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Taking Inspiration from Biology to Explore New Chemical Reactivity

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Biomimetics, Second Coordination Sphere interactions, Artificial Metalloenzymes

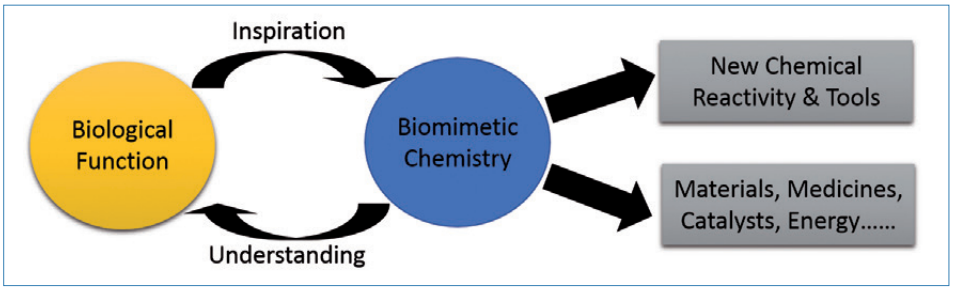


Figure 1: Flow of information in biomimetic chemistry: from biological systems to applications

Nature has evolved methods to perform complicated chemical processes with high selectivity and efficiency as part of the basic pathways necessary for life. This ranges from specific molecular recognition across multiple size regimes, to efficient high turnover activation of small molecules (H_2, O_2, H_2O, CO_2, etc…), to the synthesis of large stereochemically complex natural products. These are functions that not only hold high value for application towards human health and sustainable energy development, but also represent areas where better fundamental understanding of the basic chemical processes involved can provide significant advances. One approach to accomplish this is by using the ways that biomolecules perform these functions as inspiration for developing new molecules and chemical reactivity. In doing so, we not only test and increase our fundamental understanding of the mechanisms behind these functions, but, with success, open up the potential to match or even exceed the capabilities of biological systems, Figure 1. This is the essence of biomimetic chemistry [1].

1. Biomimetics

The term biomimetic was coined in the 1950's to describe technology, engineering, scientific approaches, etc… that are inspired by or attempt to imitate the mechanisms and functions found in Nature [2]. However, the idea of trying to replicate natural processes has, without doubt, existed for a much longer time. One can imagine early humans observing birds flying, crafting the first artificial wings, climbing to the top of a cliff, flapping their arms wildly, and then proceeding to leap to their death. This example, beyond being a cautionary tale for the importance of safety in experimental design, also introduces two important aspects of biomimetics:

-First, *Mimicking does not mean exactly duplicating*. To use the often quoted example, a jumbo jet is not just a giant pigeon [3]. The success of manmade flight derives from using concepts inspired from studying birds and combining these with the tools available.

-A *detailed understanding of the mechanisms involved in the biological process is essential*. It is not solely the flapping of wings that lets birds fly, but rather multiple components working together. Understanding the role of each and separating out what is and is not crucial for achieving function is important for design.

2. Biomimetic Chemistry

The above concepts hold equally true for biomimetic chemistry. The structural complexity of biomolecules, which gives rise to their various functions, represents a significant synthetic challenge. Trying to compete using the same limited set of building blocks (amino acids, nucleic acids, etc…) available to biology would be a near impossible task. Rather, chemists focusing on trying to mimic these systems, use the best approximations for achieving similar function without the complexity of an entire biomolecule. This can be observed in the examples shown in Figure 2.

The first three examples highlight a continuing goal of biomimetic chemistry, the development of artificial enzymes, Figure 2A-C [4]. One of the early approaches for this was inspired by substrate binding sites in enzymes. Numerous groups showed that synthetic host molecules appended with reactive groups could interact with a target molecule and promote diverse reactions including deacetylation, transacylation, aminolysis, etc. This strategy continues to be exploited, notably with inorganic systems, where cavitands have additionally been used to provide site isolation and hydrophobic environments for metal sites in order to mimic the analogous effects in metalloenzymes without having to use an entire protein [5].

The use of abiological components is also wide spread in structural biomimetics. Foldamers [6], synthetic molecules that adopt stable higher ordered structures in solution through non-covalent interactions, have been synthesized that mimic helices or β-sheets and even tertiary or quaternary structures. Often these molecules contain monomer units, such as the aromatic heterocycles pictured in Figure 2D [7], that do not resemble those found in biopolymers. As our abilities to design more complex synthetic architectures continues to advance, the possibility to expand on the

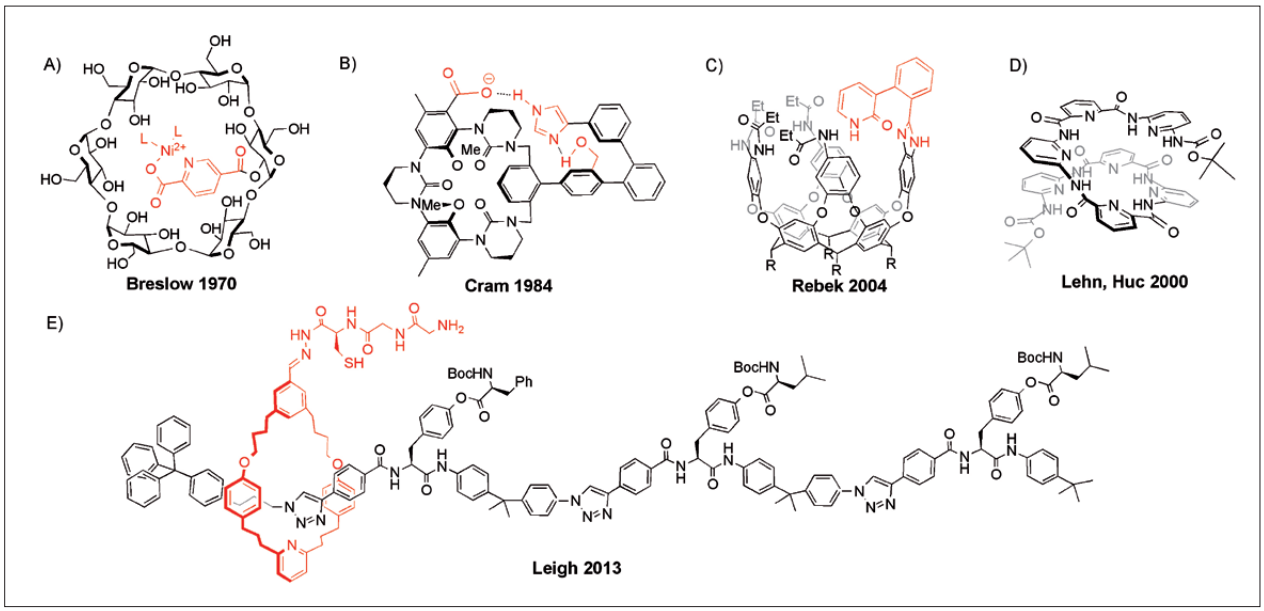


Figure 2: Examples of biomimetics using synthetic components for mimicking biological features/reactivity. A) Cyclodextrin based artificial enzyme with a substrate recognition site (black) and a reactive site (red) that catalyzes deacetylation; [4a] B) Chymostypsin mimic incorporating a catalytic triad (red) on a recognition site; [4b]C) Resorcinol cavitand with convergent functional group (red); [4c] D) Structural biomimetic of helices found in nature using an aromatic oligoamide sequence [7]; E) A rotaxanne based biomimetic of ribosomal peptide synthesis [8].

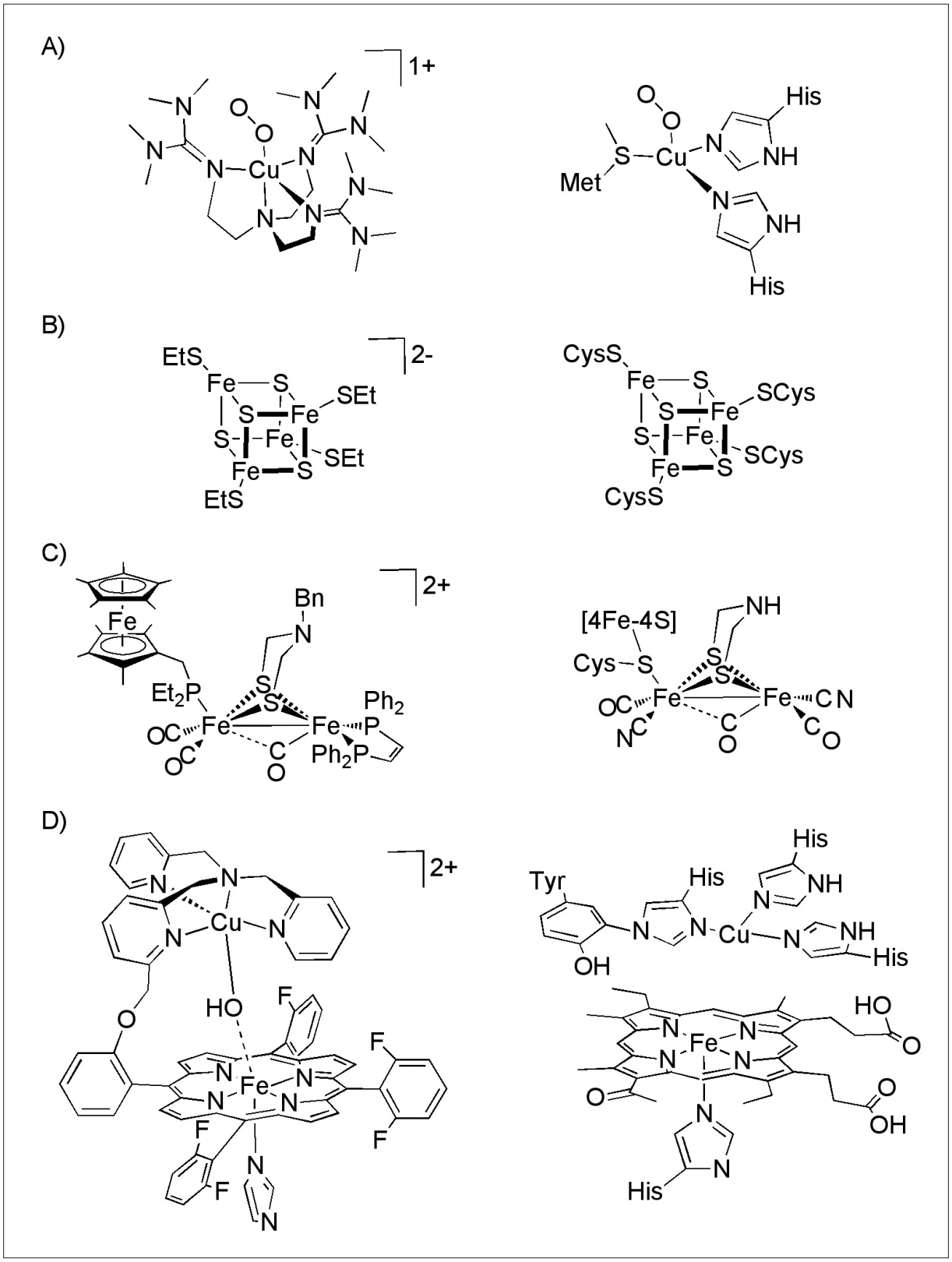


Figure 3: Model Complexes (left) of biological metal sites or interactions (right)[10-11] A) Oxygen activation by copper in monooxygenase (example: peptidylglycine-a-hydroxylating monooxygenase), B) 4Fe4S clusters; C) Active site of [FeFe]-Hydrogenase; D) Cytochrome c oxidase

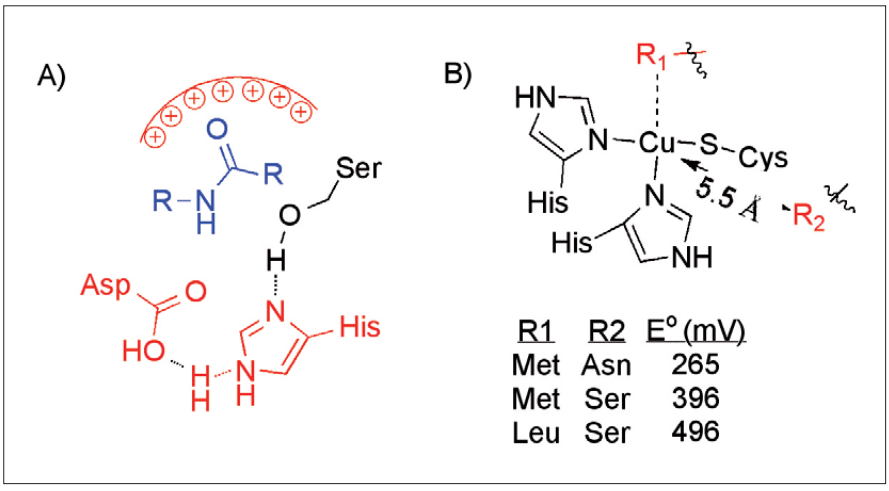
design of artificial enzymes or reactive biomimetics has the potential to lead to intricate systems designed to perform specific syntheses. This is well illustrated by the recent example from Leigh and co-workers of a rotaxane based molecular machine, Figure 2E, that can synthesize short peptides in a sequence specific manner, mimicking ribosomal peptide synthesis [8].

For the second concept of biomimetics listed above, understanding the mechanistic nuances of a biological reaction or function is also important for the design of functional biomimetic molecules. However, rather than just taking inspiration from this understanding, as is the case in most other areas of biomimetics, biomimetic chemistry is unique in the ability to additionally provide a means to study biological functions and reactions. Due to the common molecular nature of chemistry and biology, the application of biomimetic molecules, notably model compounds – molecules that aim to reproduce a specific feature of a biomolecule or reaction intermediate, can aid in testing mechanistic hypotheses or confirming the presence of certain structural features in a biomolecule [9].

One area that has made extensive use of this approach is bioinorganic chemistry, where numerous model complexes have been synthesized as a way to better understand the reactivity of metalloenzymes. Figure 3 shows a few examples of these systems as well as the biological components they model. These range from providing information about metal-substrate interactions (Figure 3A) [10] to understanding cooperation in the more complex multi-metallic sites found in redox cofactors or active sites, Figure 3B-D [11]. These models can serve as spectroscopic probes and aid in the understanding of the electronic structures of metalloenzyme active sites. In this way, while the design of these molecules benefits from advancements in other areas including biochemistry, structural biology, and chemical biology, they also provide valuable chemical tools for advancing understanding in these fields as well.

3. Trying to match the reactivity of enzymes

While biomimetic chemistry encompasses a range of topics in chemistry, one of largest areas of focus is the development of biomimetic catalysts that can match the efficiency and selectivity of enzymes. For the biomimetics or model complexes described above, many are capable of performing the same reaction as their natural analogue, however they typically display significantly lower levels of reactivity. This disparity indicates that, while the concepts or structural components that these systems mimic are important, additional aspects must be considered in the design of reactive biomimetics. Part of the challenge in replicating enzyme activity is that the active site is not an isolated feature separate from the surrounding protein. Rather numerous non-covalent and cooperative interactions surrounding the active site act to facilitate and tune reactivity. These interactions comprise a second coordination sphere (SCS) [12] (for substrate and/or for metal atoms in the case of metalloenzymes) that is essential for observed reactivity. This can be well illustrated by the catalytic triads present in some proteases. The example in Figure 4a shows a Asp-His-Ser triad in the active site [13]. The residue directly



R1	R2	E° (mV)
Met	Asn	265
Met	Ser	396
Leu	Ser	496

Figure 4: Active sites of A) a Asp-His-Ser protease [13] and B) Azurin [14] showing the primary coordination sphere or active residues (Black) and residues in the second coordination sphere that influence reactivity (Red). For B) values for the Cu(II/I) reduction as a function of amino acids present in the second coordination sphere are given below the structure.

responsible for cleaving the peptide bond (shown in black) is the serine. However, this is part of a hydrogen bonding network in the SCS (shown in red) that includes the His and Asp groups. The effect of this network is an increase in the nucleophilicity of the serine. Additionally, a collection of positively charged groups in the SCS near the oxygen of the amide carbonyl additionally the formation of the negative charge.

The type 1 Cu site in Azurin is also an excellent example of the influence these interactions can have on the properties of a metal site. Lu and co-workers have shown that the SCS can be used to tune the reduction potential for copper by over 700 mV. Interestingly, variation in SCS groups almost 6 Å from the copper center can affect the Cu(II/I) reduction potentials as much, or more in some cases, as groups that interact more directly with the Cu [14].

With such effects possible, it is intriguing to think that the perfect combination of such interactions in synthetic complexes could generate new and highly active catalysts. The best illustration of this possibility is the recent report on the insertion of a synthetic model complex, $[(\mu\text{-}SCH_2NHCH_2S)[Fe(CO)_2(CN)]_2^{2-}$, of the [FeFe]-hydrogenase active site into the apo-hydrogenase enzyme [15]. The apo-enzyme is the perfect SCS for this model complex. Upon inclusion of the unreactive model system, a reactive enzyme, equivalent to the natural system, is obtained. Perhaps, more impressive is that fact that an optimized SCS might be generally applicable to less than ideal complexes. Even when a hydrogenase model complex is less approximating of the active site, inclusion in the apo-enzyme can still give residual activity that is higher than the synthetic complex by itself [16].

The idea that a SCS can be orthogonally optimized for a reaction in parallel to the catalyst could be a powerful new approach for synthetic chemistry. However, to accomplish this, a better fundamental understanding of how these SCS interactions influence reactivity and methods for including them in synthetic complexes must be developed. This is one of the main focuses of our research group. To do this, we combine synthetic and supramolecular approaches to develop multi-functional ligand environments for understanding SCS interactions in metalloenzymes and for studying metal/metal and metal/ligand cooperativity. A brief description of some of the projects in this area, on-going in our laboratory, is given below.

4. On-going projects

4.1. Developing scaffolds for mimicking multi-layered coordination environments

A major obstacle for studying SCS interactions in synthetic complexes is the availability of methods for generating organized, asymmetric, and easily modifiable functional group arrays around a reactive center. A host scaffold needs to be large enough to surround the active site, functionalized to allow tuning of the environment, and structurally ordered for specific placement of functional groups. The combination of the above traits in a single system is rare. Indeed, large size, ordered structure and asymmetry, while common in biology, are generally conflicting traits when it comes to synthetic feasibility. Our group is exploring two different approaches to overcome this problem:
1) The use of structural biomimetics, specifically aromatic oligoamide foldamers, that fold into stable secondary structures in order to provide pre-organized metal binding sites, Figure 5A&B. These scaffolds allow us to precisely control the positioning of first coordination sphere ligands and to add functional groups at multiple locations for studying non-covalent modification of metal based reactivity and properties. Using these features, we are developing multi-layered mimics of cupredoxins [14] and lytic polysaccharide monooxygenases [17], Figures 4C & 5C, in order to better study the role of structural features and SCS interactions in these enzymes that are not easily accessible by standard small molecule approaches.

2) The use of host-guest systems based on molecular capsules and tweezers with specific sites for recognition of metal complexes, Figure 5D&E. Planning asymmetric environments based on host-guest approaches poses a challenge. In order to predictably provide specific SCS interactions the number of orientations that the metal complex can adopt upon interaction with the host must be limited. By designing parts of the host molecules to include single sites that directly interact (through hydrogen bonding, metal coordination, π-stacking, etc….) with the first coordination sphere ligands on the guest complexes,

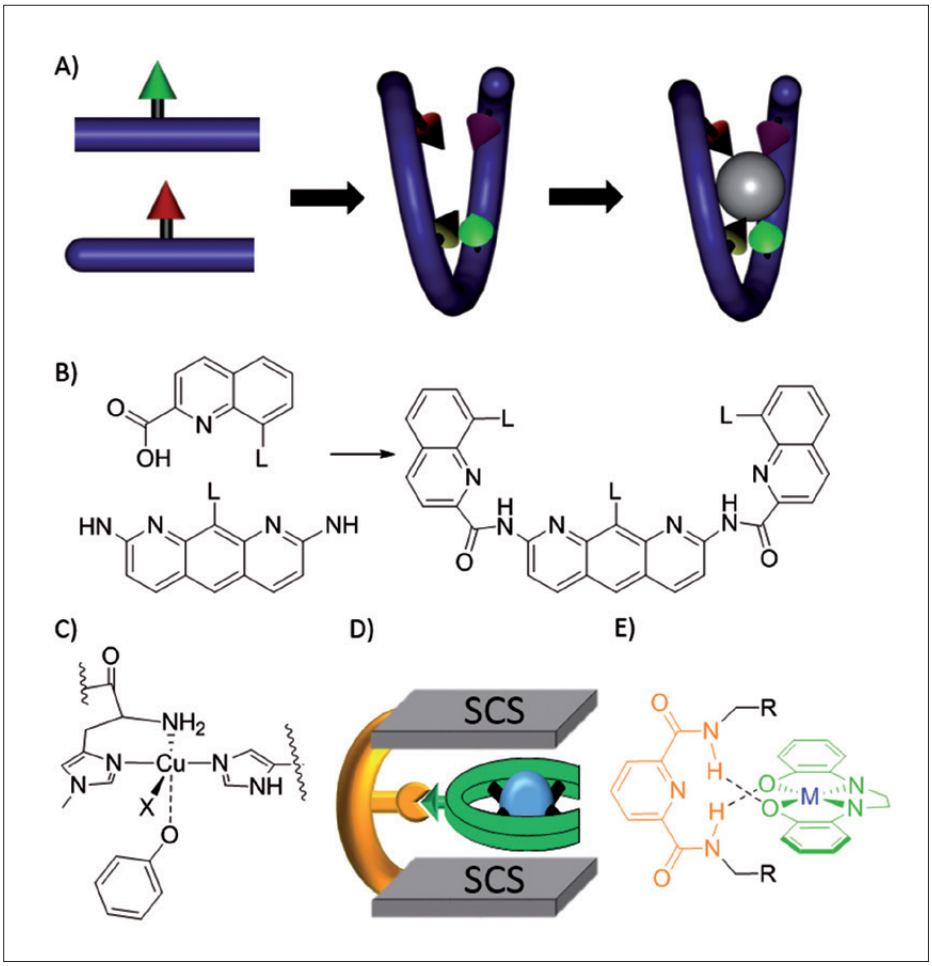


Figure 5: A) Cartoon representation of the SCS design process. Functionalized Monomers are linked together to give an oligomer that folds into a stable structure with a preorganized binding site for a metal atom. B) Heteroaromatic monomers can be used to design curved sequences with convergent functional groups for this purpose. C) The active site of lytic polysaccharide monooxygenases is an initial target for mimicking with the folded multi-layered coordination approach. D) Cartoon representation of the host molecule with a bound guest and E) a general chemdraw structure showing the interactions between the host and guest. The metal complex is represented in green. The SCS can be modified through modification of the R groups.

we can control the way the guest is aligned within the host. Using this approach we have developed multiple host molecules that can bind planar metal complexes in a specific orientation with association constants up to 1 x 10^5 M^{-1}. By including similar matching sites into the first coordination sphere of other metal complexes, the SCS hosts can be easily applied to a variety of different metal complexes. This can help provide insights on test the general applicability of an optimized SCS on the reactivity of different catalysts.

4.2. Electron reservoirs for cooperative redox reactivity

Within the coordination spheres found in metalloenzymes, multiple components exists that also participate more directly in the catalytic reactions. An impressive example is the use of electron reservoirs for the multi-electron activation of small molecules.

Biological systems use base metals to perform multi-electron transformations. This is in sharp contrast to synthetic chemistry, where reactions requiring multiple electrons are routinely performed with noble metal catalysts. While the later readily undergo two electron oxidations or reductions, typical redox couples for base metals involve single electron oxidation and reduction events. Natural systems overcome this issue through the use of additional electron donating/accepting sites present near the active site or as part of an electron transport chain, Figure 6A&B. These sites act as molecular capacitors, storing and providing electrons for reactions in order to avoid energetically unfavorable states at reactive centers.

To mimic this aspect, we are developing a wide variety of highly asymmetric ditopic ligands, Figure 6C, containing hexadentate and mono/bidentate sites, and using them to synthesize multinuclear base metal complexes capable of multi-electron chemistry. In doing so, our goal is to develop cheap and efficient catalysts that can readily perform the oxidative addition and reductive elimination steps needed for cross-coupling reactions.

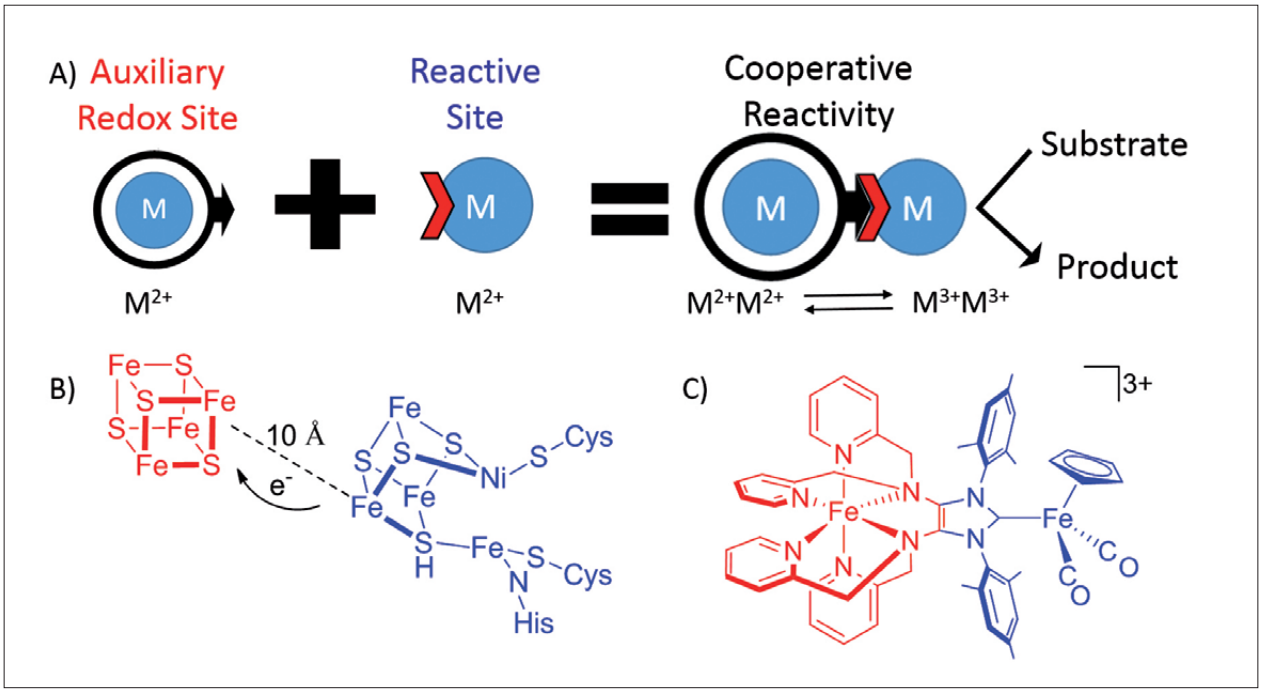


Figure 6: A) Schematic representation of the project; B) Active site of carbon monoxide dehydrogenase showing one of the iron-sulfur clusters involved in the electron transport chain.[18] C) Example of the complexes targeted in our group for cooperative redox reactivity. Reactive sites are shown in blue. Redox auxiliary sites are shown in red.

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