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Designing Enzyme-like Catalysts: A Rhodium(II) Metallopeptide Case Study

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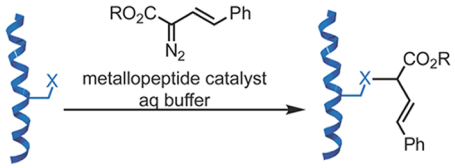
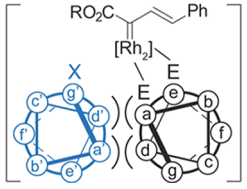
CONSPECTUS

Chemists have long been fascinated by metalloenzymes and their chemistry. Because enzymes are essential for biological processes and to life itself, they present a key to understanding the world around us. At the same time, if chemists could harness the reactivity and selectivity of enzymes in designed transition-metal catalysts, we would have access to a powerful practical advance in chemistry. But the design of enzyme-like catalysts from scratch presents enormous challenges. Simplified, designed systems often don't provide the opportunity to mimic the complex features of enzymes such as selectivity in polyfunctional environments and access to reactive intermediates incompatible with bulk aqueous solution.



Site-specific modification is possible at amino acids comprising >40% of protein space:
X = Phe, Tyr, Asn, Gln, Asp, Glu, Trp, Arg, Ser, Lys, His



Extensive efforts by numerous groups have led to remarkable designed metalloproteins that contain complex folds, including well-defined secondary and tertiary structure surrounding complex polymetallic centers. These structural achievements, however, have not yet led to general approaches to useful catalysts; continued efforts and new insights are needed. Our efforts have combined the attributes of enzymatic and traditional catalysis, bringing the benefits of polypeptide ligands to bear on completely nonbiological transition-metal centers. With a focus on designing useful catalytic activity, we have examined rhodium(II) carboxylates, bound to peptide chains through carboxylate side chains. Among other advantages, these complexes are stable and catalytically active in water.

Our efforts have centered on two main interests: (1) understanding how Nature's ligand of choice, polypeptides, can be used to control the chemistry of nonbiological metal centers, and (2) mimicking metalloenzyme characteristics in designed, nonbiological catalysts. This Account conveys our motivation and goals for these studies, outlines progress to date, and discusses the future of enzyme-like catalyst design.

In particular, these studies have resulted in on-bead, high-throughput screens for asymmetric metallopeptide catalysts. In addition, peptide-based molecular recognition strategies have facilitated the site-specific modification of protein substrates. Molecular recognition enables site-specific, proximity-driven modification of a broad range of amino acids, and the concepts outlined here are compatible with natural protein substrates and with complex, cell-like environments. We have also explored rhodium metallopeptides as hybrid organic–inorganic inhibitor molecules that block protein–protein interactions.

Introduction

The chemistry of metalloenzymes has long been a source of inspiration to chemists. Metalloenzymes have a variety of attributes that do not exist in traditional transition-metal catalysis: Site-specific catalysis in a polyfunctional environment is common; highly reactive intermediates are formed which are incompatible with the aqueous environment of bulk solution; complex feedback and regulatory networks allow precise and reversible switching of enzyme activity. These properties would be extremely valuable in synthetic catalysts as well, but are difficult to design. We have become fundamentally interested in how to borrow ideas from natural systems to create useful catalysts with enzyme-like properties. In the process, we believe that designing useful enzyme-like catalysts is an alternative route to understand the natural world. The widely cited Feynman quote, "What I cannot create, I do not understand,"[1] is a mantra for enzyme design; our ideas about enzyme function are validated if they can be effectively reproduced and tested in nonbiological systems.

Reported examples of designed metalloproteins, aided significantly by modern computational methods, exhibit

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remarkable structures, including multiple metal ions, complex tertiary structure, and well-controlled assembly of multiple polypeptide chains.[2−8] Many of these ideas and successes have had a significant impact on our thinking. The link between structure and catalytic function, however, is a challenging one. Mimicking the local environment and spectroscopy of natural enzymes is a formidable enough challenge in many cases; creating new catalytic function that approaches the efficiency and selectivity of natural systems has proven exceptionally challenging.[9−13] In our work with rhodium, we have made a conscious decision to focus on the latter, less studied challenge: designing useful reactivity and catalytic function.

Selective catalysis in a polyfunctional environment is a fascinating and remarkable challenge that natural systems overcome with ease: site-selective tyrosine phosphorylation in a sea of phenol groups; chemoselective methylene C−H hydroxylation of long alkyl chains. In many cases, these reactions require overriding inherent chemical reactivity to achieve reactivity at less reactive sites or functional groups in the presence of inherently reactive ones.[14] As chemists, we have extremely limited tools to mimic these reactions. Studies of steroid C−H functionalization by Breslow and co-workers established the possibilities of combining molecular recognition with transition-metal catalysis.[15−17] A cyclodextrin bound to a metal catalyst served to bind hydrophobic appendages and thus to localize a steroid molecule near the catalyst. Substrate recognition can be more straightforward for nucleic acid targets, and transition-metal approaches to site-specific DNA oxidative damage have been reported.[18,19] Nickel(II) metallopeptides catalyze oxidative strand scission of DNA and RNA; selective oxidation of RNA loop regions has been noted. Although not employing transition metals, Miller and co-workers have developed selective chemistry on several (quite structurally distinct) complex natural products.[14,20−22] In a key study, Miller examined acylation of a polyhydroxylated, macrolide natural product and found a peptide catalyst that overrides the inherent preference for deoxy sugar acylation in favor of selective macrocycle acylation.[14] Recently, the group reported extension of these ideas to selective polyolefin oxidation.[22]

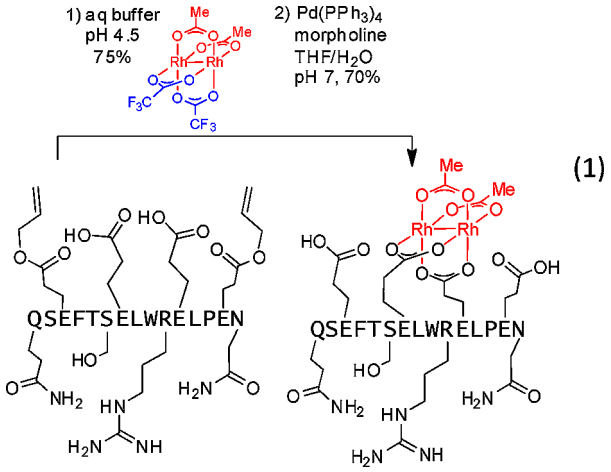
There are practical advantages to using peptides rather than traditional ligands. The modular structure makes them amenable to automated, high-throughput optimization methods. Also, peptides facilitate the design of molecular order over large length scales (several nanometers or more), which is necessary for molecular recognition, precise functional-group positioning, and many other aspects of enzyme function. Incorporating both practical and theoretical considerations, it is possible to describe our own efforts as centered on two main interests: (1) new capabilities that are possible by combining nature's ligand of choice (polypeptides) with nonbiological metal centers, and (2) new function made possible by mimicking characteristics of metalloenzymes in nonbiological catalysts.

There is, of course, no reason that metalloenzyme-like function should require the building blocks of biological systems (polypeptides). However, the vast literature of protein folding makes polypeptides a well-understood context in which to develop new function. Thus, our goal became to fuse knowledge from bioinorganic peptide ligand design with entirely nonbiological metal centers and, thus, completely nonbiological catalytic function.[10,23−28] A final practical point merits comment: the choice of the dirhodium center has proved useful in many ways, both fortuitous and planned. Designing a stable ligand environment around a metal center while retaining catalytic activity is itself a significant challenge. Generally speaking, well-defined and stable peptide−metal constructs are closed-shell, substitution-inert species and thus of limited value for catalysis. The differentiated coordination environment of dirhodium(II) complexes nicely addresses these challenges. The equatorial ligands are strongly held and are kinetically inert under biological conditions, even for monodentate equatorial ligands such as acetonitrile. This fact implies that a dirhodium−peptide adduct would be stable even in a complex biological milieu. At the same time, the trans effect of the Rh−Rh bond labilizes the axial sites.[29,30] Only weak, readily exchanged axial coordination is observed, which allows access to open coordination sites and thus to catalytic activity even in the presence of strong donors (cysteine, histidine, etc.).

Synthesis, Stability, and Structure of Rhodium(II) Metallopeptides

We spent a considerable amount of time examining potential methods for rhodium metallopeptide synthesis before settling on heteroleptic trifluoroacetate complexes as precursors (eq 1). Under appropriate conditions, trifluoroacetate is readily displaced by carboxylates, but not by carboxamides, guanidines, imidazoles, or thiols. In the end, two general protocols were developed: Method A, in 2,2,2-trifluoroethanol (TFE) with Hünig's base, is effective for organic-soluble substrates without free primary amines. Method B, in water buffered to pH 4−5, is effective for water-soluble substrates and those with free primary amines.[31] A protecting-group strategy permits the synthesis

of metallopeptides with free carboxylates (eq 1);[32] the metalation reaction behaves as an irreversible, kinetic process, and reaction of $Rh_2(OAc)_3(tfa)_1$ with biscarboxylate sequences typically provides a statistical mixture of all possible metallopeptides. That the carboxylate ligand is readily available as a natural side chain is an additional benefit of this approach.



The rhodium(II) metallopeptides are almost indefinitely stable in aqueous buffer at pH 2−7 but decompose readily at pH ≥ 8. Rather than disrupt folding, rhodium chelate binding could also enforce helical structure for sequences with carboxylates in *i*, *i* + 4 (or in some cases *i*, *i* + 3) spacing (Figure 1).[33] Even sequences with no evidence of helical structure could become strongly helical upon metal binding. On the other hand, metal binding could be used to obliterate helical structure in sequences with carboxylate spacing incompatible with helix formation, such as *i*, *i* + 7.[31,33] High concentrations of acetate (1 M) accelerate demetalation, an observation that we used early on as a switch of molecular assembly.[31] Upon treatment with acetate, a "caged" metallopeptide, with biscarboxylate chelate binding that prevents helical structure by the topology of rhodium attachment, released free peptide, which assembled with a complementary coil in situ.[31]

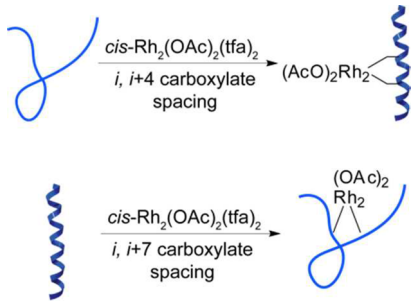


FIGURE 1. Control of helix formation through chelate aspartate coordination. Carboxylate-containing aspartate (D) or glutamate (E) side chains bind to rhodium(II) centers. Metal-binding to appropriately spaced residues results in helical structure.[31] Sequences: (top) YGK-AAAADAGADKAAAAK, (bottom) YGKAAAADAADAKAAAAK.

Developing a Minimalist Peptide Ligand for Asymmetric Catalysis

Fairly early on we set out to study the use of rhodium metallopeptides for asymmetric catalysis. Remarkably, polypeptides remain little used as chiral ligands, though peptides have found important uses in asymmetric organocatalysis.[34−36] The exchange-inert nature of rhodium−carboxylate bonds obviates nonselective reactivity of unbound, free metal, a significant benefit of our system. We examined a few simple biscarboxylate nonapeptides with *i*, *i* + 4 spacing, based on the naïve notion that stable secondary structure (in this case, helicity) is more likely to lead to effective chiral discrimination. In silane insertion reactions, we saw modest enantioselectivity with mono-peptide catalysts, (Rh_2(peptide)$(OAc)_2$). Metalation of precursor $Rh_2(tfa)_4$ with 2 equiv of peptide resulted in the corresponding bis-peptide catalysts, $Rh_2(peptide)_2$, one of which was an efficient silane insertion catalyst (Figure 2).

Two isomers are possible for bis-peptide catalysts, due to a parallel or antiparallel orientation of the peptide chains (see Figure 2 for an example of antiparallel orientation). Metalation reactions produce a mixture of the two isomers, which were separated by RP-HPLC before testing. Establishing the isomeric structure of the two separable isomers was challenging. A key difference between mono-peptide and

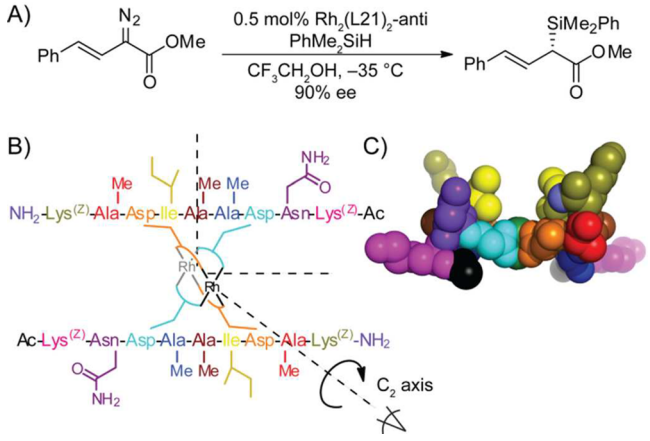


FIGURE 2. (A) Enantioselective silane insertion with a metallopeptide catalyst.[37] (B,C) Structure of the optimized catalyst, $Rh_2(L21)_2$-anti, with selected side chains shown explicitly. The suffix "anti" refers to the antiparallel isomer of peptide orientation. Adapted from ref 38 by permission of The Royal Society of Chemistry.

bis-peptide catalysts is that the mono-peptide structures have C_1 symmetry and thus two chemically distinct rhodium sites. The bis-peptide structures have C_2 symmetry and thus (only in the case of parallel isomers) two identical rhodium sites. The antiparallel isomers, with a different axis of C_2 symmetry, also have two chemically inequivalent rhodium sites (see Figure 2). Because structures with multiple unique catalytic sites seemed likely to be less selective, we had tentatively assumed that the parallel isomers would be necessary for selectivity. Sometime later, this tentative assumption was proven incorrect, as pyrene excimer fluorescence allowed us to conclusively establish by the most selective catalyst, indeed, had antiparallel orientation (Figure 2).[38] To us, this result points to the difficulty of designing chiral ligands and the benefit of high-throughput peptide ligand screens.

A different metallopeptide from the initial library, derived from the L16 sequence, functioned as an effective catalyst in cyclopropanation reactions (Figure 4, top path). Access to the opposite enantiomer is often limited when chirality is derived from natural sources, so we set out to find alternative sequences of natural (L)-amino acids that would furnish the opposite (1*S*,2*R*) stereochemistry. Our original library provided no lead structures, so we designed approaches to increase throughput in the hopes of finding new regions of chiral space. Our initial screening used parallel, automated peptide synthesis followed by HPLC purification, rhodium metalation, and a second HPLC purification of the final metallopeptide, requiring roughly 2 months to screen about 35 peptides. We next developed techniques to metalate the peptides and screen the resulting metallopeptides directly on resin.[39] This method eliminates purification steps, and one researcher in our lab synthesized and screened a 96-well plate in less than one week (Figure 3). On-bead screening only allows access to monopeptide catalysts (i.e., Rh_2(peptide)$(OAc)_2$), which are generally less selective, and so we screened two generations of 96-well plates, chose the best sequences (affording 40−55% ee), and synthesized the

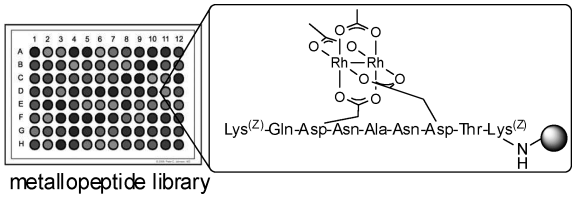


FIGURE 3. Libraries of metallopeptides Rh_2(peptide)$(OAc)_2$, were grown on solid support using a parallel synthesis strategy in 96-well plates. Reaction screening on bead allows a 96-member library to be synthesized and screened in less than 1 week.

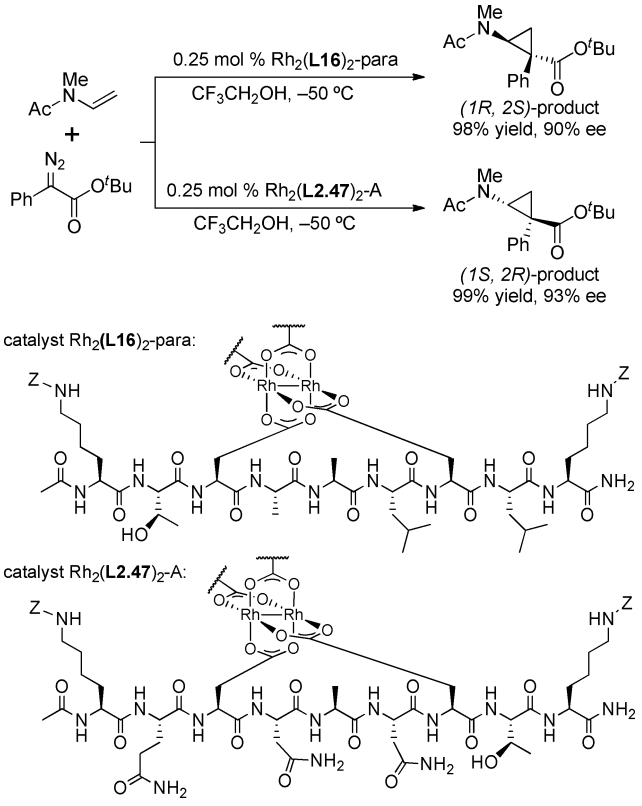


FIGURE 4. A solution to the "enantiomer problem." The complementary product enantiomers are accessible with completely distinct peptide sequences, each composed of only natural (L)-amino acids. The suffix "para" refers to the parallel isomer of peptide orientation. The suffix "A" is an arbitrary identification of orientational isomers.

bis-peptide variant, Rh_2(peptide)$_2$, separately in solution. This process identified a new catalyst to produce products with the opposite sense of enantioinduction (Figure 4, bottom path).

Site-Specific Protein Modification: Catalyzing Reactions That Override Inherent Reactivity

Site-specific protein modification is an interesting and unsolved chemical problem that we thought might be addressable with metallopeptides catalysts. Enzymes, of course, catalyze numerous chemistries on protein substrates. Some of these enzymatic reactions can be engineered to allow useful, sequence-specific modification in a laboratory, and a few chemical approaches to site-specific modification exist as well.[40,41] However, current methods generally require recombinant engineering of the protein substrate and have other drawbacks relative to chemical methods; general methods to modify natural proteins are an unmet need.

Conceptually, a peptide ligand would serve as a molecular recognition element, and the rhodium center as the

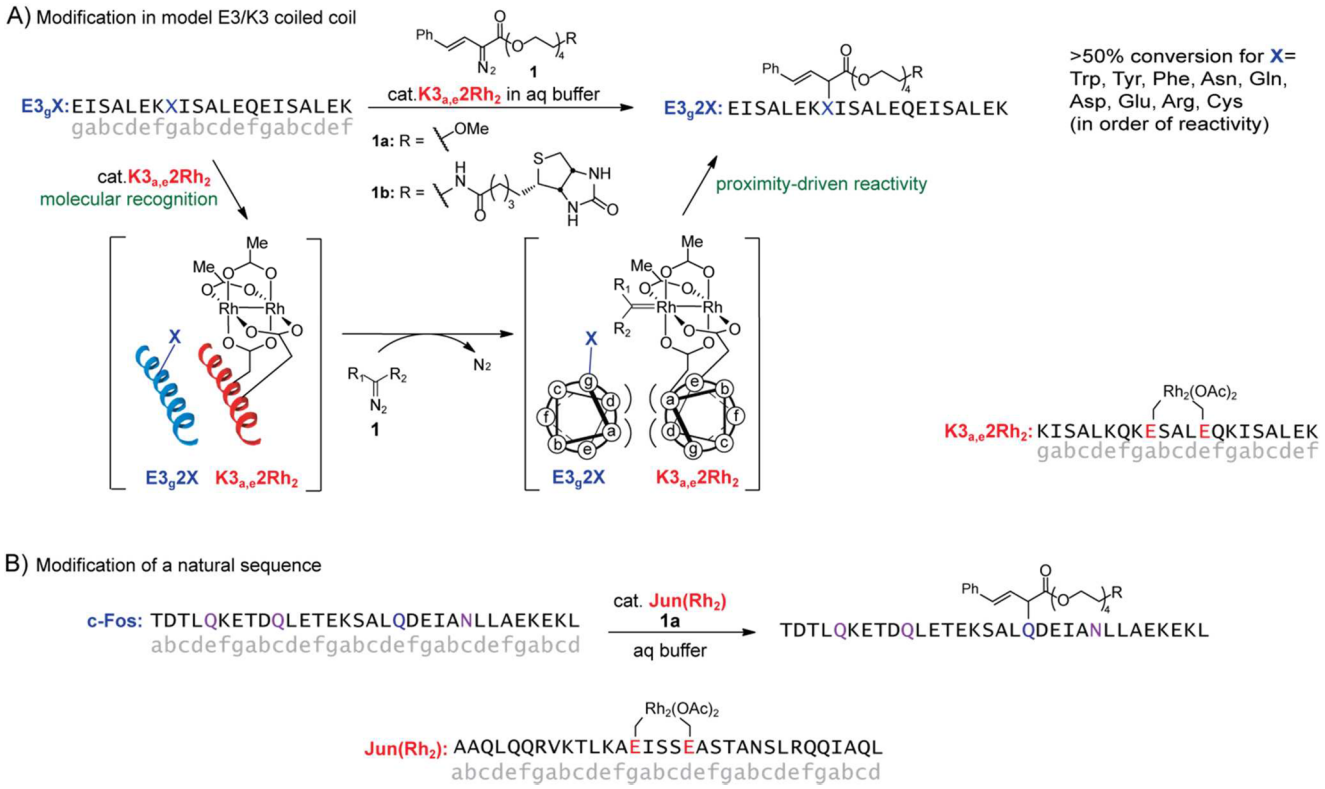


FIGURE 5. Proximity-driven modification of designed (A) and natural (B) coiled coils.[44,45] Letters in gray below each sequence indicate location on a helical-wheel model of a coiled-coil dimer.

modification catalyst. Binding of the peptide ligand to a protein target would localize the rhodium center near specific side chain(s), which would then be selectively modified as the substrate of a rhodium-catalyzed reaction (Figure 5). The modular nature of this design makes it possible to address recognition and reactivity separately.

We first examined dimeric coiled coils as a simple and well-studied model of protein–peptide interactions. Francis had reported modification of tryptophan indole side chains with a diazo reagent catalyzed by the rhodium complex $Rh_2(OAc)_4$.[42,43] In our design, a tryptophan residue at position *g* of a coiled coil (Figure 5, X = W) would be close in space to a rhodium catalyst, bound through carboxylates at positions *a* and *e*, on a complementary coil. In competition reactions of the designed tryptophan-containing substrate and a randomized control sequence, the designed substrate was far more reactive. Conversion > 90% was possible with 2 mol % catalyst (0.4 μM), and rate acceleration was $>10^3$ relative to background intermolecular reactions.

The true enabling power of proximity-driven catalysis has been the modification of otherwise completely unreactive side chains. Tryptophan is the only amino acid side chain for which modification was observed with simple small-molecule catalysts. However, when the residue at position *g* of the substrate coil is changed, many amino acids, comprising >40% of natural protein space, are effectively modified.[44,45] Although modification of other amino acids is inherently much less efficient than tryptophan modification, proximity-driven catalysis makes it possible to modify these less-reactive amino acids in the presence of tryptophan, even within the same molecule (Figure 6). This broad amino-acid scope implies that protein modification can be driven by molecular recognition regardless of the specific amino acids present near the binding interface. For several of these side-chain functionalities, such as phenyl (phenylalanine) and carboxamide (asparagine, glutamine), chemical modification is not possible by other chemical means.

The coil-driven modification provides a good testing ground to assess design capabilities. We were interested in developing several orthogonal substrate-catalyst pairs for the independent modification of multiple proteins. Another focus was the extent to which ground-state assembly successfully predicts catalytic activity. An exhaustive study of the reactivity and selectivity of all six possible tryptophan substrates within the E3 coil (all combinations of three sites along the coil axis and each of two faces: front and back; see Figure 7), in reactions catalyzed by each of six corresponding

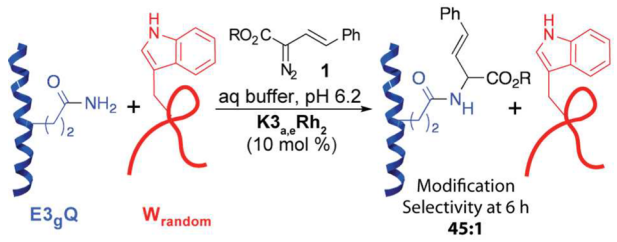


FIGURE 6. Catalyzing chemical reactions that override inherent chemical reactivity. The inherent reactivity of tryptophan (W) residues is estimated to be 12× that of glutamine (Q) residues, yet molecular recognition results in 45:1 selectivity in favor of the *less* reactive glutamate carboxamide functional group.[45] Reproduced from ref 45 by permission of The Royal Society of Chemistry.

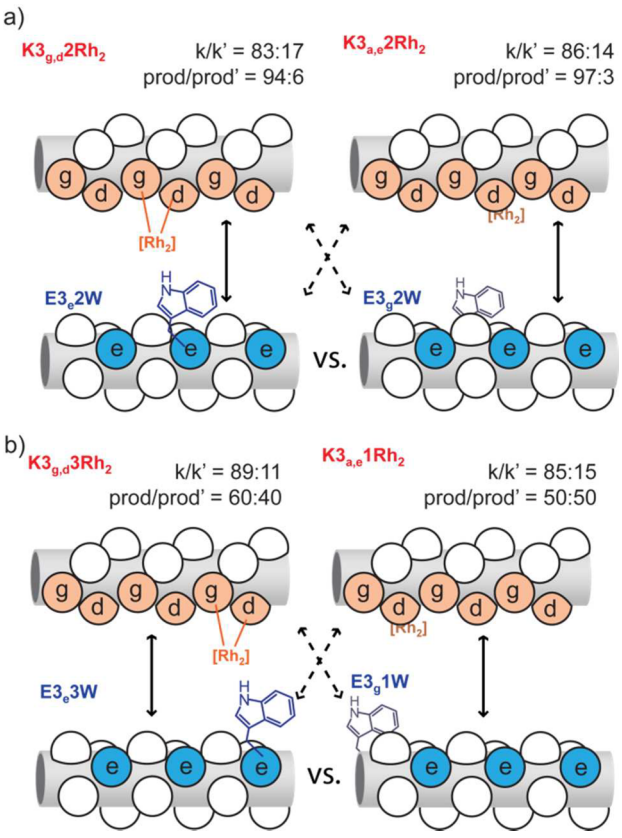


FIGURE 7. Measures of selectivity in template-driven modification of coiled coils. For each catalyst, two measures of selectivity are presented for reactions involving the two substrates below it: a "matched" substrate (directly underneath, solid arrows) and a mismatched one (broken arrows). Numbers indicate the relative modification rate observed in separate kinetic experiments (k/k′, matched/mismatched) and relative conversion observed in direct competition reactions involving a mixture of two peptides (prod/prod′, matched/mismatched).[46] (a) A representative highly orthogonal pair. (b) A catalyst pair that is nonselective in pairwise competition reactions.

catalysts, improved our understanding of the system.[46] The general conclusion of these studies was that designed catalysts are indeed capable of differentiating small sequence differences among mixtures of peptide substrates (Figure 7a).[46] The ability to distinguish among similar substrates with identical functional groups is an important metalloenzyme property replicated here.

Although most designed pairs were quite selective, it is worth mentioning an example that points out limitations in our understanding. Selectivity of a given catalyst was measured in two different ways: first, comparing product formation rates in separate reactions with matched and mismatched substrates (k/k′) and, second, comparing product ratios in direct competition experiments (prod/prod′). The selectivity of direct competition experiments (prod/prod') is generally superior to the rate ratio in separate, single-substrate reactions (k/k′) (e.g. Figure 7a). This fits with an intuitive (although not particularly rigorous) notion that unintended reactions are less likely in the presence of a matched substrate. The $E3_e3W/E3_g1W$ pair is an outlier (Figure 7b). Single-substrate reactions (k/k′) indicated a significant preference for the matched substrate (89:11 and 85:15, respectively), similar to that seen with other pairs (cf. Figure 7a). Yet, the competition experiment (prod/prod′) is almost completely nonselective for both peptides (60:40 and 50:50). Indeed, modification of the "mismatched" substrate pair occurs *faster* in the presence of the "matched" substrate. These results imply that simple, ground-state heterodimer explanations are insufficient to explain transition states for catalytic reactions; transient higher-order assemblies are apparently at play here. This, of course, is a fundamental problem in designing enzyme-like reactivity: selectivity is determined by transition state energy, yet we are largely limited to designing ground-state interactions.

Protein Modification

Orthogonal catalyst–substrate pairs could effectively modify two different proteins independently in a complex mixture. A recombinant form of maltose-binding protein (MBP) fused to the designed peptide substrate, $E3_g2W$, was selectively modified in crude cell lysate.[47] We then expressed a second protein (GST) fused to a separate peptide, $E3_e2W$. Treatment of lysate mixtures with diazo reagent allowed the two proteins to be individually and orthogonally modified, depending on the choice of metallopeptide catalyst (Figure 8).[46] This result demonstrates several enzyme-like features: First, specific catalysis is possible in a complex, functional-group-rich environment. Second, catalysts select for specific substrates based on catalyst sequence.

Moving from recombinant to natural protein substrates is a logical and important next step. The realization of

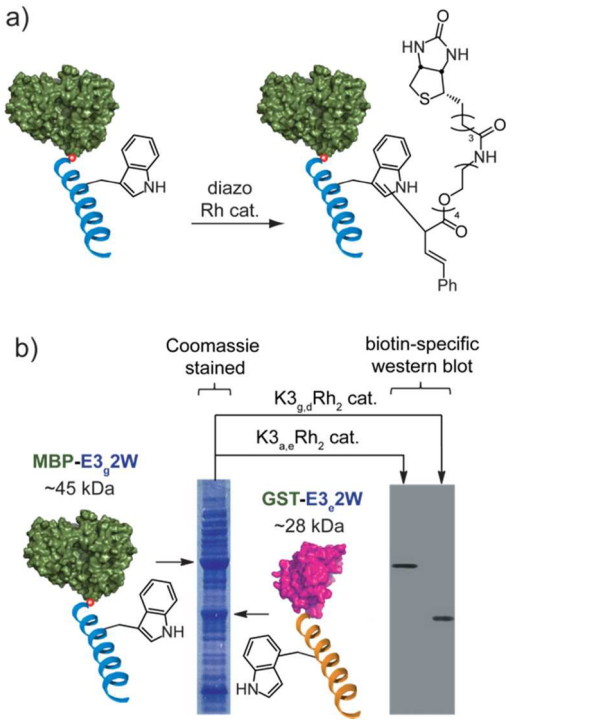


FIGURE 8. (a) Proximity-driven modification for site-specific modification of proteins at fused coil domains. (b) Catalyst-dependent, orthogonal biotinylation of two different proteins in lysate. Modification of different proteins with a biotin–diazo reagent is controlled by choice of rhodium metallopeptide catalysts.[46] (Adapted from ref 46. Copyright 2012 American Chemical Society.) Maltose binding protein (MBP) and glutathione S-transferase (GST) were expressed as fusions to the tryptophan-containing helices, $E3_g2W$ and $E3_e2W$, respectively. Visualization of protein modification was conducted with biotin-specific Western blot.

site-specific rhodium catalysis on recombinant (tagged) proteins is a powerful demonstration and a potentially useful tool. However, the promise of modifying natural proteins, based solely on molecular recognition without the need to engineer tagging sequences, sets these concepts apart from other approaches. We first examined c-Fos modification based on the Jun–Fos bZip domain.[48] This natural DNA-binding unit is a direct analogy of the designed coiled coils studied previously, and modification at a specific glutamine proceeded generally as expected using a rhodium analogue of Myc (Figure 5).[45] The Jun–Fos dimer exhibits weak dimerization ($K_d \sim 20\ \mu M$), and the rhodium analogue, Jun(Rh_2), binds with marginally weaker affinity ($K_d \sim 100\ \mu M$). The ability to perform selective chemistry at low concentration (1–20 μM) based on weak, transient assemblies is another common feature of enzymes replicated in this work.

SH3 domains bind short, proline-rich peptides in an extended PPII conformation, play key roles in human biology, and provide a useful platform to assess modification of a natural protein based on an entirely new recognition motif.[49] The Fyn SH3[50] (Figure 9a), like many other SH3 domains, contains several aromatic residues near the binding pocket that might serve as sites for modification, including two tryptophan residues. We were able to design and synthesize several variants of a proline-rich peptide known to bind Fyn, VSLARRPLPLP ($S2E^{Rh}$, $L3E^{Rh}$, $R2E^{Rh}$), that position a rhodium center near the binding pocket without disrupting binding. Modification is highly efficient with all three of the designed metallopeptides and is absent in the negative controls (Figure 9b).[46] Modification in this case occurs at tryptophan residues, of which there are two near the binding pocket. The different metallopeptide catalysts do have somewhat different reactivity and selectivity, with $S2E^{Rh}$ doing the best job ensuring only single modification at Trp42. From a practical perspective, it is encouraging that all three metallopeptides function as intended, indicating that it is not necessary to spend inordinate effort to find a "sweet spot" for each new protein of interest.

The SH3 domain is one member of a family of protein folds that recognize short peptide sequences. This set includes SH3, SH2, WW, and PDZ domains, among others. These domains represent some of the most attractive targets for study with proximity-driven catalysis. Because the ligand peptide is relatively small, synthesis of the requisite metallopeptide is relatively straightforward. All data obtained thus far points to the metalation reaction as a kinetic phenomenon: it is not possible to equilibrate the rhodium center among several carboxylates. Because of this, currently metallopeptide synthesis is limited to peptides. New methods are needed to modify protein domains that recognize proteins via large-surface-area contacts containing complex tertiary structure. Fortunately, calculation and screening methods are becoming adept at discovering small ligands for a given protein of interest.

Function beyond Catalysis: Hybrid Organic–Inorganic Protein Inhibitors

The low reactivity of histidine-containing peptides was one curious observation from our initial coiled-coil studies; phenylalanine, for example, is much more reactive. After conducting thermal unfolding studies and examining UV–vis spectra, it became clear that positioning histidine near the rhodium center led to coordination to the rhodium axial site. Because the rhodium atom has only a single open coordination site, this coordination inhibits catalysis.[51] Thermal unfolding studies revealed a ~20 °C increase in T_m due to histidine binding (to 66 °C).[52] Fitting the unfolding curves

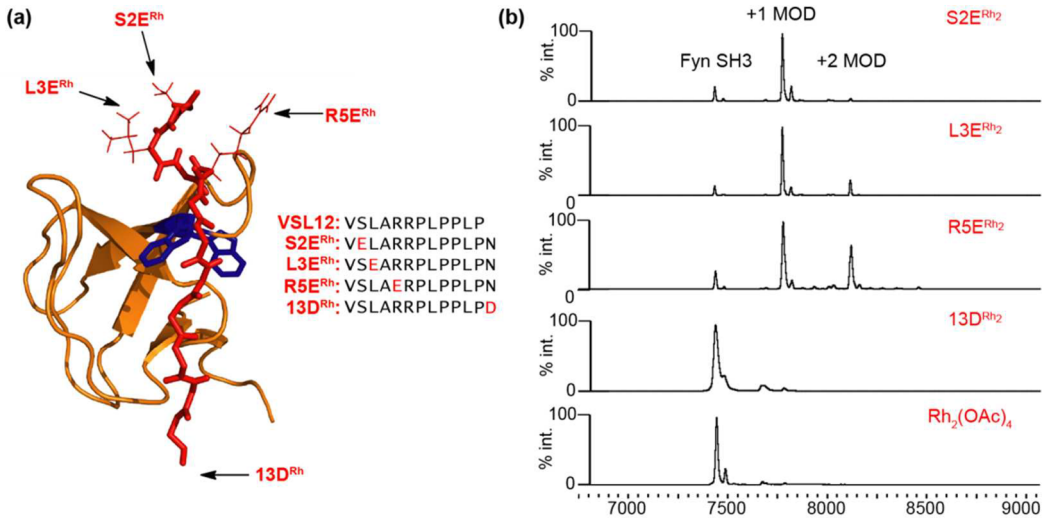


FIGURE 9. (a) Structure of the Fyn SH3 domain (orange). Arrows indicate rhodium placement along a Fyn-binding peptide (red) in the metallopeptide sequences given. (b) Results with various rhodium(II) metallopeptides for Fyn modification, demonstrating site-selective, proximity-driven modification of a natural protein. The two bottom entries are negative controls.[46] (Reprinted with permission from ref 46. Copyright 2012 American Chemical Society.)

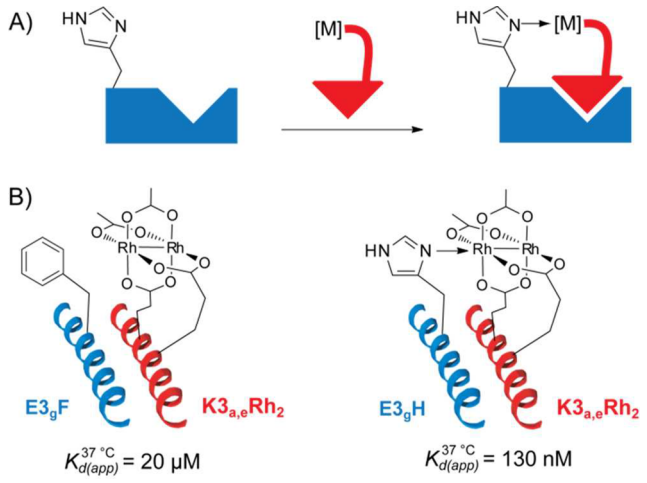
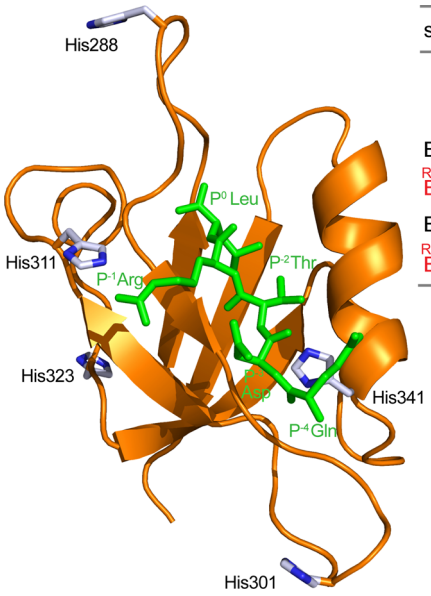


FIGURE 10. (A) Conceptual depiction of hybrid organic–inorganic inhibition, taking advantage of metal-side-chain coordination peripheral to a ligand-binding site to enhance the affinity of an otherwise weak inhibitor. (B) Specific rhodium–histidine interactions significantly improve the affinity of a coiled coil.[52]

gave roughly linear plots of log(*K*) vs *T* and allowed estimation of an apparent dissociation constant ($K_{d(app)}$). The coiled-coil stabilized by rhodium–histidine coordination exhibits an apparent dissociation constant of 130 nM, significantly lower than that of the parent coiled coil (20 μM, Figure 10B).[52]

Rather than a coil-histidine inhibiting a metallopeptide, we began thinking of a metallopeptide inhibiting a histidine-containing biomolecule (Figure 10). Many validated protein targets are resistant to inhibitor development with traditional small molecules. From our perspective, cooperativity benefits might result in improved potency if an organic inhibitor could be conjugated to a transition-metal capable of interacting with nearby Lewis-basic side chains, such as a histidine, cysteine, or methionine. Although conceptually straightforward, the idea has been challenging to put into practice.[53] Substitution-inert, covalent-like bonding is needed between the transition metal and the organic fragment: substitution-labile coordination is unlikely to be of use in a living cell, where transition-metal availability is tightly regulated.[54] While a substitution-inert linkage to the organic fragment is necessary, readily reversible binding to a side chain, such as histidine, on the protein target is required to prevent irreversible off-target activity. This requirement of a heterogeneous coordination environment is nicely satisfied by the tight equatorial and weak axial binding in a dirhodium complex. At an early stage of these studies, a few rhodium analogues of MDM2-binding peptides were synthesized and found to retain affinity for MDM2.[32] The study also provided the opportunity to develop synthetic approaches to carboxylate-containing metallopeptides, the native peptide sequence contained carboxylate residues that we wanted to preserve, but there was no affinity enhancement due to rhodium.[32]

Working with peptide and metallopeptide inhibitors of the CFTR-associated ligand (CAL) PDZ domain[56] brought significantly more success (Figure 11). The cystic fibrosis transmembrane conductance regulator (CFTR) is an ion channel protein, and its membrane localization is controlled by interactions of the CFTR C-terminal region with PDZ-containing proteins. Inhibiting one of these proteins (CAL)



sequence	K_i (μM)
VQDTRL	320
VQ^{Rh}DTRL	>6
EWPTSII	65
^{Rh}EWPTSII	1.7
EVQSTRL	42
^{Rh}EVQSTRL	0.56

FIGURE 11. Potent inhibitors of the CAL PDZ domain. Left: Structure of the CAL PDZ (orange, histidine residues shown explicitly) bound to a peptide, VQDTRL (green). Right: Inhibition constant (K_i) for peptide inhibitors, with and without rhodium. When a rhodium(II) center is conjugated to the P^{6-} residue, the metallopeptides are potent inhibitors of CAL PDZ based on specific coordination with the His301 residue.[55] (Adapted with permission from ref 55. Copyright 2012 Wiley-VCH.)

has been shown to increase CFTR function,[57] but developing a potent CAL inhibitor remains an unsolved problem. We screened metallopeptide variants of several known CAL-binding sequences before arriving at the metallopeptide E^{Rh}VQSTRL. This peptide binds CAL PDZ with $K_i = 0.56$ μM, 75-fold more potent than the parent peptide, EVQSTRL, and the most potent CAL PDZ inhibitor yet reported. Other sequences with the rhodium bound in the same P^{6-} position (such as E^{Rh}WPTSII) have shown similar gains in affinity, indicating a general effect of rhodium at that position. In addition, CAL PDZ mutagenesis experiments indicated that most of the affinity gain could be tied to a single histidine, H301, on CAL. The metallopeptide binds the H301A mutant with significantly increased K_i (9.2 μM), while the affinities of the parent peptide for the mutant and wild-type proteins are within 2-fold of one another (80 and 42 μM, respectively).[55] Affinity pull-down inhibition experiments demonstrated that CALP interactions are more effectively inhibited in cell lysate as well.[55]

Outlook

The prospects for designing useful catalysts with enzyme-like selectivity are bright and exciting. At the same time, progress has been slow and hard-earned; significant problems remain. Chemical research has naturally progressed toward increased molecular size and molecular complexity, and this increased complexity means that the development of catalysts with selectivity that rivals that of natural enzymes is increasingly important. We are only now beginning to understand how to design macromolecular structure, and designing macromolecular function, in the sense of reactivity, is an even more challenging problem that we simply do not yet have general methods to address. Compared to the beautiful achievements in protein structure design, the examples of designed reactivity in this work and by others appear crude by comparison. In addition, improved methods for high-throughput catalyst discovery, and their creative implementation in new contexts, are needed. Remarkable advances in bioengineering have made it possible to assess libraries of thousands or millions of compounds to identify aptamers, antibodies, or enzyme mutants, to name a few. These techniques are often made possible through affinity pull-down methods or through in vivo reactivity screens linked to survivability or some other readily observable outcome. Applying very high throughput approaches to new bond formation or enantioselectivity is challenging when cell viability or visible readout is not possible. Continued improvement in high-throughput screening techniques, at both a scientific and an engineering level, are likely go hand-in-hand with improved design methods.

For small-molecule catalysis, we are convinced of the benefits that the modular peptide ligand structure provides. In moving beyond rhodium(II) centers, a key challenge will be to build well-defined metal-binding sites for diverse metals. In addition, there are probably catalytic processes with which peptide ligands are incompatible. It is interesting to speculate what other modular polymers could be used as the basis of high-throughput ligand screening, including peptoids and foldamers with entirely nonpeptidic backbone structure. A next step in the small-molecule area is the development of general methods for molecular recognition that might enable site-specific chemistry to override inherent reactivity. For protein and nucleic acid substrates, a huge body of work has provided a framework for thinking about molecular recognition and assembly. For small molecules, this understanding is lacking and in some cases the challenges more daunting. Although a few approaches to this problem exist, truly general, useful frameworks for positioning small molecules at a catalyst site remain a formidable challenge.

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Zachary T. Ball is an assistant professor of Chemistry at Rice University. He grew up in Columbus, OH, and earned an A.B. in in Chemistry from Harvard University (1999), where he worked in Gregory Verdine's laboratory. He moved to Stanford University to study transition-metal catalysis in Barry Trost's lab, obtaining a Ph.D. in 2004. He then moved to UC–Berkeley as a Miller Fellow working on photo- and electroactive polymers in the lab of Jean Fréchet until 2006, when he moved to Houston to take up his current position at Rice.

FOOTNOTES

The author declares no competing financial interest.

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