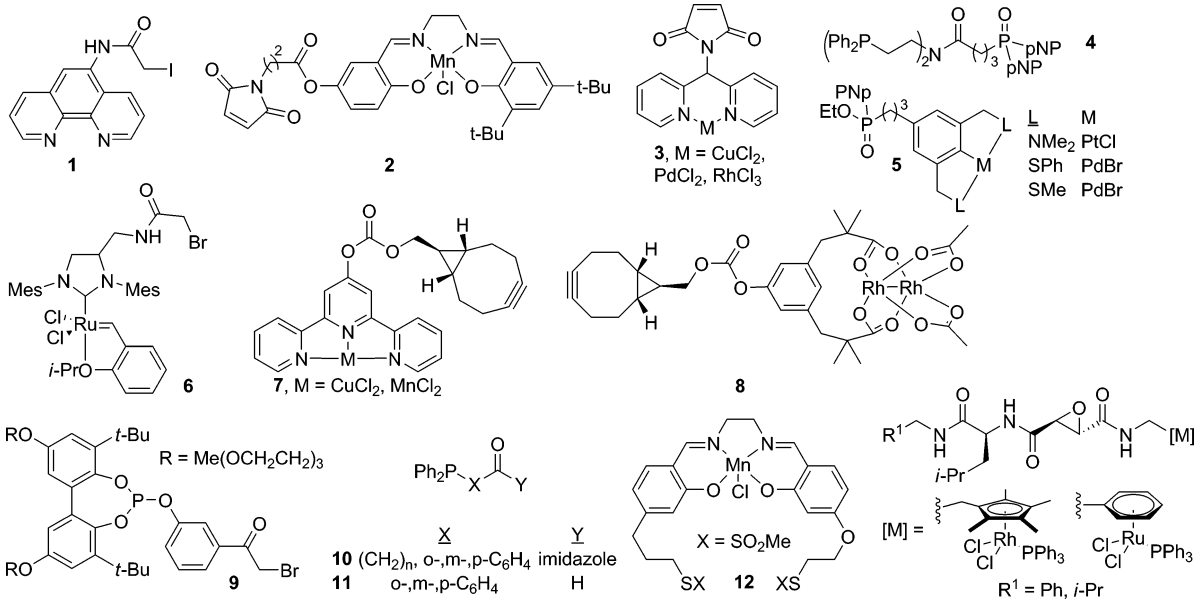
Table 1. A Summary of Bioconjugation Methods Used To Covalently Link Synthetic Cofactors to Protein Scaffolds

Entry	X (residue)	Y	X-Y
1[89,90]	R–SH (cysteine)	R'–Br	R–S–R'
2[92,99,102,112,113]	R–SH (cysteine)	R'–C(=O)–X; X = Br, I	R–S–C(=O)–R'
3[105]	R–SH (cysteine)	R'–S(=O)–S–CH3	R–S–S–R'
4[94,95,109,110]	R–SH (cysteine)	N-substituted maleimide	thiosuccinimide
5[103]	R–SH (cysteine)	R'–C(=O)–N(imidazole)	R–S–C(=O)–R'
6[104]	R–SH (cysteine)	succinimide–NHNH2, then R'–CHO	R–S–succinimide–HN–N=CH–R'
7[91]	R–NH2 (lysine)	2-iminothiolane, then R'–C(=O)–X	R–NH–C(=NH2+)–S–CH2–CH2–C(=O)–R'
8[94,96,97]	R–OH (serine hydrolase)	R'–P(=O)(pNP)(OAk/pNP); pNP = *p*-nitrophenol	R–O–P(=O)(OAk/pNP)–R'
9[100]	R–N3 (*p*-azidophenylalanine)	strained alkyne	triazole

Scheme 8. Representative Cofactors and Ligands Used To Generate ArMs via Covalent Scaffold Modification



showed that bioconjugation could be used to generate hybrid catalysts and paved the way for incorporation of metal cofactors. One of the earliest examples of covalent ArM formation involved modifying the primary amines (4 lysine residues) on the *E. coli* Trp repressor protein (*trp*) with iminothiolane followed by sulfur alkylation with 5-iodoacetamide-1,10-phenanthroline (**1**, Scheme 8) and metalation using Cu(II) (Table 1, entry 7).[91] The resulting ArM-catalyzed site-specific cleavage of a DNA fragment containing the *aroH* transcription unit naturally recognized by the *trp* in the presence of Trp and 3-mercaptopropionic acid in a manner analogous to the later work described above (Figure 6B). Since this example, a number of related efforts have utilized haloacetamide-substituted phenanthroline ligands (subsequently metalated with Cu(II)) or similarly substituted Fe(III)−EDTA complexes (either preformed or generated

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following bioconjugation) to alkylate various proteins that bind specific nucleic acids or proteins.[85] Bioconjugation was typically conducted using either 2-iminothiolane-modified lysine residues as outlined above or, more commonly, using genetically encoded cysteine residues. The resulting ArMs uniquely enabled site-specific cleavage of DNA, RNA, and protein substrates and clearly illustrate the potential for substrate recognition imparted by protein scaffolds to impact reaction selectivity and substrate specificity.[82]

Following these successes, researchers began to utilize covalently generated ArMs for organic synthesis. Distefano and co-workers established that adipocyte lipid binding protein (ALBP), a small globular protein containing a unique cysteine residue within a large (600 Å^3) cavity, could be used as a scaffold for covalent attachment of **1** (Scheme 8; Table 1, entry 2).[92] The resulting conjugate was metalated with Cu(II) and used to catalyze amide bond hydrolysis or kinetic resolution of amino acid esters via ester bond hydrolysis. Although up to 86% ee was observed in the hydrolytic resolution of L-tyrosine methyl ester, a maximum of only 7.6 turnovers was possible. Analysis of the ArM crystal structure indicated that the phenanthroline ligand was *completely buried* within the globular protein and that little structural perturbation resulted from cofactor bioconjugation (Figure 3).[93] A broad range of

Figure 3. Crystal structure of ALBP−phenanthrene bioconjugate showing location of the phenanthrene ligand deep within the protein interior, sequestered from bulk solvent.[93]

chemistries have since been examined in different ArM constructs. Reetz demonstrated that maleimide-substituted Mn-salen (**2**) and Cu-, Pd-, and Rh-bipyridine (**3**) cofactors could be linked to papain[94] and tHisF[95] via cysteine alkylation (Scheme 8; Table 1, entry 4), but low enantioselectivities (<10% ee) were reported for unspecified epoxidation and hydrogenation reactions catalyzed by these ArMs. Both Reetz[94] and van Koten[96,97] demonstrated that phosphonate-substituted cofactors, including bisphosphine ligand **4** and Pt− and Pd−pincer complexes **5** (Scheme 8), could be covalently linked to various serine hydrolases in analogy to known inhibitors of these enzymes,[98] but no catalysis was reported. Ward and Hilvert demonstrated that the G41C mutant of a small heat shock protein from *Methanococcus jannashii* could be alkylated using the substituted Grubbs−Hoveyda catalyst **6** (Scheme 8; Table 1, entry 2) to generate ArMs for olefin metathesis (up to 33 TON).[99] We have also demonstrated that unnatural amino acid mutagenesis can be used to incorporate *p*-azidophenylalanine into proteins to enable bioorthogonal click reactions of bicyclononyne-substituted cofactors via strain-promoted azide−alkyne cycloaddition (SPAAC; Table 1, entry 9).[100] The bioorthogonality of this reaction eliminates the need to remove native amino acid residues in the scaffold and should facilitate ArM formation under a variety of reaction conditions.[101] Indeed, we demonstrated that BCN-substituted Mn− and Cu−terpyridine (**7**) and dirhodium tetracarboxylate (**8**) cofactors could all be incorporated into different scaffold proteins using this approach (Scheme 8). The dirhodium ArMs catalyzed cyclopropanation of *p*-methoxystyrene using ethyl diazoacetate and carbene insertion into the Si−H bond of diphenylmethylsilane using methyl phenyldiazoacetate.

Given the importance of phosphorus-based ligands in transition metal catalysis, covalent modification of proteins using substituted phosphine ligands and complexes has received significant attention. In addition to the reports from Reetz noted above (**4**), Feringa and co-workers demonstrated that papain could be alkylated with phosphite **9**, which could subsequently be metalated with $[Rh(COD)_2]BF_4$ (Scheme 8).[102] The resulting ArM-catalyzed hydrogenation of methyl 2-acetamidoacrylate, but low enantioselectivities (<10% ee) were reported for all reactions studied. More recently, Kamer has shown direct modification of the unique cysteine residue in photoactive yellow protein (PYP) using phosphines (**10**, Scheme 8) substituted with a 1,1′-carbonyldiimidazole-activated carboxylic acid moiety (Table 1, entry 5).[103] Phosphine-substituted PYP was metalated with $[PdCl(\eta^3\text{-}C_3H_5)]_2$, and the resulting ArM-catalyzed allylic amination of 1,3-diphenylprop-2-enyl acetate with benzylamine, but no enantioselectivity was reported. Because of the reactivity of phosphine ligands toward the maleimide anchor often used for covalent cysteine modification, these researchers also developed a two-step protocol involving initial bioconjugation of a hydrazine-substituted maleimide cross-linker followed by imine formation between the hydrazine and aldehyde-substituted phosphine ligands (**11**, Scheme 8), but no catalysis was reported (Table 1, entry 6).[104]

These examples show that whereas ArMs generated via covalent modification of scaffold proteins have been used for selective biomolecule cleavage, selective reactions on small-molecule substrates have proved challenging. Low ArM selectivity has been rationalized in terms of poor control over cofactor/scaffold (secondary coordination sphere) interactions, resulting from large active site volumes,[37] linker length,[100,102] and linker flexibility.[105] In most cases, wild-type proteins or cysteine mutants thereof were used as scaffolds, and few attempts were made to improve selectivity via scaffold mutagenesis, which Reetz pointed out would likely be required for selectivity (vide infra). However, different linkage strategies have been explored to correct these problems. For example, although all the examples outlined above involve single covalent bond between cofactor and scaffold, Lu found that a dual-point attachment of the doubly methane thiosulfonate-substituted Mn−salen complex **12** to a cysteine double mutant (L72C/Y103C) of apo-myoglobin (apo-Mb) led to improved selectivity (51% ee) for thioanisole sulfoxidation relative to the analogous single-point mutant (Y103C, 12% ee).[105]

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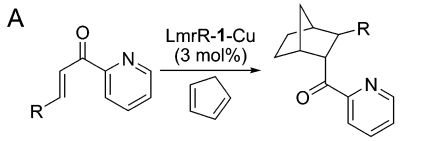
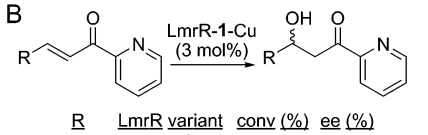
Subsequent studies using Mb-T39C/L72C-**12** showed that improved enantioselectivity (up to 60% ee) and exclusive selectivity for sulfoxidation over sulfone formation could be achieved.[106] Polar, protic residues in the ArM active site were proposed to increase sulfoxidation efficiency based on solvent effects observed for small-molecule Mn−salen-catalyzed reactions. The hydrophobicity of the channel leading to the active site was believed to inhibit sulfoxide entry and thus reduce sulfone formation, and this conclusion was supported by increased levels of sulfone formation upon introduction of polar residues in the channel. Examining the effect of pH on this reaction revealed that the protein scaffold improved sulfoxidation rates at low pH relative to free cofactor and provided further increases in ArM enantioselectivity (up to 67% ee).[107] These effects were rationalized by invoking the involvement of specific active site residues (i.e., His-64) in catalysis via hydrogen bonding. This proposition was supported by mutagenesis studies and again highlights the potential for control over secondary coordination sphere effects using ArMs.

Eppinger explored the use of cofactors substituted with both noncovalent recognition elements (amino acids) and a reactive electrophile (epoxide) to better position substituted d_6-transition metal piano stool cofactors (**13**, Scheme 8) within papain for enantioselective ketone transfer hydrogenation.[108] Notably, the cofactors were *not* chiral at metal (vide infra), and previous studies by Salmain using only covalent attachment of such cofactors to papain[109,110] led to low enantioselectivities (up to 15% ee).[111] In Eppinger's work, different amino acids appeared to play only a minor role in modulating enantioselectivity, but an 82:18 enantiomeric ratio was observed in the reduction of *p*-chloroacetophenone. Finally, Roefles demonstrated that LmrR, a dimeric transcription repressor, could be alkylated at cysteine residues installed at the hydrophobic dimer interface using phenanthroline **1** (Table 1, entry 2, Scheme 8).[112] This approach allows for cofactor encapsulation within the scaffold complex. The apo-scaffolds were metalated with Cu(II), and the resulting ArMs catalyzed the Diels−Alder reaction between azachalcones and cyclopentadiene with up to 97% ee (Scheme 9A). Interestingly, an ArM derived from a bipyridine analogue of **1** provided the opposite product enantiomer in the single example reported,

Scheme 9. (A) Diels−Alder and (B) Hydration Reactions Catalyzed by LmrR Covalently Modified Using 1 and Subsequently Metalated with Cu(II)[112]



R	LmrR variant	conv (%)	ee (%)	endo/exo
Ph	M89C,V15A	89	97	96:4
m-MeOPh	M89C	56	93	96:4
Me	M89C	97	<5	-



R	LmrR variant	conv (%)	ee (%)
i-Pr	M89C	67	77
t-Bu	M89C	80	84
n-pent	M89C	57	67
i-Pr	M89C,D100A	28	<5

and in several reactions, significant rate increases were observed in ArM-catalyzed reactions relative to those catalyzed by Cu(II)−phenanthroline. These same ArMs catalyzed hydration of azachalcones with up to 84% ee, demonstrating that different reactions may be feasible using a given class of ArMs in analogy to small molecule catalysts (Scheme 9B).[113] Substrate-dependent scaffold acceleration was also observed in these reactions, and the importance of an active site aspartic acid (Asp-100) to ArM efficiency suggested that this residue plays a critical role in catalysis, perhaps as a general base to activate water for nucleophilic attack or as a ligand for Cu. These results stand among the highest selectivities reported to date using covalent scaffold modification and, along with the improved selectivities observed for dual-point cofactor attachment, suggest that additional cofactor−scaffold interactions can better position cofactors substituted with flexible linkers within ArM scaffolds for selective catalysis.

3.3. Noncovalent Linkage of Metal Catalysts and Ligands (Cat⊂Scaffold). Noncovalent interactions have also been extensively used to incorporate metal cofactors into scaffold proteins without the need for direct coordination of the metal by the scaffold (Scheme 2D). This approach eliminates the need for covalent scaffold modification while still allowing the use of diverse cofactors for ArM formation, but requires specific scaffold−cofactor interactions that restrict the range of scaffold proteins that can be used. Binding both to catalysts directly or to pendant anchor groups on the desired catalyst has been used (Scheme 10). Although cofactor synthesis is also

Scheme 10. Noncovalent ArM Formation via Binding (A) Directly to a Catalyst or (B) to a Ligand (blue)-Substituted Catalyst; Red Denotes Scaffold−Catalyst/Ligand Binding Interactions

required, this is often less challenging than the preparation of cofactors for covalent scaffold modification because reactive functional groups are not required.

Heme proteins, particularly myoglobin (Mb), have enjoyed a long history as ArM scaffolds because of the ability of their heme-binding pockets to accommodate synthetic metal catalysts, including substituted porphyrin and Schiff base complexes.[114,115] In native heme proteins, the heme cofactor is tightly bound via extensive hydrophobic interactions with the porphyrin ring, hydrogen bonding to heme carboxylic acid substituents, and coordination of the metal atom by an axial ligand (e.g., His-93 in Mb), and all of these can be exploited to incorporate synthetic cofactors. Watanabe and others have reported extensive studies on the peroxidase activity of Mb mutants[116] and ArMs derived from Mb via reconstitution with chemically altered heme cofactors.[108,110,111] Watanabe then demonstrated that reconstitution of Mb with Mn(III)− and Cr(III)−salophen complexes was also possible (**14**, Scheme 11) and that Cr(III)salophen⊂Mb ArMs catalyzed thioanisole sulfoxidation in the presence of hydrogen peroxide with up to 13% ee (Scheme 11).[117] Similar studies on Mn− and Cr−salen (**15** and **16**, Scheme 11),[118] Fe−salophen and −Schiff base,[119] and Ru−phebox complexes[120] have since been reported (Scheme 11), in some cases specifically investigating the

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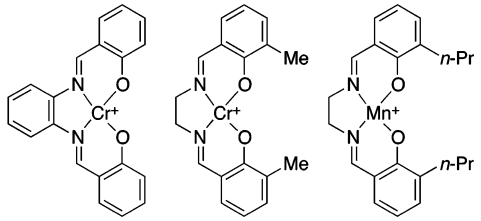
Scheme 11. Cofactor Structures Used To Generate Cofactor⊂Mb ArMs, and Results for ArM-Catalyzed Thioanisole Sulfoxidation Reactions[107,117]



	14	15	16
Mb variant	H64D,A71G	H64D,A71G	A71G
ee (%)	8-13 (*S*)	33 (*S*)	27 (*R*)
rel. rate	83	210	2724

impact of cofactor substituents[107,121] on ArM stability and activity. Up to 33% ee for thioanisole sulfoxidation is possible using such systems (Scheme 11),[107] and crystal structure analyses[119,120] have revealed that the desired His coordination is observed and little perturbation of the myoglobin scaffold occurs, despite sometimes dramatically different cofactor orientations (Figure 4).

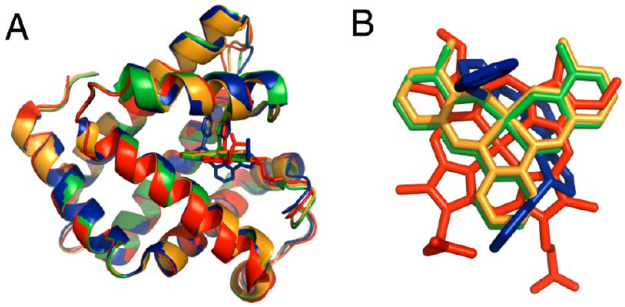
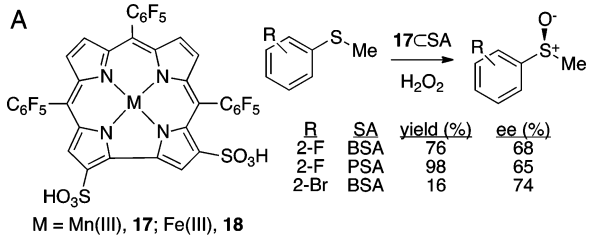


Figure 4. Crystal structures of representative cofactor⊂Mb ArMs illustrating (A) the conserved Mb fold and (B) the relative cofactor positions within the scaffold (red, heme⊂Mb, 4MBN;[120] orange, Cr(Me_2−salophen)⊂MbA71G, 1J3F;[118] green, Fe(Me_2−salophen)⊂MbA71G, 1UFJ;[119] blue, Rh(phebox−Ph)⊂MbA71G, 2EF2[120]).

Other scaffold proteins have been used to bind various metal complexes to generate ArM oxygenases with improved selectivity. For example, human serum albumin (HSA) forms 1:1 complexes with Mn(III)−corrole complexes,[122] and ArMs prepared from a range of serum albumins and either Mn(III)− or Fe(III)−corrole complexes catalyzed enantioselective thioether sulfoxidation with good yields and up to 74% ee (Scheme 12A).[123] HSA was used to generate an ArM using Mn(III)−salen complexes, and although no enantioselectivity was observed, complete selectivity for sulfoxide over sulfone formation could be achieved, reversing the reactivity of the cofactor alone (Scheme 12B).[124] ArM-active site hydrophobicity and Mn coordination by active site residues were proposed to account for this selectivity, in analogy to Lu's work. Mahy and co-workers found that 1:1 Fe(II)- and Mn(III)-tetrakis(*p*-carboxyphenyl)porphyrin⊂xylanase ArMs showed peroxidase activity[125] and catalyzed enantioselective thioanisole sulfoxidation (up to 40% ee and 85% yield)[126] and enantioselective styrene epoxidation (up to 80% ee and 16% yield).[127] Spectroscopy and modeling studies suggest that xylanase residue(s) may bind to the cofactor Fe and that positively charged residues in the xylanase active site hydrogen

Scheme 12. Selective Sulfoxidation Reactions Catalyzed Cofactors Bound to SA Proteins[123,124]



R	SA	yield (%)	ee (%)
2-F	BSA	76	68
2-F	PSA	98	65
2-Br	BSA	16	74

cat.	%SO:%SO_2	conv. (%)
19	1:58	59
19⊂HSA	97:0	97

bond to the (required) carboxylate groups on the cofactor to present a single face of the cofactor for catalysis.

The well-known binding abilities of antibodies have also been exploited for ArM formation. Schultz found that monoclonal antibodies specific for *N*-methylmesoporphyrin IX bind Fe(III)− and Mn(III)−mesoporphyrin and that the resulting ArMs catalyzed the oxidation of pyrogallol, hydroquinone, and *o*-anisidine, in analogy to natural peroxidases.[128] Similar results have been described for antibodies elicited against various substituted porphryins.[129−131] Fujii reported that haptens for known antibodies could be substituted to enable incorporation of organic moieties into antibodies for chemical catalysis (Scheme 10).[132] Along these lines, Mahy employed the antiestradiol antibody 7A3 to bind estradiol-substituted Fe−porphyrin complexes (**20**, Scheme 13A).[133] The resulting porphyrin⊂7A3 ArMs displayed increased peroxidase activity (ABTS oxidation) relative to unbound cofactor.

Ligands for other proteins other than antibodies have also been substituted with metal catalysts to enable ArM formation (Scheme 10). For example, Ward demonstrated that *p*-arylsulfonamide-substituted d_6-Ir-piano stool complexes bind to Zn-containing human carbonic anhydrase II (hCA) via a Zn-sulfonamide linkage with K_d values as low as 15 nM (e.g., **21** and **22**, Scheme 13B).[134,135] An ArM derived from chiral at metal cofactor **21** catalyzed enantioselective transfer hydrogenation of the cyclic imine salsolidine with up to 70% ee (Scheme 13B), and X-ray crystal analysis of **22**⊂hCA confirmed the overall design of the ArMs (Figure 5). In a similar fashion, Salmain has demonstrated that *β*-lactoglobulin binds d_6-transition metal piano stool complexes bearing long chain alkyl substituents (e.g., **23**, Scheme 13C) to form ArMs that catalyze enantioselective transfer hydrogenation of trifluoroacetophenone with up to 26% ee.[136] These examples illustrate the range of scaffold binding elements that can be employed for ArM formation and suggest that a range of different metal-substituted ligands could be useful in this regard.

By far and away, the most studied approach to generate ArMs for organic catalysis via noncovalent interactions (or perhaps via any means) involves binding biotin-substituted cofactors to avidin (Avi) or streptavidin (Sav) scaffolds, collectively (strept)avidin (Scheme 10B).[137−139] This approach is facilitated by the tight binding of biotin to (strept)avidin (K_d = 10^{-12}−10^{-15} M), which ensures rapid and essentially quantitative ArM formation, and the ease with which biotin can be attached to a range of metal complexes. In 1978,

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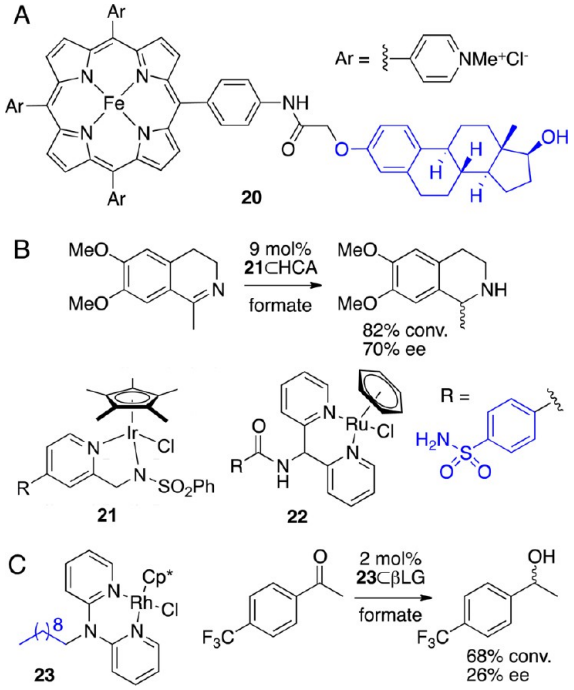
Scheme 13. Enantioselective Catalysis Using ArMs Generated from Scaffolds Binding Known Ligands Substituted with Metal Catalysts[a]



[a](A) Estradiol−Fe(porphyrin) conjugate used to bind to an antiestradiol antibody.[133] (B) sulfonamide-substituted complexes used to bind to human carbonic anhydrase.[134,135] (C) Alkane-substituted complex used to bind to β-lactoglobulin.[136] Blue portions denote scaffold binding elements as in Scheme 10.

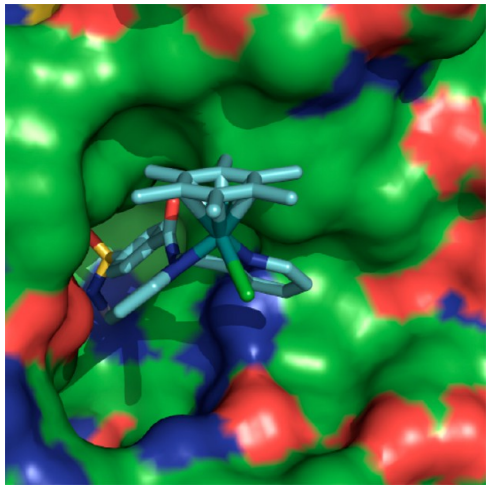
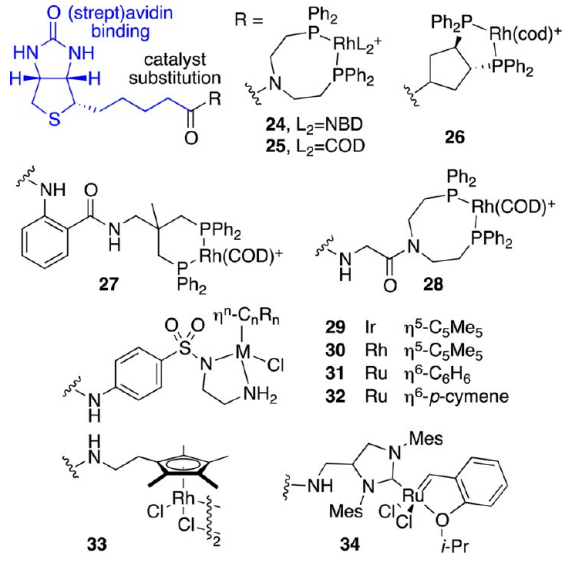


Figure 5. Crystal structure showing location of cofactor **22** in **22**⊂hCA (PDB ID 3PYK).[127]

Whitesides demonstrated the first example of enantioselective ArM catalysis using biotinylated Rh−bisphosphine complex **24** (Scheme 14) bound to Avi for hydrogenation of 2-acetamidoacrylate (~40% ee, *S* enantiomer).[140] Chan later demonstrated that Avi binding could alter and even invert the selectivity of chiral biotinylated Rh−Pyrphos complexes (**26**) toward hydrogenation of itaconic acid.[141] More recently, Ward showed that the COD derivative of **24** (**25**), when bound to Sav, catalyzed hydrogenation of 2-acetamidoacrylate with 92% ee (*R*) and quantitative conversion.[142] This was improved to

Scheme 14. Representative Biotinylated Cofactors Used To Generate (Strept)avidin-based ArMs



96% ee using Sav variant S112G, Ser-112 being located proximal to the bound metal catalyst, and this same variant, in combination with cofactor **27**, provided 57% ee for the *S* product enantiomer.[143] The *S* selectivity of Avi-based ArMs was also improved to (up to 80% ee) using cofactor **28**. Further improvements in selectivity for both product enantiomers and substrate scope were realized through the use of cofactors containing enantiopure amino acid linkers.[144] Kinetic analysis of representative ArM-catalyzed reactions established that the (strept)avidin scaffolds accelerate these reactions relative to those catalyzed by cofactor alone, and this was attributed to a hydrophobic effect conveyed by the ArM active site.[41]

Ward then established that a range of biotinylated diamine-d_6 transition metal piano stool complexes (Ru, Rh, and Ir)[145,146] could be used to generate analogous ArMs for ketone transfer hydrogenation.[147] Examining a number of cofactor (Scheme 14) and scaffold (Ser-112 mutants) combinations led to the identification of ArMs that catalyzed selective reduction of a range of substrates, including dialkyl ketones, with moderate to excellent enantioselectivity (Scheme 15A).[148] Additional improvements to ketone transfer hydrogenation efficiency were made by introducing point mutations at scaffold sites (Lys-121 and Leu-124) proximal to the metal catalyst based on analysis of the crystal structure of **31**⊂Sav-S112K.[149] Furthermore, Ir-based ArMs **29**⊂Sav-S112X also catalyzed transfer hydrogenation of salsolidine with good to excellent enantioselectivity for both *R* (86% conv., 96% ee) and *S* (quant., 78% ee) product enantiomers.[150] Related piano stool cofactor⊂(strept)avidin ArMs were used by the Ward group to catalyze alcohol oxidation[151] and (in collaboration with Rovis) enantioselective benzannulation (**33**, Scheme 15B),[152] and a biotin-substituted Grubbs catalyst (**34**) was used to generate an ArM for olefin metathesis[153] (Scheme 15C). The latter two examples highlight the potential utility of ArMs for C−C bond formation, which remains a relatively unexplored.

Given the success of (strept)avidin-based ArMs for enantioselective catalysis, it is worth considering what structural features might impart their efficacy and whether these features might inform ArM design more broadly. The Sav quaternary structure is a dimer of dimers in which the biotin-binding

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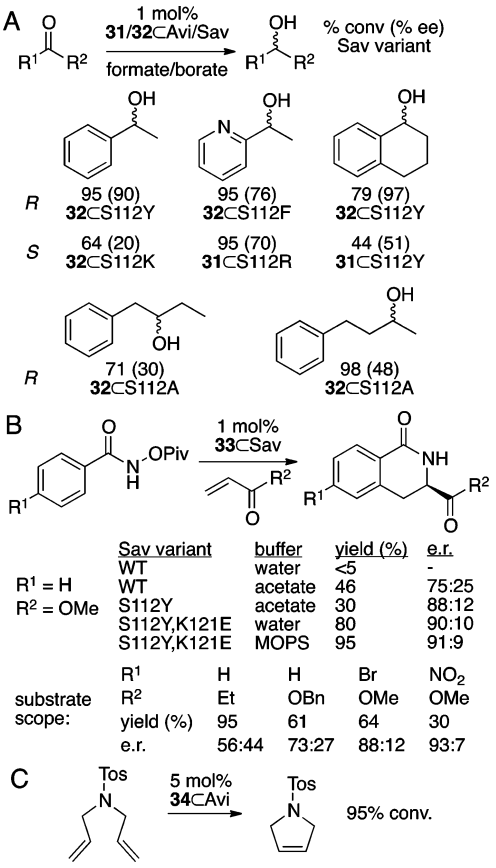
Scheme 15. Cofactor⊂(Strept)avidin-Catalyzed Enantioselective (A) Transfer Hydrogenation of Ketones[148] and (B) Salsolidine,[150] (C) Hydroarylation of Olefins,[152] and (D) Olefin Metathesis[153]



pockets from alternating pairs of Sav monomers face one another (Figure 6A).[154] Biotin binds deep within these pockets such that suitably designed biotin-substituted metal complexes typically project from the Sav scaffold via only a couple rotatable bonds (Figure 6B,C).[149,150] The tetrameric Sav structure also leads to a proximal orientation of biotin-substituted complexes in the corresponding ArMs when all binding sites are occupied, and consistent with this proximity, cofactor loading can impact ArM selectivity.[41,144] The relative orientation of the piano-stool arene (Cp*, benzene, etc.) and diimine ligands varies in the different structures: in some cases the arene is projected toward the solvent, and in others, it is projected toward the scaffold (Figure 7). Perhaps most importantly, the metal complexes are *solvent-exposed* and lie in a shallow cleft formed at the dimer interfaces (Figure 6B,C). These structural features suggest that relatively subtle scaffold/cofactor/substrate interactions, rather than substrate binding deep within the scaffold in analogy to many natural metalloenzymes[50] and some ArMs,[93] could play a significant role in the selectivity of Sav-based ArMs.

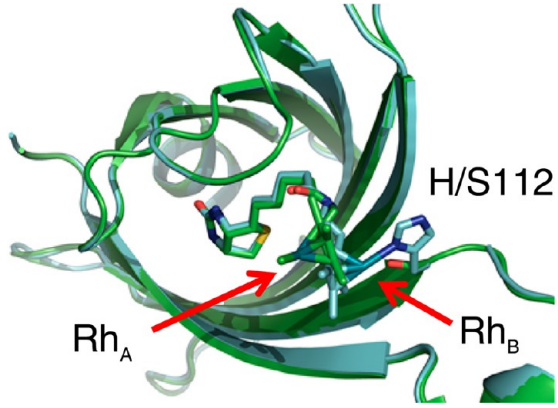


Figure 7. Overlay of structures for (A) **33**⊂Sav (green carbon atoms) and (B) **33**⊂Sav-S112H (cyan carbon atoms) showing differing orientations of metal fragment as a result of histidine ligation (PDB IDs 4GJS and 4GJV).[156]

Much of Ward's work has focused on biotin-substituted cofactors derived from either fluxional, bidentate bisphosphine−Rh(I) complexes or racemic, readily racemized, chiral-at-metal d^6 transition metal piano stool complexes (Scheme 14).[137] In the former case, a relay of chirality from the scaffold to the bisphosphine ligand to generate a chiral Pd(II)−bisphosphine complex was used to explain the enantioselectivity of ArM-catalyzed hydrogenation of acetamidoacrylate.[142] A similar mechanism was invoked to rationalize the enantioselectivity of ArMs employing analogous Pd(II)−bisphosphine

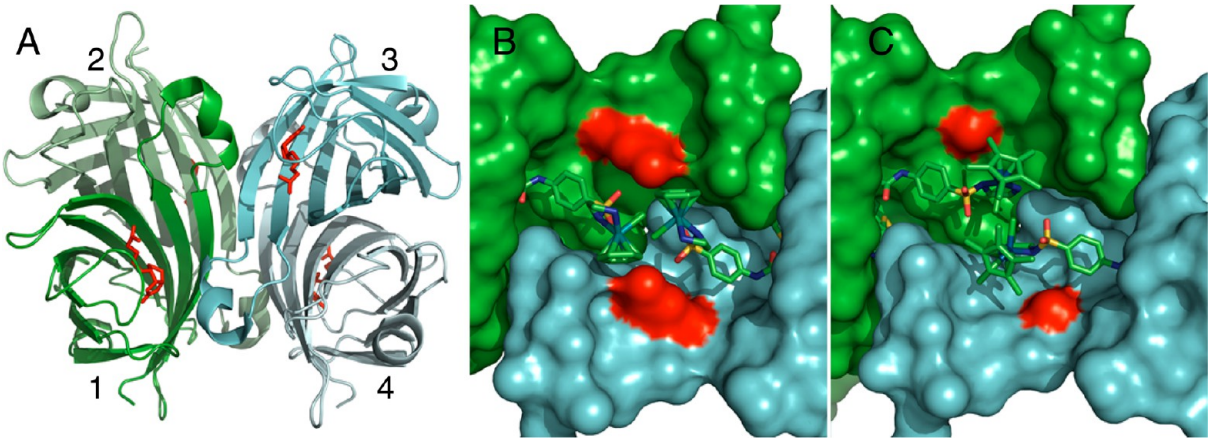


Figure 6. (A) Ribbon structure of tetrameric WT Sav with 1 biotin (red) bound to each monomer (PDB ID 1STP).[154] Alternating subunits (e.g., 1 and 3) possess proximal biotin binding sites. (B) Proximal subunits of **31**⊂Sav-S112K (PDB ID 2QCB) with Lys-112 highlighted in red.[149] (C) Proximal subunits of **29**⊂Sav-S112A (PDB ID 3PK2).[150]

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cofactors for allylic substitution based on an induced Cotton effect exhibited by the Sav-bound Pd(II)−bisphosphine cofactor.[155] In the latter case, crystal structures for ArMs containing chiral-at-metal piano stool complexes each show only a single cofactor enantiomer (Figure 6B,C), again suggesting that the scaffold acts to impart chirality to metal complexes and can even resolve stereogenic metal centers.[149,150] Furthermore, the crystal structure of **31**⊂Sav-S112K showed that the *S* catalyst enantiomer was formed, and this ArM provided the same sense of induction for ketone reduction as the *S* enantiomer of the corresponding small-molecule catalyst.[149] Assuming the static structure is relevant to the catalytically active ArM, this provides evidence that the scaffold was inducing metal-based chirality rather than imparting selectivity via interactions with the substrate. Notably, metal-based chirality was not possible for the related piano stool complexes used by Eppinger and Salmain for covalent scaffold modification, which could contribute to the lower selectivities observed for those ArMs.[108,111]

On the other hand, Ward has shown that altering both cofactor and scaffold structure can cause significant and substrate-dependent changes in enantioselectivity, overriding and even inverting the natural sense of induction preferred by small molecule catalysts.[148,150] Thus, it seems that the selectivity of Sav-based ArMs can arise either from a relay of chirality from Sav to the metal center or from the influence of scaffold residues on the orientation of the substrate within the ArM active site. This distinction is important because selectivity induced by relay of chirality implies that manipulating the selectivity of rigid metal complexes less susceptible to conformational distortion could be challenging using Sav. Regardless of the means by which Sav conveys enantioselectivity to biotinylated cofactors, Ward and Rovis showed that secondary coordination sphere effects can play a direct (as opposed to relayed) role in manipulating cofactor reactivity. Specifically, installing a glutamic acid residue (K121E) in the active site of **33**⊂S112Y led to a marked rate increase in ArM-catalyzed hydroarylation reactions, presumably by facilitating C−H activation via concerted metalation−deprotonation (Scheme 15B).[152] Interaction of Sav residues with the cofactor primary coordination sphere has also been used to impact reaction selectivity. For example, designed coordination of Rh cofactor **33** by a histidine residue (S112H) introduced into Sav was used in a dual noncovalent/metal coordination anchoring strategy to improve selectivity for salsolidine reduction using cofactors lacking diimine ligands.[156]

Together, studies on ArMs generated via noncovalent methods highlight a range of subtle synergies that can arise between cofactors and scaffolds, each of which must be taken into account when selecting and designing ArM components. From a design perspective, these examples provide some of the most detailed information described to date on the induction of chirality using ArM catalysts and the means by which catalyst activity and selectivity can be improved using protein scaffolds.

4. METALLOPEPTIDES

The ArMs developed to date show that scaffold−cofactor interactions can alter the reactivity and selectivity of metal catalysts to enable regio-, enantio-, substrate- and site-selective reactions, some of which are not readily achieved using small molecule catalysts. The efficacy of the most successful ArMs, all of which contain well over 100 residues (e.g., ALBP, 131; Sav, 183; papain, 345), raises the question of how large polypeptide scaffolds must be to readily achieve these interactions in a general sense. Amino acids and short peptides (1−3 residues) have been extensively employed as ligands in asymmetric catalysis because of their ability to impart a well-defined primary coordination sphere to metals ions,[157] but their small structures cannot achieve the secondary coordination sphere effects possible using ArMs. Between these two extremes lie metallopeptides of intermediate length that could also possess a range of enzyme-like properties, particularly substrate binding, for selective catalysis.[45] Exploring this space touches on not only fundamental aspects of molecular recognition in catalysis, protein design, and protein folding,[158] but also the many potential benefits of peptide catalysts,[159] including their modular primary structures, defined secondary structures, and automated parallel solid phase synthesis. These catalysts can also be used under cryogenic conditions to improve their selectivity. However, the development of metallopeptide catalysts has somewhat lagged behind the use of naturally occurring proteins as scaffolds for catalyst incorporation. Kaiser suggested that this resulted not only from the ease with which some proteins could be expressed in the laboratory, but also from the difficulty in predicting the structures of small peptides.[160] Without such predictive abilities or established folds, developing well-defined peptide catalysts, let alone metallopeptides, was actually more difficult that working with proteins. Despite these challenges, researchers have developed a range of approaches to prepare metallopepdide catalysts by exploiting naturally occurring secondary structural elements (e.g., α-helix, β-turns, etc.) or meeting the challenge of de novo design. To date, only coordination of metal catalysts by peptides (Scheme 2B), some of which include synthetic ligands, and covalent modification of peptides with substituted metal complexes (Scheme 2C) have been utilized to catalyze organic transformations, although heme-binding peptide bundles have been prepared as oxidoreductase models (Scheme 2D).[161] Given these examples, the success of nonmetalated peptide catalysts,[162] and the range of additional metallopeptides that have been prepared but for which catalysis has not yet been demonstrated,[163−170] this area seems ripe for further development.

4.1. Coordination of Metal Ions and Complexes.

Dervan provided one of the earliest examples in which a natural peptide fragment with a conserved fold was modified with a synthetic metal-binding moiety to generate a metallopeptide catalyst.[171] This work involved modifying a sequence-specific DNA-cleaving peptide with EDTA in analogy to earlier work on ArM formation from DNA binding proteins (e.g., Scheme 6B).[91] Specifically, a synthetic C-terminal DNA binding domain of Hin recombinase, which adopts a helix−turn−helix structure that binds to the DNA major groove, was synthesized and covalently linked to EDTA via amide bond formation at the peptide N-terminus. Following metalation, the Fe(II)-substituted peptide catalyzed oxidative cleavage of DNA at Hin binding sites in the presence of a reducing agent, such as dithiothreitol (Scheme 16A). Following this initial report, Dervan also reported that a GlyGlyHis tripeptide, the consensus sequence for the copper-binding domain of serum albumin, could be fused to the N-terminus of the Hin recombinase DNA binding domain to generate a similar site specific DNA-cleaving peptide following metalation with Cu(II).[172] More recently, Roelfes modified bovine pancreatic polypeptide (bPP), a 36-mer that adopts a type II helix−turn−helix tertiary structure and an antiparallalel homodimer

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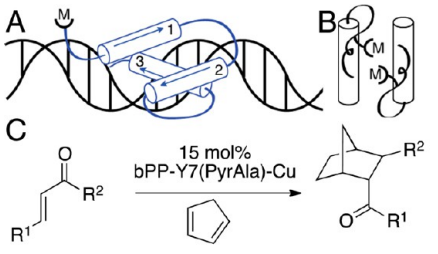
Scheme 16. (A) Cartoon Representation of Helix−Turn−Helix Metallopeptide Catalyst Binding to DNA for Sequence-Specific Cleavage by Metals Bound to Covalently Linked Ligands, Fe(EDTA)[171] or Cu(phenanthroline);[172] (B) Cartoon Representation of the bPP Helix−Turn−Helix Metallopeptide Dimer; and (C) bPP−Cu-Catalyzed Diels−Alder Reaction of Azachalcones and Cyclopentadiene[173]



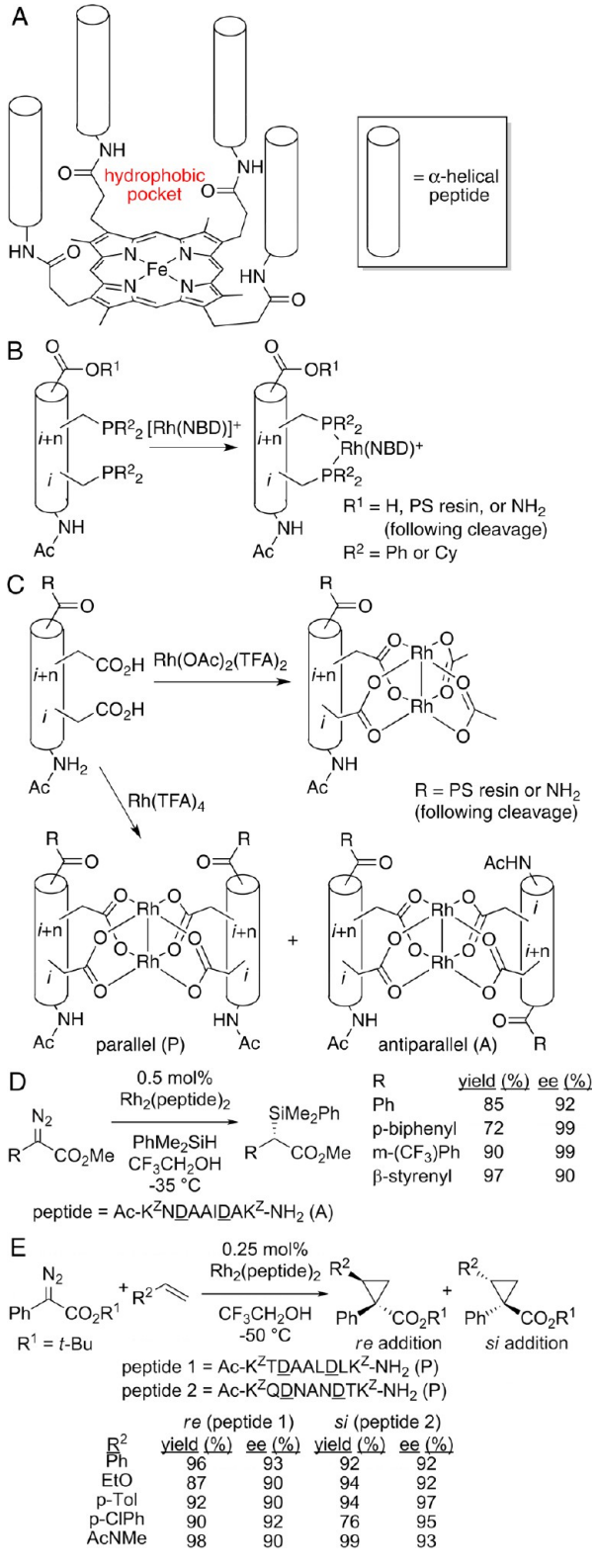
R^1	R^2	conv. (%)	ee (%)
Ph	2-pyridyl	73	83
Ph	2-*N*-methylimidazolyl	quant	65
p-MeOPh	2-*N*-methylimidazolyl	3	62
Me	2-*N*-methylimidazolyl	quant	<3

quaternary structure, to serve as a metallopeptide catalyst (Scheme 16B).[173] The natural peptide was truncated to 31 residues, and metal-binding amino acids, including histidine and 3- and 4-pyridylalanine (PyrAla), were incorporated into the peptide 7-position using solid phase synthesis to generate bPP-Y7 variants. Spectrophotometric analysis indicated that monomeric bPP-Y7(3-PyrAla)−Cu metallopeptides formed in the presence of Cu(II), and these species catalyzed the Diels−Alder reaction of azachalcones and cyclopentadiene with good levels of enantioselectivity (up to 83% ee with full conversion, Scheme 16C). Only the peptides containing the 3-PyrAla residue provided significant levels of enantioselectivity, and a 3.5-fold rate acceleration was observed in the presence of the best catalyst. These same metallopeptides also catalyzed the Michael addition of dimethylmalonate to azachalcones, and up to 86% ee with 85% conversion was observed using bPP-Y7(3-PyrAla)D10E−Cu.

Extensive studies on the structures of natural α-helices, the most common secondary structure in proteins, have led to the development of rules for preparing synthetic α-helices,[174] which have figured prominently in the design of metallopeptide catalysts.[45,175] Indeed, among the earliest examples of metallopeptide catalysts was Kaiser's helichrome catalyst,[176] which was generated by covalently linking the N-termini of four 15-residue peptides to a porphyrin substituted with four tetrahydroxysuccinamide esters. The peptides were designed such that when folded into α-helices, hydrophobic residues would be projected toward the region directly above the central porphyrin in analogy to P450 enzymes (Scheme 17A). This design both promotes helix formation and generates a hydrophobic substrate-binding pocket. Circular dichroism measurements indicated that although the peptide fragments themselves were disordered, the helichrome construct exhibited strong α-helical character. Following metalation, the imidazole adduct of the corresponding Fe(III)-substituted helichrome catalyzed hydroxylation of aniline to *p*-aminophenol following Michaelis−Menten kinetics and was inhibited by superoxide dismutase, in analogy to native heme enzymes.

Gilbertson developed synthetic methods to synthesize and incorporate diphenyl-[177] and dicyclohexylphosphinoserine[178] residues into peptides using solid phase synthesis and prepared

Scheme 17. (A) Kaiser's Helichrome Metallopeptide Catalyst;[176] (B) Metallation of peptides containing phosphinoserine Residues;[177,178] (C) Metallation of Peptides Containing Aspartic Acid or Glutamic Acid Residues;[184] (D) Examples of Dirhodium Metallopeptide-Catalyzed Si−H Insertion Reactions;[186,186] and (E) Examples of Dirhodium Metallopeptide-Catalyzed Cyclopropanation[189]



R	yield (%)	ee (%)
Ph	85	92
p-biphenyl	72	99
m-(CF_3)Ph	90	99
β-styrenyl	97	90

	re (peptide 1)		*si* (peptide 2)	
R^2	yield (%)	ee (%)	yield (%)	ee (%)
Ph	96	93	92	92
EtO	87	90	94	92
p-Tol	92	90	94	97
p-ClPh	90	92	76	95
AcNMe	98	90	99	93

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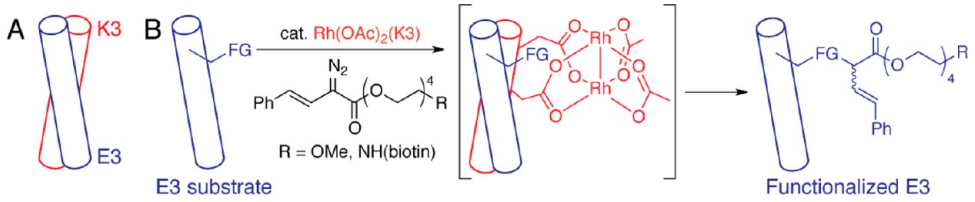
Scheme 18. Cartoon Representation of the K3/E3 Coiled Coil[191] and (B) Sequence-Specific, Site-Selective $Rh(OAc)_2(K3)$-Catalyzed Carbenoid Insertion into E3 Substrates.[192]



peptides designed to form α-helices that contained these residues at proximal positions (e.g., i and i + 1, 3, and 4)[179] within the helix structures (Scheme 17B). Treating the peptides with $[RhCl(NBD)]ClO_4$ led to the formation of helical peptide–Rh complexes, as judged by NMR spectroscopy and X-ray crystallography.[180] The initially prepared mixed phosphine complex catalyzed hydrogenation of 2-acetamidoacrylate with low enantioselectivity (8% ee, full conversion),[178] but combinatorial optimization of the peptide sequence on solid support[181] (THF solvent) provided significant increases in selectivity (up to 38% ee).[179,182] Interestingly, the optimal metallopeptides provided poor enantioselectivity in analogous homogeneous hydrogenation reactions, but selectivities similar to those observed on solid support were observed when the reactions were conducted in water, suggesting that aggregation effects could be impacting metallopeptide structure and selectivity.

More recently, Ball demonstrated that carboxylate residues (Asp/Glu) positioned at the i and i + 4 positions (and later, i + 3) of suitably designed nonapeptides were efficiently metalated[183] by $Rh_2(OAc)_2(TFA)_2$ or $Rh_2(TFA)_4$ to generate $Rh_2(OAc)_2$(peptide) or Rh_2(peptide)$_2$ complexes, respectively,[184] with induced α-helical structures[185] (Scheme 17C). The resulting metallopeptides catalyzed enantioselective carbenoid insertion into the Si–H bond of $PhMe_2SiH$ using methyl phenyldiazoacetate (Scheme 17D), and the antiparallel (A) bispeptide complexes, which must be separated from the corresponding parallel (P) isomers by HPLC,[186,186] generally provided enantioselectivity superior to either the parallel isomers or monopeptide[187] complexes. A survey of ∼40 bispeptide (A) complexes[188] containing different amino acids in the $i \pm 1$, +3, and +5 positions relative to the i and i + 4 Asp/Glu residues led to the identification of a highly selective catalyst (92% ee) for the aforementioned model reaction, and this complex catalyzed Si–H insertion using a broad range of aryl, vinyl, and alkyl diazoacetate carbenoid precursors. The P isomer of one of these catalysts also provided high enantioselectivity (93% ee) for the asymmetric cyclopropantion of styrene using phenyldiazoacetate (Scheme 17E). To access the other enantiomer of this product, these researchers developed a high-throughput protocol for synthesizing and evaluating the catalytic activity of monopeptide complexes on solid support.[189,190] A total of 94 unique peptides, including substitutions at all positions along the nonapeptide except 3 and 7 (i and i + 4 Asp residues), were prepared and evaluated, which led to the identification of a peptide that provided the opposite product enantiomer in >25% ee. A second-generation focused library based on this improved sequence led to the identification of 10 peptides that provided >45% ee on bead. Evaluating the corresponding bispeptide complexes (both parallel and antiparallel) revealed a catalyst that provided 83% ee of the desired product enantiomer, despite a lack of correlation between the selectivities of the catalysts on solid support (monopeptide complexes) and in solution (mono- and bispeptide complexes).[189] Broad scope was again demonstrated with respect to the alkene substrates, and good to excellent enantioselectivity for either product enantiomer was possible using the two optimized catalysts.

Ball has also exploited the ability of suitably designed α-helix pairs to form heterodimeric coiled coil structures to enable selective functionalization of residues in a range of α-helix-containing peptide and protein substrates via dirhodium catalysis. Initial effort focused on modified variants (each 21 residues long) of the known coiled coil E3/K3 α-helix pair (Scheme 18A). Residues in K3 were substituted with glutamic acid for installation of a dirhodium center to generate $Rh(OAc)_2(K3)$ metallopeptide catalysts, and residues in E3 were substituted with tryptophan to generate (E3W) substrates for carbenoid insertion.[191] $Rh(OAc)_2(K3)$ catalyzed sequence-dependent and residue-selective carbenoid insertion into E3W with a nearly 10^3-fold rate acceleration over the same reaction catalyzed by $Rh_2(OAc)_4$ as a result of substrate binding (Scheme 18B). This proximity-induced rate acceleration enabled carbenoid insertion into a range of other amino acids (11 total) suitably placed within the E3 helix.[192] Furthermore, fusing the E3 peptide to maltose-binding protein enabled selective protein modification on the E3 tag using a biotinylated diazo carbene precursor and $Rh_2(OAc)_2(K3)$, even in cell lysate,[193] and modification of both E3 and $Rh_2(OAc)_2(K3)$ peptides enabled improved bioconjugation efficiency and specificity.[194] This method was extended to enable residue-specific functionalization of a 31-residue segment of the leucine zipper domain from the protein c-Fos using a similarly sized Rh_2-substituted variant of the natural c-Fos binding protein, c-Jun.[192] The high efficiency of the Fos/Rh_2(Jun) system despite the weak binding measured for the two peptide components suggests that a range of selective transformations exploiting weak interactions, including structures other than coiled coils,[194] could be possible.[188]

Nonhelical secondary structures have also been exploited for metallopeptide catalysis. For example, Gilbertson demonstrated that different turn-inducing motifs,[163,195] including Pro-D-Yyy, where D-Yyy is a D-amino acid, can be used to construct peptides containing Xxx-Pro-D-Yyy-Zzz, where Xxx and Zzz are phosphinoserine residues. These peptides can be metalated using $[PdCl(\eta^3\text{-}C_3H_5)]_2$ to generate bisphosphine[196] metallopeptide catalysts for asymmetric allylic alkylation (Scheme 19A).[197] Optimizing peptide structure using a 96-member peptide library on solid support[197] and varying phosphine substitution[198] led to the identification of a peptide catalyst that provided up to 95% ee for the reaction of 3-acetoxycyclopentene with dimethylmalonate. Ball and Albrecht have also described metallopeptide catalysts based on metal-induced induced turn structures.[187,199] Breit has demonstrated that

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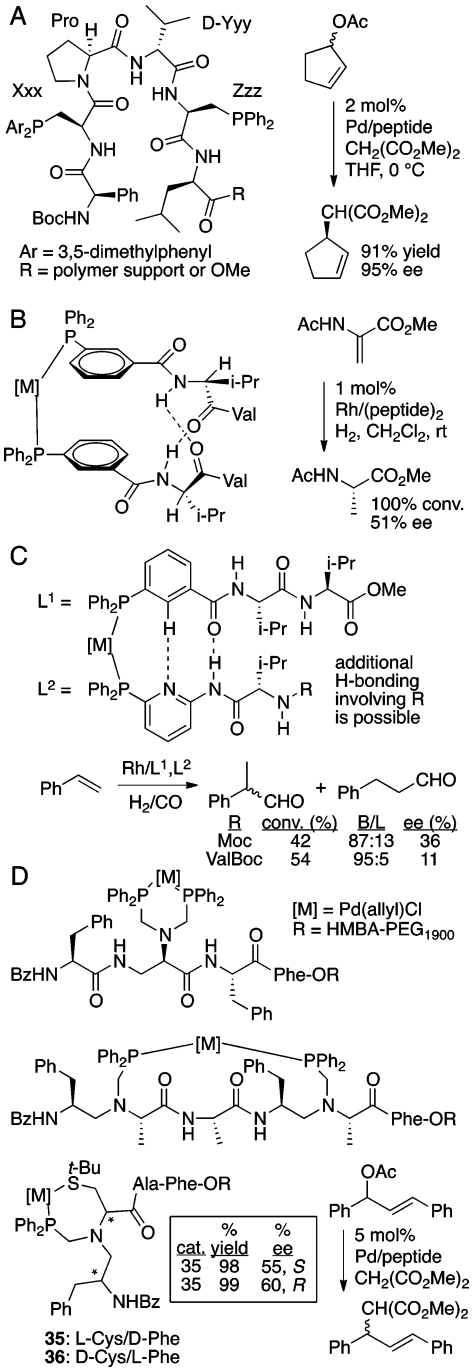
Scheme 19. A) General Structure of Phosphinoserine-Containing β-Turn Peptides, Optimized Peptide, and Asymmetric Allylic Alkylation Reaction Catalyzed by Pd–Peptide Complex;[197] (B) Hydrogen-Bonded Bisphosphine Metallopeptide Catalyzed Hydrogenation;[192] (C) Antiparallel β-Sheet Bisphosphine Metallopeptide Catalyzed Hydroformylation;[193] and (D) Phosphinomethyl-Substituted Metallopeptide Catalyzed Allylic Alkylation[202,203]



phosphine-substituted peptides can self-assemble into hydrogen-bonded bisphosphine complexes or phosphine-substituted antiparallel β-sheet structures in the presence of Pt and Rh.[200,201] In both architectures, peptide chirality could be relayed to an otherwise achiral primary coordination sphere, and different ligand combinations could be used to vary the composition of the bismetallopeptide complexes. These complexes catalyzed enantioselective hydrogenation of methyl 2-acetamidoacrylate (up to 51% ee, excluding examples with enantiopure phosphites)[200] and hydroformation of styrene (up to 36% ee, 87:13 branched/linear)[201] (Scheme 19B, C). Meldal demonstrated that peptides containing a single primary amine substituent or two secondary (backbone) amines but *lacking* any predefined secondary structure could be alkylated with hydroxymethylphosphines to generate the corresponding *N,N*-di(phosphinomethyl)amine- or bis *N*-(phosphinomethyl)-amine-substituted peptides, respectively (Scheme 19D).[202] The resulting peptides were reacted with $[PdCl(\eta^3\text{-}C_3H_5)]_2$ to generate metallopeptides that catalyzed allylic alkylation of dimethylmalonate with 1,3-diphenylpropenyl acetate in up to 21% ee. Later work showed that secondary amine modification using Ph_2PCl could be achieved and that P–S chelation was possible for metallopeptides derived from either phosphine- or phosphinomethyl-substituted amines containing *t*-Bu-methionine residues.[203] These metallopeptides catalyzed the same allylic alkylation reaction, providing either product enantiomer in >55% ee (Scheme 19D).

Finally, building on impressive progress in the field of de novo metallopeptide/protein design, Degrado and Lombardi have reported a series of studies on the development of a family of 48-residue peptides that fold into helix–loop–helix motifs. These structures were designed to project two glutamate residues and a histidine residue toward a putative *C*2 symmetric dimetal binding site along the peptide dimer interface. Addition of various metal ions, including Fe(II), Co(II), and Zn(II), led to the predicted dimeric dimetallopeptides (Figure 8A). The di-

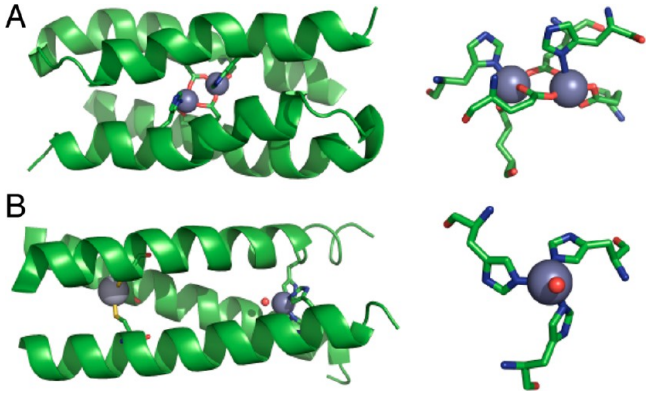


Figure 8. (A) NMR structure of a designed dimeric carboxylate-bridged di-iron helix–loop–helix metallopeptide catalyst, including an expanded view of the catalytic $[HisAsp_2Fe]_2$ center (PDB ID 2KIK).[204] (B) X-ray crystal structure of a three-helix CoilSer metallopeptide catalyst containing Cys_3Hg and His_3Zn sites. An expanded view of the catalytic His_3Zn site with water bound is shown (PDB ID 3PBJ).[206]

Fe(II) structure was found to react with O_2 to form an oxo-bridged di-Fe(III) species in analogy to a number of natural oxidase enzymes. Most relevant to the current discussion was the design of a substrate binding pocket into the di-Fe(II) structure,[204] which improved a previous design[205] and catalyst activity for oxidation of 4-aminophenol to the corresponding benzoquinone monoimine. Michaelis–Menten kinetics were observed, and up to 50 turnovers were possible.

Pecoraro described the design of a carbonic anhydrase (CA) model consisting of three helix bundle of TRI peptides with distinct metal binding sites for Hg(II) and Zn(II) built from

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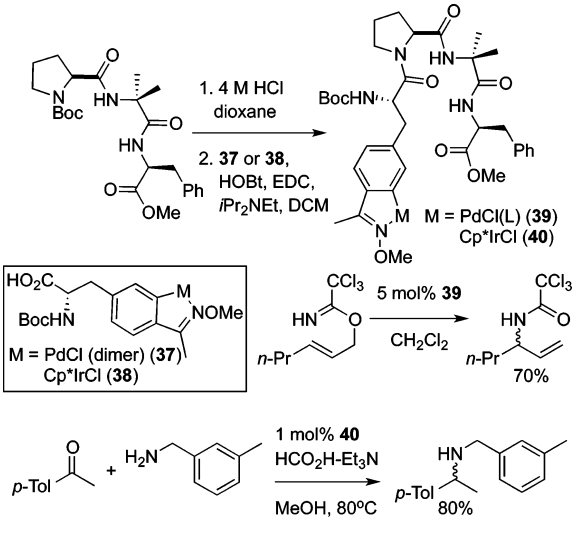
three cysteine and three histidine residues (one of each on each peptide), respectively.[206] Analysis of a crystal structure for an analogous bundle built from functionally similar CoilSer peptides confirmed the expected metal binding design and indicated the presence of a Zn-bound water or hydroxyl ligand (Figure 8B), which closely mimics the carbonic anhydrase active site. Accordingly, the TRI peptide bundle catalyzes hydrolysis of *p*-nitrophenyl acetate following saturation kinetics and provides rates 33 times greater than any small molecule CA model systems and just under 100 times less than the maximum efficiency of native CAII. Furthermore, this system also catalyzes CO_2 hydration 70-fold faster than any reported CA model system and within 500 fold of CA II. Despite the relatively small number of reports of de novo-designed catalytic metallopeptides, these examples clearly illustrate the significant power of incorporating metal centers into peptide scaffolds. More broadly, the selectivity of several metallopeptide catalysts discussed above suggests that relatively simple structures may capture many of the unique properties of ArMs and will certainly lead to a number of exciting advances in catalysis.

4.2. Covalent Linkage of Metal Complexes. Relatively few examples of covalently linking preformed organometallic catalysts (rather than only ligands) to peptides have been reported. As previously noted, this approach allows for introduction of complexes that cannot be formed in the presence of the peptide scaffold. Yaghi demonstrated that amino acids with Pt−terpyridine-substituted side chains could be coupled to peptides on solid support, but these were not used for catalysis. We developed an approach to prepare and couple amino acids with organometallic side chains to peptides using standard peptide bond forming conditions.[207] Both palladacycle- and iridicycle-based amino acids were compatible with the method, and the resulting metallopeptides catalyzed allylic alkylation and transfer hydrogenation (Scheme 20), but no enantioselectivity was observed. As with many systems, we presume that our simple β-turn mimics did not provide sufficient interactions with the metal centers or the substrates to impart selectivity, and subsequent work will involve exploration of alternate sequences to improve these catalysts.

Scheme 20. Structures and Reactions of Peptide Catalysts 39 and 40 Prepared via Covalent Attachment of Iridicycle and Palladacycle Catalysts 37 and 38[207]



5. SUMMARY AND OUTLOOK

5.1. New and Improved Methods for Hybrid Catalyst Preparation. The ArMs and metallopeptides outlined above highlight the unique structures and functions of hybrid catalysts that have been developed to date. The combination of inorganic/organometallic and biopolymer components provides materials with beautiful, three-dimensionally defined architectures not possible using small molecule ligands. The improving efficiency of these catalysts attests to our growing understanding of the principles required for their design, preparation, and optimization. However, from a practical perspective, a catalyst must ultimately be judged by how well it performs, whether it performs better than all other options, and whether the margin of improvement is worth the catalyst preparation effort. Preparing hybrid metal−peptide catalysts is not necessarily any more difficult than preparing conventional metal/ligand catalysts, but a number of challenges remain.

The ease of protein expression, peptide synthesis, and chemical ligation methods can make preparing peptide scaffolds simple relative to small molecule ligand syntheses. The challenge then becomes identifying and optimizing scaffolds for the desired catalytic function. As previously noted, this requires linking the peptide and metal components in a manner that allows the peptide to influence the primary or secondary coordination sphere of the metal.[38] In the case of ArMs, this has been accomplished by incorporating catalysts into vacant space within proteins and between protein complex interfaces,[37] whereas metallopeptide catalysts typically exploit secondary structural motifs as active sites.[188,195] X-ray crystallography studies suggest that the conformations of individual residues can be altered as a result of metal incorporation, but the overall scaffold folds and secondary structures are typically maintained, even following covalent modification with large metal complexes.[92,208] Despite the integrity of these constructs, poor selectivity remains problematic for the majority of hybrid catalysts. Researchers have cited metal binding to multiple (nonselective) scaffold sites,[73] cofactor movement within vacant space due to linker flexibility or poor cofactor binding,[105] and cofactor projection from scaffolds[100,102] as potential explanations for these selectivity problems, and in many cases, X-ray crystallography supports these conclusions. Improved methods to predict metal placement within scaffolds, particularly via computational design of metal binding sites for *catalytically active* metal ions[53,204,206] and molecular dynamics simulations to predict cofactor placement and linker design[156,209] will be highly useful in this regard.

In general, any native catalytic activity of the scaffold is not necessarily relevant to the desired hybrid catalyst activity, so scaffolds can be selected for properties that facilitate their preparation and application, including cofactor or substrate binding, high expression levels (ideally in *E. coli*)/ease of synthesis, thermostability, and organic solvent tolerance. The extent to which this functional decoupling is possible depends on the approach taken for metal installation (metal coordination, noncovalent, or covalent). For example, metalation of peptide scaffolds via metal coordination or noncovalent binding of either metal ions (e.g., scaffold−Cu(II)) or metal complexes (e.g., hemeCscaffold) requires discrete binding sites within the scaffold. Although such scaffold−cofactor binding interactions can potentially be designed,[79,158,188,195] most examples reported to date utilize scaffolds that already possess

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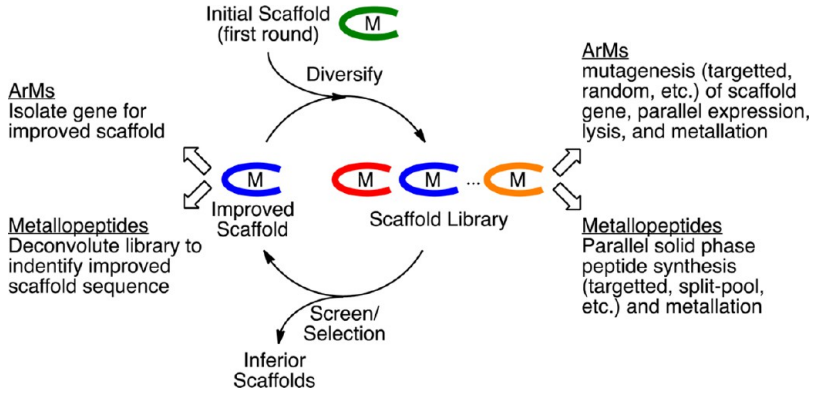

Figure 9. Conceptual overview of scaffold evolution for ArMs and metallopeptide catalysts.

ligand-binding capabilities. Similar restrictions apply to cofactors linked to scaffold proteins via noncovalent anchoring moieties, such as biotin or sulfonamides, which must be used with (strept)avidin[137] or carbonic anhydrase,[134] respectively. Both metal coordination and noncovalent metalation strategies have the advantage of rapid catalyst formation, but this low barrier to metalation can come at the expense of tight metal/cofactor binding, and dissociation can be problematic, depending on the scaffold. Covalent attachment eliminates the possibility of cofactor dissociation during catalysis, but requires fast and selective bioconjugation reactions.[87] Perhaps most importantly, this approach can potentially be used to link any desired metal complex with any desired peptide scaffold, which enables researchers to fully exploit the diversity of protein and peptide scaffolds available. Although cysteine alkylation provides a convenient starting point for covalent modification, new methods, such as our SPAAC approach,[100] would greatly improve the scope and utility of covalent ArM formation and facilitate further developments in ArM catalysis.

A corollary to the independence of ArM and metallopeptide activity on scaffold activity is a complete dependence on the nature of the metal ion or cofactor employed. Whereas metal coordination involves direct bonding of either metal ions or complexes to scaffold residues, cofactor-based approaches for ArM formation pose a number of unique challenges that must be addressed. Most importantly, cofactors substituted with either noncovalent protein-binding elements (e.g., biotin) or reactive handles for covalent scaffold modification (e.g., α-bromoacetamide) must be synthesized. Such syntheses can be challenging, particularly if reactive functional groups must be installed, and new methods and strategies for creating such bifunctional cofactors are needed. Because these cofactors are themselves catalytically active (but nonselective) species, strategies must also be devised to remove any unreacted cofactor from hybrid catalyst. This has been accomplished through the use of excess scaffold to ensure that all cofactor is consumed,[210] but most commonly, some form of chromatography (e.g., size exclusion) or affinity separation (e.g., avidin support) is required. Several researchers have reported scaffold-accelerated catalysis,[41] and if such relative reaction rates can be further improved, the need to separate excess cofactor could be eliminated in analogy to small molecule ligand accelerated catalysis. The prospect of designing scaffolds to explicitly activate metal catalysts so that only scaffold-bound cofactors are active is also highly intriguing.[88]

These considerations suggest that hybrid catalysts could be most readily applied toward fine chemical synthesis, late stage functionalization of complex molecules, or biomolecule functionalization, in which the value of their potential substrate recognition capabilities outweigh catalyst cost and scale issues. Indeed, a number of ArMs and metallopeptide catalysts have been developed for selective biomolecule functionalization reactions that are not possible using small molecule catalysts or natural enzymes.[82] However, most of the small molecule reactivity explored to date involves reactions that already have very well characterized and highly selective small molecule catalyst systems (e.g., transfer hydrogenation, thiol oxidation, etc.).[105,137] Although these reactions make reasonable starting points for catalyst development, established small molecule and enzyme catalysts stand as challenging benchmarks against which to evaluate the unique capabilities of hybrid catalysts. Furthermore, with some impressive exceptions,[112,137,188] most of the examples discussed above and a number of unpublished results from our own laboratory indicate that even in these reactions, ArM and metallopeptide-catalyzed reactions often provide only low-to-moderate enantioselectivities. Thus, despite the amazing progress made to date, much work will be required before ArMs can be regarded as a catalyst of first choice for organic synthesis. Focusing on reactions that have proven challenging to address with small molecule catalysts[50,211,212] will help to establish the utility of hybrid catalysts.

5.2. Catalyst Libraries and Directed Evolution. Early on, Reetz pointed out that simply introducing a metal catalyst into a protein would by no means guarantee a selective ArM and that, just as with natural enzymes, scaffold evolution would likely be required to improve the utility of these catalysts (Figure 9). Similarly, one of the often-cited benefits of metallopeptide catalysts is the ease with which peptide libraries can be prepared (iteratively) to enable catalyst optimization. Most of the highly selective ArMs and metallopeptide catalysts developed to date resulted from significant scaffold optimization efforts, but relatively few sustained efforts toward this end have been reported. Notable exceptions include the extensive mutagenesis of streptavidin (Sav) by Ward and Reetz to generate the highly selective ArMs for hydrogenation and transfer hydrogenation that were detailed above.[144,148,149,210] Central to both of these works was expressing Sav variants in concentrations sufficient for ArM formation and catalysis. Reetz isolated Sav variants grown in 150 mL cultures, a relatively low-throughput approach, and used limiting amounts of biotinylated cofactor to reduce cofactor background catalysis.[210] Ward exploited the tetrameric Sav structure to both immobilize Sav variants from crude lysate on biotinylated sepharose and subsequently bind biotinylated cofactor so that excess cofactor

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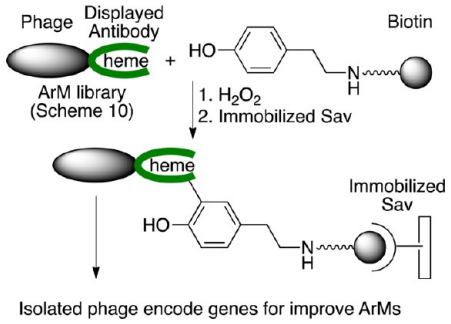
could be washed away and the immobilized ArMs could be more readily screened.[149] To further augment the utility of evolutionary approaches to ArM optimization, Ward emphasized the importance of simultaneously optimizing both cofactor and scaffold structure,[213] so-called chemogenetic optimization,[85] and Reetz developed methods aimed at evolving ArMs derived from covalent modification.[95]

Although impressive, these efforts involved targeted point mutations or single-residue saturation mutagenesis rather than less-targeted approaches that have proven highly beneficial and more generally useful for enzyme optimization.[23,25] Along these lines, Schultz developed a selection scheme to evolve peroxidase activity in a heme⊂antibody ArM (Scheme 21).[214]

Scheme 21. Cartoon Representation of Selection Scheme for Optimizing Heme⊂Antibody ArM Peroxidase Activity[214]

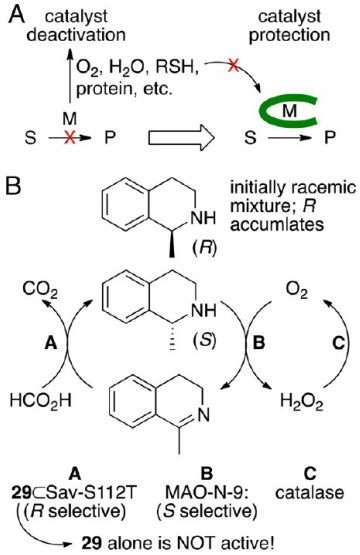


In this system, ArM formation involves heme binding to the parent antibody (7G12) displayed on M13 phage. ArM-catalyzed oxidation of a biotin-linked phenol generates a reactive intermediate that covalently modifies the ArM and enables isolation of active catalysts (and their sequences encoded in the phage) on a Sav support without the need for ArM isolation or purification. Libraries of ~10^7 7G12 variants were generated via either error-prone PCR or saturation mutagenesis of multiple targeted residues screened to identify variants with improved activity as a result of increased expression, heme affinity, and increased catalytic efficiency.

In the area of metallopeptide catalysts, both Gilbertson[179,182,195] and later Ball[189] demonstrated that library peptide scaffolds could be readily prepared on solid phase synthesis. The corresponding metallopeptides could also be generated on solid support, and the residual metal precatalyst washed away so that libraries of ~100 metallopeptides could be readily screened. In both cases, significant improvements in enantioselectivity were obtained, with Ball demonstrating >90% ee for a range of metallopeptide-catalyzed cyclopropanation reactions and Gilbertson reporting similar enantioselectivity for the allylic alkylation of 3-acetoxycyclopentene with dimethylmalonate.[189,195] It should be noted that only Reetz and Ball described iterative rounds of mutagenesis and screening, a hallmark of conventional directed evolution efforts.[189,210]

On the basis of the successes outlined above, it is clear that routine directed evolution of ArMs and metallopeptides must be achieved to unlock the potential of these catalysts (Figure 9). In the case of ArMs, this will require advances in high-throughput scaffold expression, ArM assembly, and strategies to differentiate ArM and unassociated metal catalysis as outlined in the previous section. Ideally, *E. coli* would be suitable for scaffold expression to facilitate both genetic variation and protein expression. Furthermore, ArM assembly and catalysis[194,215] should be compatible with *E. coli* lysate to eliminate the need for extensive ArM purification.[216] This can be challenging because of the presence of various metal-binding small molecules and reducing agents, such as glutathione, in cell lysate, but suitable scaffold design could be used to protect metal catalysts from poisoning by cellular components in analogy to natural metalloenzymes and even some synthetic systems (Scheme 22A).[70,106] In the case of metallopeptides,

Scheme 22. A) Small Molecule Catalysts Are Deactivated by Various Species, But Scaffolds Can Block These Reactions to Protect Catalysts,[70,106] and (B) MAO/29⊂Sav-S112T-Catalyzed Tandem Double Deracemization of Imines[220]



solid phase synthesis is more than capable of generating scaffold diversity required, but robust methods to install active metal catalysts onto the peptide scaffold are needed. In both cases, high-throughput methods for catalyst evaluation will be required, but these have been extensively reviewed elsewhere.[217,218]

5.3. Future Directions. As directed evolution of ArMs and metallopeptides becomes more practical, so, too, will engineering these catalysts to control catalyst selectivity through primary and secondary coordination sphere effects. Initially, this control may provide researchers with a means to improve selectivity in cases, specific substrates for example, in which small molecule ligands prove ineffective.[25] Hybrid catalysts could also be used to control catalyst selectivity toward similarly reactive functional groups on a substrate or to override substrate control and enable reaction of less reactive functional groups.[211,219] Although much of this work will use cofactors based on known, chemoselective small molecule catalysts, one could also envision designing metal cofactors with the intent that their selectivity be controlled not through relative reactivity of functional groups but through molecular recognition.

Beyond improving cofactor selectivity, scaffold evolution could potentially enable new cofactor reactivity not possible in the absence of the scaffold. Proximity-induced functionalization of peptide residues catalyzed by $Rh_2(OAc)_2$(peptide) catalysts but *not* by small molecule dirhodium catalysts show that new reactions can, indeed, be enabled by the hybrid catalysts.[188] Scaffold acceleration also holds great promise for enabling new

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reactions.[41] Enhancing such effects on one substrate may improve catalyst reactivity to enable new reactions on new substrates, following a substrate walking approach that has already been demonstrated for natural enzymes.[50] To date, scaffold acceleration has most often been ascribed to a general hydrophobic effect in which organic substrates favorably bind proximal to metal catalysts within scaffolds. Studies aimed at firmly establishing the nature and origins of these effects will inform efforts to improve their impact via directed evolution. Scaffolds can also alter metal reactivity directly through coordination, hydrogen bonding, electrostatic, or steric effects.[107] Indeed, scaffold modulation of metal redox potential and even oxidation state was used to generate an ArM for O_2 reduction from an inactive cofactor;[88] evolving this activity could allow the use of activated (e.g., reduced) cofactors for reactions of organic substrates. Subsequent efforts could even enable novel metal reactivity possible only in the presence of the scaffold in analogy to natural metalloenzymes.[56]

Scaffold evolution could also be used to control the ability of species in solution to access cofactors. Researchers have demonstrated that protein scaffolds can extend the lifetimes of catalysts prone to decomposition via dimerization or reaction with water, oxygen, or other catalyst poisons.[70,107] Given the sensitivity of many transition metal catalysts to such species, evolving this ability could provide a general means to improve catalyst lifetimes (Scheme 22A). ArMs have also been used to enable concurrent reactions with two catalysts that would otherwise prove mutually incompatible (Scheme 22B). For example, monoamine oxidases (MAOs) stereoselectively oxidize α-chiral amines to imines and can be used in conjunction with reductants (e.g., $NaBH_4$) to deracemize amines.[220] Utilizing a chiral catalyst for reduction would improve this process via double deracemization, and although small molecule Ir (e.g., cofactor **29**) is incompatible with MAO, **29**⊂Sav-S112T enabled efficient double deracemization.[221] Extending this concept to multicatalyst systems could be used to generate reaction sequences analogous to natural metabolic pathways.[27,222] Ultimately, this level of control could enable in vivo applications of ArMs. In vivo incorporation of both unnatural heme complexes into heme proteins[223] and a ferrocene-substituted amino acid into human superoxide dismutase[224] have been reported, and catalysis by small-molecule metal complexes in living systems has been explored.[215] Continued work toward this end could allow efficient cofactor incorporation into protein scaffolds to enable new transformations on naturally occurring metabolites and other biomolecules in living organisms.

ArMs and metallopeptides thus have great potential as catalysts, arising as a result of the unique capabilities of their peptide and metal components and augmented through directed evolution. To date, great progress has been made in developing methods for preparing and characterizing hybrid catalysts, but further advances in catalyst design, protein engineering, and synthetic biology will be required to facilitate their use in synthetic chemistry. These challenges will undoubtedly drive exciting developments impacting all of catalysis science.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

ArM, artificial metalloenzymes; CPA, carboxypeptidase A; hCA, human carbonic anhydrase; SA, serum albumin; BSA, bovine serum albumin; HSA, human serum albumin; Mb, myoglobin; Sav, streptavidin; Avi, avidin; CAP, catabolite activator protein; COD, cyclooctadiene; NBD, norbornadiene; UAA, unnatural amino acid; Bpy, bipyridyl; Pyr, pyridyl; TFA, trifluoroacetate; ROS, reactive oxygen species

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