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The Interface of Inorganic Chemistry and Biology

This *JACS* Select issue covers 21 Communications and Articles at the interface of inorganic chemistry and biology.[1−21] The application of metals to treat human ailments dates back at least to the fifth century B.C., and the study and mimicry of metals in biology is a vibrant subject today. It was difficult to limit the selection of articles to those covered here, but they are representative of the panoply of publications on this topic in the journal over the past decade.

Typical inorganic elements, especially metals, are responsible for important biological functions, including cell signaling, metabolism, energy production, and the immune response. The introduction of inorganic probes of biological structure and function and the expanding role of metals in medicine constitute another major component at the interface between inorganic chemistry and biology. Communications and Articles in the *Journal of the American Chemical Society* at this interface reflect the creative spirit with which chemists approach their science. Many report the invention of molecules that mimic aspects of a metalloenzyme active site with the aim of providing geometric and mechanistic fundamentals to guide the interpretation of results obtained by direct studies of the natural systems. The present bioinorganic *JACS* Select issue is dominated by such contributions, for they accurately reflect publishing activity in the area. Other selected articles provide insight into how the natural systems import metal ions or metal-based drugs, assemble cofactors, or achieve catalytic chemistry. Additional publications describe novel analytical probes of metal ions in biology. Although fewer in number, this last group represents an important and growing component of contributions to the journal and are therefore included in this compendium.

Metal Entry into Cells and Active Sites

In order for a metal ion to function in biology, it must first enter cross the cell membrane. Bacteria acquire iron with the assistance of specialized ligands known as siderophores, which provide hexadentate coordination, often via three chelating catecholate units. The details of how this process is managed by bacillibactin in Gram-positive bacteria and by enterobactin in Gram-negative bacteria have been sought for many years, culminating recently in a reasonably

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complete picture.[1] Once the iron-coordinated siderophore enters the cell, a specific esterase is required to hydrolyze the backbone of the ligand to promote release of the iron. The chirality of the ligand backbone and tris-chelate metal center is an important feature of the process.

Once metal ions enter cells, they must find their way to metalloenzyme active sites or, in certain cases, be converted into specialized cofactors. An example of the latter is Moco, a molybdopterin construct that is synthesized from guanosine-5′-triphosphate (GTP). The first step in this process is catalysis by the enzymes MoaA and MoaC. The former contains two $\{4Fe\text{-}4S\}^{+/2+}$ clusters, one of which binds *S*-adenosylmethionine (SAM) to generate a radical required for the chemistry, and the other of which binds to the GTP substrate. Although X-ray crystallography was unable to elucidate the details of the latter interaction, $^{14}N/^{15}N$ ENDOR spectroscopy of a triple-mutant MoaA enzyme, engineered to remove the catalytic SAM-binding cluster and simplify data analysis, revealed the nature of GTP binding.[2] The primary interaction occurs between deprotonated N1 of the guanine ring and a corner of the iron−sulfur cluster. Metal-induced tautomerization of the purine ring may play a role in subsequent chemistry leading to the synthesis of the pterin ring.

Understanding Naturally Occurring Metalloenzymes

Three contributions in our selection present studies of naturally occurring metalloenzymes. Two of these enzymes are catalysts for the synthesis of small inorganic molecules, O_2 and NO. The former is essential for aerobic life as we know it on Earth, and the latter serves as an immune system component and biological messenger. Most life processes that involve these molecules, from their synthesis to their translocation in living organisms and to their utilization, involve bioinorganic chemistry.

The formation of dioxygen from water at a polymanganese/calcium cluster in photosystem II (PSII) is accompanied by deprotonation events. With the use of isotope-edited Fourier transform infrared (FTIR) spectroscopy, it has been possible to monitor proton release during this process.[3] The Kok cycle in PSII involves four flashes of light to trigger the steps in water oxidation, and FTIR spectral analysis of the process reveals that proton release from substrate (H_2O) occurs with a 1:0:1:2 stoichiometry for the $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ steps. These values put limits on the chemical species corresponding to intermediates, which has led to a schematic picture of the water oxidation process at the polymetallic center in PSII.[3]

The biological synthesis of nitric oxide from L-arginine is a complex process, involving oxidation by O_2 of the guanidinium group of the amino acid arginine at an iron porphyrin center with the assistance of NADPH as a reducing agent to prime the center for dioxygen activation and with the participation of tetrahydrobiopterin as a cofactor. N^G-Hydroxy-L-arginine (NHA) is a stable intermediate in the process.[4] A ferric peroxo intermediate has been postulated in the step(s) that convert NHA to the final products, NO and L-citrulline. This hypothesis is now supported by studies of site-directed mutants of inducible nitric oxide synthase (iNOS) and the use of fluorinated substrate analogues.[4] In this study, H_2O_2 was used to mimic the natural, O_2-dependent pathway. The ability of bioinorganic chemists to manipulate the parameters of complex metalloenzymes in this manner to obtain detailed mechanistic information is one of the hallmarks of the field.

The third publication in this section reports the results of a study of AurF, which utilizes O_2 to oxidize *p*-aminobenzoate (PABA) to *p*-nitrobenzoate, a building block of the antibiotic aureothin.[5] This enzyme contains a diiron center similar to those in non-heme enzymes that oxidize hydrocarbons and a variety of other substrates by activating dioxygen reductively. In the case of AurF, a diiron(III) peroxo intermediate of unusual longevity ($t_{1/2} = 7$ min in the absence of substrate) was characterized and found to effect the first and possibly subsequent steps in the overall six-electron oxidation of PABA. The mechanism was postulated to resemble steps in the oxidation of aromatic substrates by toluene/*o*-xylene monooxygenase.[22]

Biomimetic Chemistry

The size and complexity of metalloenzyme active sites have inspired many bioinorganic chemists to prepare synthetic analogues designed to mimic the structures, physical properties, and functions of the natural systems. Success in this endeavor has the potential not only to provide insights in the workings of the enzymes but also to produce new chemical catalysts for promoting difficult reactions under mild conditions now often referred to as "green". The *Journal of the American Chemical Society* attracts arguably the best research articles of this kind, and it is no surprise that this *JACS* Select would highlight several of them. In this section we reprise 12 such articles ranging in chemical diversity from proton-coupled electron-transfer

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reactions, which underlie many biological redox protein mechanisms, to the synthesis and chemical reactivity of models for enzymes that fix, bind, and/or activate simple inorganic molecules including N_2, H_2, CO, and NO.

Molybdenum nitrogenase is one of the most complex metalloenzyme systems, containing two proteins with iron−sulfur clusters that deliver electrons and protons in a chemically choreographed molecular dance in which N_2 is converted catalytically into NH_3, with H_2 forming as a byproduct. The Mo protein contains two clusters, a so-called *P*-cluster that delivers electrons and an iron−molybdenum cluster, or cofactor (FeMoco), where dinitrogen is reduced to ammonia. The chemical compositions of these clusters are complex, and it has been difficult to reproduce them outside the enzyme system. In a significant step toward resolving this problem, two routes to forming the [8Fe-7S] core structure of the *P*-cluster have been devised.[6] From detailed physical studies of the model complexes, the authors were able to assign details of the electronic structure of the cluster that should help to interpret the properties of the analogous unit in the enzyme.

Of equal if not greater interest is the iron−molybdenum cofactor, the site at which N_2 is converted to NH_3. Although the presence of Mo in this cofactor has long focused attention on molybdenum as the potential site of dinitrogen binding and activation, recent studies have suggested interaction along an Fe−Fe edge of the [7Fe-Mo] FeMoco cluster to be a reasonable alternative.[23] This work has inspired the synthesis of thiolate-bridged diiron complexes and studies of their interaction with dinitrogen and hydrazine or diazene compounds that might mimic intermediates in the mechanistic pathway leading to N_2 reduction. This *JACS* Select issue contains one such report, namely, the preparation and catalytic N−N bond-cleaving properties of [Cp*Fe(μ-SR)$_2$(μ-η^2-R′N═NH)FeCp*] complexes.[7] Studies of this kind establish precedence for proposed enzyme intermediate chemistry, albeit with ligands that are not biologically relevant.

One kind of biological interaction that has been contemplated for over four decades[24] but only recently realized is that between a metal cation and the π-cloud of an aromatic residue. Following on a report of a Cu(I)−π interaction involving a tryptophan side chain in the copper-trafficking factor CusF,[25] one of our *JACS* Select articles now describes a Cu(II)−π interaction and its signature circular dichroism spectrum in the bioactive neuromedin C peptide NMC, Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2, where the last NH_2 designation refers to N-terminal amidation.[8] The well-known participation of copper and the indole side chain of tryptophan in biological electron-transfer reactions suggests that metal ions may one day be found to modulate this function in metalloproteins, now that the interaction has been identified in two systems.

The activation of dioxygen in metalloenzyme active sites, typically those of Mn, Fe, and Cu, is an essential component of many catalytic biological oxidations. Logically, this multielectron process is expected to proceed in single-electron steps, with an adduct between the metal ion in the $(n + 1)$ oxidation level and the superoxide ion, O_2^-, being the initial species formed in the process. Capturing a superoxo complex in biomimetic chemistry has been a challenge, which has been met in the case of copper by a structural mimic of the copper(II)−superoxo intermediate in peptidyl-glycine *R*-hydroxylating monooxygenase and dopamine β-monooxygenase.[9] The synthetic model complex also shares many of the physical and chemical properties of the enzyme active sites, including aliphatic hydroxylation chemistry.

Intermediates downstream of the superoxo species formed during the activation of dioxygen are more prevalent in kinetic studies of metalloenzymes, but identifying their geometric and electronic structures can be challenging. Synthetic analogues are helpful by providing spectroscopic signatures for comparison to the natural systems. Of particular interest is the diiron(IV) oxo intermediate Q, which forms in the methane monooxygenase catalytic reaction. Q, for which a definitive structure has yet to be obtained, converts CH_4 selectively to CH_3OH. Mössbauer spectroscopic analysis of biomimetic diiron(IV) complexes, generated by addition of H_2O_2 to diiron(III) precursors and trapped at low temperatures and frozen, provides a valuable benchmark for comparison with physical studies of the enzyme intermediate.[10] The teasing out of Mössbauer parameters for the diiron(IV) unit of interest, in the midst of signals from other species present in the frozen solution, requires careful analysis and many repeated measurements. Theoretical analyses and comparison with the results of other methods such as EXAFS spectroscopy add further confidence in the approach.

We have already seen how electron transfer (ET) primes FeMoco during N_2 reduction, but ET and proton-coupled ET (PCET) similarly are important for closing a catalytic cycle in the

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reductive activation of O_2 for hydrocarbon oxidation. Fundamental studies of PCET continue unabated, and the results are critical to forming the foundation of knowledge necessary to interpret biological ET. A *JACS* Select Communication takes our knowledge of how the sites of electron and proton transfer in PCET can be separated physically by long distances, >10 Å, and still maintain the coupling required for efficient PCET.[11] Reactions of this kind have been reported in several metalloenzymes.[26]

Hydrogenase enzymes interconvert H_2 and ($2H^+ + 2e^-$) at [FeFe] or [NiFe] dimetallic centers containing ligands classically found only in organometallic and not bioinorganic catalysts, namely CO and CN^-. Because the metal ions are bridged by a dithiolate ligand, their environment is susceptible to oxidation reactions that convert thiolates (RS^-) to sulfonates ($RS{=}O^-$). One of our *JACS* Select articles reports the synthesis, characterization, and reactivity of diiron complexes with *S*-oxygenated bridging ligands that provide valuable parallels to the oxygenated forms of [FeFe] diiron hydrogenase active site cores.[12] Intriguingly, the oxygenation damage can be repaired under appropriate reductive conditions.

There has been no dearth of model chemistry for the [NiFe] version of hydrogenases. Of significance is a Communication reporting the first such analogue with a hydride ligand in a bridging position between Ni and Fe atoms in the dinuclear complex $[(dppe)NiFe(pdt)(H)(CO)_3]^-$, where dppe is 1,2-bis(diphenylphosphino)ethane and pdt is propylene-1,3-dithiolate.[13] Aspects of the structure match those in the nickel−iron hydrogenase. Electrochemical studies revealed that the complex is an active catalyst for the reduction of protons to H_2.

I have long been convinced that synthetic inorganic chemists are often defined by their ligands. A good ligand can provide routes to stable biomimetic complexes which, under appropriate conditions, display chemistry relevant to that at the active sites of metalloenzymes. One such ligand class is the sterically hindered β-diketiminate ions. Three-coordinate cobalt(II) β-diketiminate chloride reacts with a source of hydride ion to form dimeric hydride complexes which, in turn, react with N_2 to form the formally dicobalt(0) dinitrogen complex $[LCoNNCoL]^{2-}$, housing a bridging $N{=}N^{2-}$ ion.[14] This interesting chemistry may one day provide precedence for the reaction mechanisms of nitrogenase and/or hydrogenase, two enzymes already discussed.

Porphyrin is possibly the ligand most utilized by biomimetic inorganic chemists. It provides great kinetic stability, numerous spectroscopic features to follow reactions, and two reactive sites in the axial positions when bound to a metal.[27] Metalloporphyrin chemistry is intimately involved in the production and utilization in proteins of small molecules such as nitric oxide. A *JACS* Select publication in this collection reports the heme-assisted coupling of two NO molecules to form $\{FeN_2O_2\}^{n-}$ intermediates of relevance to the formation of N_2O from NO in nitric oxide reductase enzymes.[15] Addition of hyponitrous acid to an octaethylporphyrin (OEP) iron complex generates the remarkable, crystallographically characterized $[(OEP)Fe]_2(\mu\text{-}N_2O_2)$ complex containing a trans Fe−O−N−N−O−Fe unit, which was then converted into N_2O, H_2O, and [Fe(OEP)Cl] upon addition of HCl.

In Nature, nitrous oxide is utilized as part of the nitrogen cycle to produce N_2, a process facilitated by transition metal complexes. This reaction has now been achieved using a $[(Me_3tacn)Cu_3S_2]^{2+}$ unit, where Me_3tacn is 1,4,7-trimethyltriazacyclononane.[16] This tricopper cation contains a disulfide ion (S_2^{2-}) bridging the three copper ions, two of which each interact with a single sulfur atom and the third of which interacts with both sulfur atoms. This unit is unique in copper chemistry. Under appropriate conditions, dinitrogen can be evolved from the complex. The chemistry has several features in common with that which occurs at the nitrous oxide reductase active site.

The juxtaposition of heme iron and copper sites in several metalloenzymes, such as cytochrome *c* oxidase (CCO), is critical for function. It has been estimated that >90% of the utilization of dioxygen in life processes on Earth passes through this site in CCO. Accordingly, several investigators have carried out biomimetic chemistry of this heme/copper unit, and a *JACS* Select Communication describes how light can eject CO or NO from one such center. These molecules then rebind with affinity and rapidity preferences that reflect the differential selectivity of these molecules for heme iron versus copper.[17] The researchers observed a higher rate for NO versus CO binding to heme, a preference that has also been observed in CCO.

Probing Metal Ions in Cells

In this final section we highlight four contributions in which clever strategies have been devised to detect toxic or essential metal ions in cells or, in one case, to probe how a therapeutic metal complex might enter the cell. All have important implications for therapeutic, toxic, and pathological aspects of medicinal applications of bioinorganic chemistry.

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(27) Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.

DNAzymes and aptamers are nucleic acids employed in bioinorganic chemistry to catalyze the transformation of, or improve the binding specificity for, a target of interest. Binding of a fluorescent module to one of these constructs provides the ability to sense a target.[18] This approach was used to detect the toxic metal ion Pb^{2+} through its known ability to promote the cleavage of DNA. Good selectivity for lead over other competing metal ions was demonstrated.

Fluorescent molecules that respond with good turn-on to metal ions have been extensively developed for diamagnetic metals such as Zn^{2+}, where they function by a photoinduced electron-transfer quenching mechanism.[28] Detecting paramagnetic ions can be more challenging, for retention of the metal at a binding site attached to the fluorophore, as occurs for many zinc sensors, introduces unfilled d-shell orbitals that can quench the photoexcited state of the fluorophore. A *JACS* Select article that avoids this problem reports "FerriBRIGHT", a catechol-BODIPY construct that reacts with $FeCl_3$ and other oxidants to oxidize the pendant catechol to the corresponding quinone, with concomitant fluorescence turn-on.[19]

Another readout of interest for biological metal ion detection is magnetic resonance imaging (MRI). Although MRI lacks the spatial resolution of optical methods such as fluorescence, it has the advantage of applicability in live animals. A class of copper-activated gadolinium MRI contrast agents has been devised using thioether ligands to convey selectivity for copper, and a gadolinium core to effect the water relaxation needed to provide the images.[20] Copper-initiated changes in the coordination sphere at the Gd^{3+} center altered the number of water molecules that could access its paramagnetic core, thus leading to the MRI response.

Cisplatin is the all-time champion inorganic compound used to treat human diseases, being applied in about half of all chemotherapy for cancer. Selective uptake into cancer cells is an important goal for any such therapeutic agent, and knowledge about the cell import pathways of the drug can help achieve this objective. In recent years, evidence has accumulated that cisplatin can enter cells via the human copper chaperone Atox1, and two crystal structures of the drug bound to this protein comprise the final entry in this *JACS* Select edition.[21] The results reveal that platinum binds to CXXC motifs, and this result supports the notion that binding at this site can lead to deleterious therapeutic consequences by sequestering the drug from DNA, its acknowledged target in the cancer cell.

In conclusion, it can be seen from these examples that understanding, mimicking, and utilizing molecular and macromolecular systems at the interface of inorganic chemistry and biology remain fertile grounds for scientific investigation. As additional frontiers emerge at the interface, chemists are sure to extend their research in pursuit of new discoveries.

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