Metalloenzymes, Structural Motifs, and Inorganic Models

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Metalloenzymes effect a variety of important chemical transformations, often involving small molecule substrates or products such as molecular oxygen, hydrogen, nitrogen, and water. A diverse array of ions or metal clusters is observed at the active-site cores, but living systems use basic recurring structures that have been modified or tuned for specific purposes. Inorganic chemists are actively involved in the elucidation of the structure, spectroscopy, and mechanism of action of these biological catalysts, in part through a synthetic modeling approach involving biomimetic studies.

Numerous chemical transformations are effected by a diverse array of active-site structures in metal-containing enzymes. Metal cores in such proteins possess distinctive ligation and coordination geometries, often with unusual colors or other spectroscopic signatures. These latter properties first spurred the interest of physical biochemists and inorganic spectroscopists because metal-containing macromolecules were viewed as having special identifiable functional groups, amenable to detailed study. Recently, aspects of reactivity have lured more inorganic chemists to this field of study, in part because of their traditional interests in industrially important small molecules such as CO, CO_2, H_2, CH_4, NO, N_2, O_2, and H_2O. All of these are also naturally occurring biological substrates or products of reactions effected by metalloenzymes. When compared with the efficiency of comparable industrial reactions (such as hydrocarbon oxidation with O_2 or dinitrogen reduction to ammonia), metalloenzyme reactions function under considerably milder conditions, effecting transformations with exceptional substrate, regio- and stereoselectivity.

Bioinorganic chemistry encompasses a variety of disciplines, ranging from inorganic chemistry and biochemistry to spectroscopy, molecular biology, and medicine. The field is undergoing a phase of explosive growth, partly because of exposure and insights obtained by increasing numbers of metalloenzyme x-ray structures (*1*, *2*). This article highlights certain of the diverse array of protein and enzyme metal coordination sites observed but also emphasizes how nature uses basic recurring structures and fine tunes them for specific purposes. Well-known active-site centers include heme (iron-porphyrin) and iron-sulfur (Fe_4S_4) cube species (*1*), but even these have recently been found to have unrealized occurrences, modifications, or functions. In these contexts and with respect to metalloenzyme structure and function, examples of the inorganic model chemistry approach will also be highlighted.

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Inorganic Model Chemistry

Because the behavior of metal ions in proteins cannot be divorced from the fundamental chemistry of the particular metal, the study of small molecule, active-site synthetic analogs is useful (*1*, *3*). The purpose of models is not necessarily to duplicate natural properties but to sharpen or focus certain questions. The goal is to elucidate fundamental aspects of structure, spectroscopy, magnetic and electronic structure, reactivity, and chemical mechanism. A synergistic approach to the study of metalloenzymes can and has yielded crucial information because synthetic analogs can be used to investigate the effects of systematic variations in coordination geometry, ligation, local environment, and other factors, often providing insights that cannot be easily attained from protein studies (Fig. 1).

Recent efforts have emphasized functional models (*4*) dealing with the mimicry of enzyme-related chemical processes or transformations. Simple examples are the discovery and characterization of synthetically derived coordination complexes that can reversibly bind O_2 or rapidly hydrolyze phosphate esters under mild conditions. Of concern is the determination of what specific metal-ion coordination properties allow for such chemistry to occur. Functional biomimics considerably supplement studies on "speculative" or "corroborative" models focusing on structure and spectroscopy. In addition to providing a basis for postulating biological reaction pathways and possible metal-complex intermediates, such modeling efforts may yield small molecule biomimetic catalysts capable of effecting transformations with practical applications, including (i) selective O_2-mediated oxidations in drug and chemical synthesis or as macromolecular probes (*5*); (ii) the hydrolysis of protein peptides or nucleic acids in biotechnological applications (*6*, *7*); or (iii) the removal of environmental pollutants such as phosphate ester pesticides (*8*) or nitrogen oxide (NO_x) compounds (*9*). The study of "artificial enzymes" has more often been applied to nonmetal-containing systems (*10*), and alternative approaches involve protein engineering (*11*) or the use of catalytic antibodies (*12*).

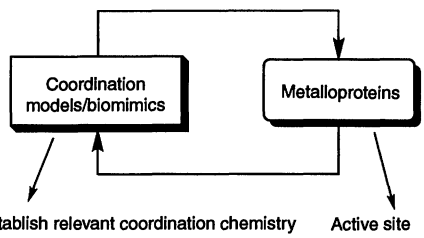


Fig. 1. The synergistic relationship between studies involving metalloprotein biochemistry and inorganic modeling. Relevant coordination chemistry: structures, spectroscopy, and reactivity as a function of, for example, *M*, redox state, and ligation; plausible intermediates; reaction competency; generation of new chemistry; and useful catalysts. Active site: structure, spectroscopy, and reaction mechanism.

Although heme and iron-sulfur protein structural cores (*1*) can exist and be synthesized and studied as well-defined entities independent of their protein matrices, numerous others cannot. They occur through the intimate interaction (ligation) with protein side chain residues such as the imidazole group from His, the carboxyl group (RCO_2^-; R = amino acid side chain) from Asp or Glu, an O ligand from Tyr, or an S donor from Cys (RS^-) or methionine ($RSCH_3$). The chemical modeling of such metalloprotein active sites represents a considerable challenge in design and synthesis.

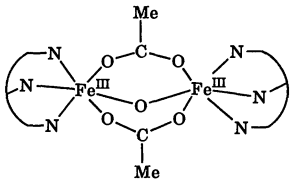
Dioxygen and Reactions with Protein-Metal Sites

Bioinorganic structural motifs and chemical modeling can be well illustrated by the diversity of proteins involved in processing O_2 and its reduced products such as superoxide (O_2^-) or hydrogen peroxide (H_2O_2). Dioxygen metabolism is crucial for aerobic organisms: O_2 serves as a primary source of

energy with its thermodynamically favorable reduction to water [standard potential ($E°$) = 0.82 V (1 atm O_2, pH = 7)]. In addition, O-atom incorporation into biological substrates occurs by functionalization through mono- or dioxygenation processes mediated by flavins (*13*) or metalloenzymes. Heme (*14*), nonheme iron (*15*), copper (*16, 17*), manganese (*18–20*), and vanadium (*21*) proteins can all effect similar types of reactions (Table 1). Their occurrence depends on the organism type and biological substrate involved.

Di-iron oxo and carboxylato proteins and complexes. These proteins have elicited excitement (*22–27*) because of their widespread occurrence and diverse nature of functions carried out, including reversible O_2 binding, alkane hydroxylation, DNA synthesis, electron transfer (*28*), phosphate ester hydrolysis (*24, 29*), and Fe storage (*30*). Depending on the protein and metal oxidation state, the two Fe atoms are separated by ~3.1 to 3.6 Å, terminally ligated by protein-derived ligands, and bridged by a combination of oxo (oxide, O^{2-}), hydroxo (OH^-), or carboxylato donors.

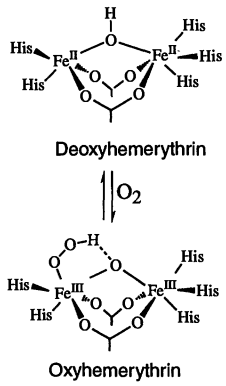
Inorganic model chemistry continues to contribute significantly to our understanding of the protein structure, physical properties, and reactivity of this structural motif (*24, 26*). Initial seminal contributions were made by Armstrong and Lippard (*31*) and by Wieghardt and co-workers (*32*), achieving the laboratory assembly of complexes **1** and **2**, which possess the basic bridging (μ-oxo)*bis*(μ-carboxylato) di-iron(III) core that is found in oxidized forms of hemerythrin. These and other species define many of the distinctive properties of the core, such as the short μ-oxo bonds (~1.8 Å), strong visible absorptions, large antiferromagnetic coupling, and sizable Mössbauer quadrupole splittings. Model compound studies have established correlations of Fe-O-Fe vibrational frequencies with Fe-O-Fe bridging angles (*33*) and aided the application of nuclear magnetic resonance, x-ray absorption, and electronic spectroscopies, which have been of both corroborative and predictive value (*24, 26, 34*).



HBpz$_3$ tacn

1 **2**

Hemerythrin. This O_2 transport protein of marine invertebrates has served as the prototype for the study of di-iron proteins. High-resolution x-ray structures are available for the unoxygenated di-iron(II) and O_2 adduct, formally a peroxo di-iron(III) species (*35*).

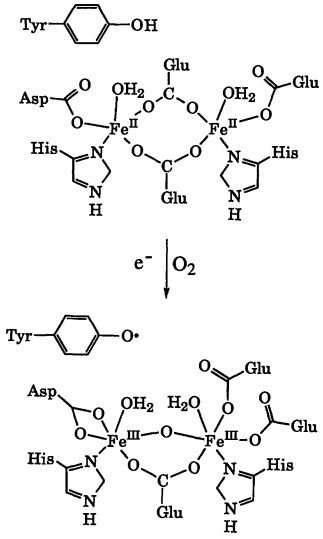


Deoxyhemerythrin

Oxyhemerythrin

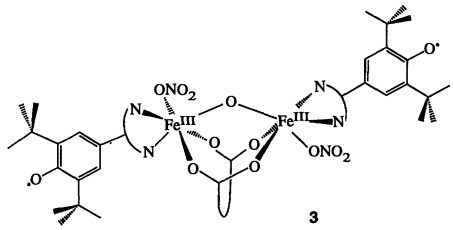
An important model compound with the essential OH^- bridged di-iron(II) deoxy structure was first described by Wieghardt and co-workers (*36*), and efforts by Que (*37*), Kitajima (*38*), and Suzuki (*39*) and their co-workers indicate that it is possible to generate metastable peroxo di-iron(III) (Fe_2-O_2) species from the low-temperature reaction of O_2 and iron(II) complexes. Future studies along these lines should aid our understanding of $Fe(II)_n$-O_2 interactions and reactivity, which are also important in other enzyme systems.

Ribonucleotide reductases. These enzymes catalyze the deoxygenation of nucleoside diphosphates to the deoxy form and so are essential in DNA biosynthesis (*40*). The enzyme from *Escherichia coli* (Fig. 2) contains a di-iron center that reacts with O_2 and oxidizes a nearby Tyr, producing a tyrosyl radical essential for protein function.



The use of x-ray investigations of (i) the oxidized protein (*41*) and (ii) a dimanganese(II) substituted form (*42*) have illuminated structural details. Physical spectroscopic studies of the di-iron proteins, calibrated by the use of model coordination compounds, anticipated the observed differences in metal coordination from that found in hemerythrin, also reflecting monodentate-bidentate "carboxylate shifts" (*43*).

A protein study by Stubbe and co-workers (*44*) indicated that the reduced enzyme reacts with O_2 to produce at least two intermediates, both capable of oxidizing the crucial Tyr residue. Lippard and co-workers (*45*) have generated an intriguing model **3**, a di-iron(III) complex with pendant-ligand phenoxyl radicals. Such efforts provide hope for a detailed understanding of the molecular events surrounding the di-iron–mediated O_2 chemistry and protein oxidation.

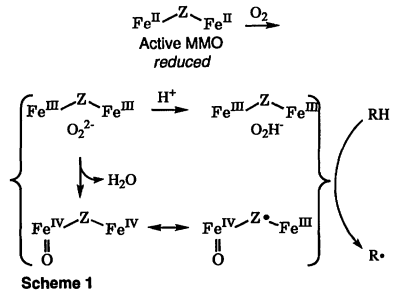


3

Methane monooxygenase. The di-iron center in this protein (*46, 47*) effects the cleavage of the C–H bond in CH_4, that is

$$CH_4 + O_2 + 2e^- + 2H^+ \rightarrow CH_3OH + H_2O$$

No x-ray structure is available, but protein and model compound comparisons suggest that the iron coordination is most similar to that of ribonucleotide reductase (*48*) but differs in detail (*22, 34, 49*). The reaction of O_2 with the active di-iron(II) form of methane monooxygenase (MMO) most likely leads to an intermediate, formally a peroxo di-iron(III) species. Most peroxo-metal complexes are not good O atom transfer agents, so the species responsible for attack of CH_4 is in question (*22, 47*). An electrophilically activated hydroperoxo species such as M_n-(OOH^-) could be involved, but reductive activation by a protein group (for example Z, giving Z·) or an Fe atom to give a high-valent iron-oxo "ferryl" species is usually considered more likely. This route follows the often quoted, but unproven, analogy to cytochrome P-450 or heme peroxidase enzymes (Scheme 1).



Active MMO *reduced*

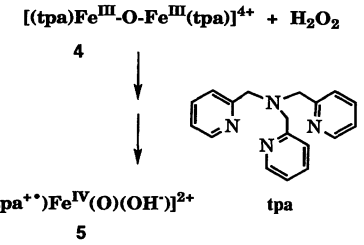
Scheme 1

Table 1. Metalloproteins involved in direct processing of dioxygen (O_2) or derivatives. Abbreviations: MMO, methane monooxygenase; *D*, stoichiometric coefficient; SOD, superoxide dismutase; *X*, *X*H, and XH_2 are substrates.

	Heme Fe	Non-heme Fe	Cu	Mn and V
Dioxygen transport				
$DM^{n+}O_2$ $\rightleftarrows M(^{n+1})_D(O_2^{D-})$	Hemoglobin* Myoglobin*	Hemerythrin* Myohemerythrin	Hemocyanin*	
Monooxygenases				
$X + O_2 + 2e^- + 2H^+ \rightarrow XO + H_2O$	Cytochrome P-450 monooxygenase*	Soluble MMO	Tyrosinase Dopamine β-hydroxylase	
	Secondary amine monooxygenase	Pteridine-dependent hydroxylases (Phe, Tyr, and Trp)	Phenylalanine hydroxylase Peptidylglycine α–amidating monooxygenase	
	NO synthase		Particulate MMO	
Dioxygenases				
$X + O_2 \rightarrow XO_2$	Indoleamine 2,3-dioxygenase	Lipoxygenase* Catechol dioxygenases*†	Quercetinase	
	Tryptophan 2,3-dioxygenase	α-Ketoglutarate–dependent dioxygenases		
	Prostaglandin H synthetase	Arene dioxygenases‡		
Oxidases				
$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$	Cytochrome c oxidase	Ribonucleotide reductase*	Cytochrome c oxidase	Ribonucleotide reductase (Mn)
			Laccase	Photosystem II§ $2H_2O \rightarrow O_2$; Mn cluster
		Clavaminate synthase Isopenicillin N synthase	Ascorbate oxidase* Phenoxazinone synthase Galactose oxidase*	
$XH_2 + O_2 \rightarrow X + H_2O_2$			Amine oxidase	
Peroxidases and catalases				
$H_2O_2 + XH_2 \rightarrow X + 2H_2O$	Catalase* Horseradish peroxidase			Mn catalase*
	Cytochrome c peroxidase*			V bromoperoxidase
$2H_2O_2 \rightarrow O_2 + 2H_2O$	Chloroperoxidase ($XH + Cl^- \rightarrow XCl$)			Mn peroxidase
	Lignin peroxidase* Prostaglandin H synthetase			
Superoxide dismutases				
$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$		Fe SOD*	Cu-Zn SOD*	Mn SOD*

*Structural data from x-ray crystallographic studies are available. †Such as protocatechuate-3,4-dioxygenase. ‡Substrates such as benzene or phthalate. §Water oxidation involves a tetramanganese cluster, also requiring Ca^{2+} and Cl^-.

If $Fe_n^{\cdot}$-OOH, Fe=O, or other complexes could be generated and characterized in model complexes, a determination of the competency toward C-H activation of alkanes could be made. A number of non-heme Fe chemical models are known to effect alkane oxidation (*22*, *50*), and high-valent, iron-oxo intermediates have been suggested as the reactive species. In support, Que and co-workers (*51*) spectroscopically observed an iron(IV)-oxo ligand radical-cation species **5**, generated from the low-temperature reaction of H_2O_2 with the oxo-bridged di-iron(III) complex **4**.



Copper-dioxygen chemistry at dinuclear centers. A large number of Cu proteins participate in a variety of O_2-processing functions (*16*, *17*) (Table 1). As examples of hydroxylation catalysts, dopamine β-hydroxylase (*52*) and peptidylglycine α–amidating monooxygenase [PAM (*53*)] have potentially considerable pharmacological import. Approximately 50% of mammalian bioactive hormones, neurotransmitters, and growth factors are peptides with a COOH-terminal carboxamide, generated by N-oxidative cleavage of a Gly-extended prohormone; this reaction is effected by the Cu enzyme PAM. An exciting new finding is that the particulate membrane-bound form of the methanogenic bacterial MMO (*p*-MMO) may possess a trinuclear Cu-cluster active site (*54*). This discovery is significant because of its implications for C-H bond activation chemistry and potential practical application to the oxidative processing of hydrocarbons.

The interplay between model and protein biophysical studies has provided considerable insight into Cu-O_2 chemistry occurring in O_2 carriers, monooxygenases, and oxidases (*16*, *17*). Arthropods and mollusks use dicopper-containing proteins (for example, hemocyanins) for O_2 transport, which has led to considerable interest in the study of inorganic complex Cu^I-O_2 interactions at dinuclear centers (*16*, *55*, *56*). Such efforts led to a triumph in biomimetic chemistry because the true O_2 binding mode occurring in oxy-hemocyanin had

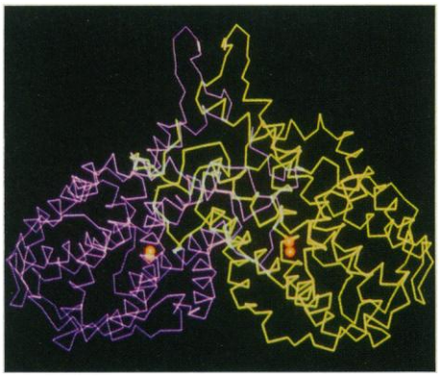
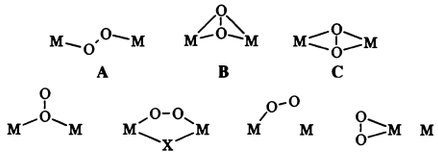


Fig. 2. The dimeric R2 subunit of *E. coli* ribonucleotide reductase, essential for DNA biosynthesis.

not been considered as a possibility until a synthetic analog revealed the actual Cu_2-O_2 coordination. Similar to the case with the di-iron protein, O_2 binding to a reduced dicopper site involves the formal reduction of O_2 to O_2^{2-}, giving a peroxo-dicopper(II) Cu_2-O_2 product. Binding could occur in a variety of ways, as shown.



The characterization of systems that mimic the reversible binding of O_2 provides detailed insights into the kind of Cu coordination environment needed for this function. Recent studies have led to the synthesis of a model complex with structure **A** (*57*), and with the use of different dinucleating ligands species suggested to have side-on ligation **B** could be generated (*58*). Most importantly, Kitajima and co-workers (*59*) crystallographically characterized a peroxodicopper(II) complex **C**, {Cu[hydro*tris*(3,5-diisopropyl-1-pyrazoyl)borate]}$_2$(O_2), **6** (Fig. 3). This complex possesses physical properties remarkably similar to the distinctive properties exhibited by oxy-hemocyanin: intense ultraviolet absorption with $\lambda_{max} \cong$ 350 nm ($\epsilon \cong$ 20,000 $M^{-1}\cdot cm^{-1}$) and $\nu_{O\text{-}O} \cong$ 750 cm^{-1}. An x-ray structure of the horseshoe crab *Limulus polyphemus* oxy form (Fig. 4) confirms this identical Cu_2-O_2 protein coordination (*60*).

Following the focus on Cu-O_2 chemistry at dinuclear centers, a model system for monooxygenation, that is, C-H activation incorporating one O atom from O_2 into a substrate, has also been developed. The dicopper(I) complex **7** reacts rapidly with O_2 (1 atm), quantitatively producing **9** [Scheme 2, 2-pyridyl (PY)]. Isotopic labeling experiments and the observed stoichiometry of reaction (that is, Cu:O_2 = 2:1) are reminiscent of biological monooxygenases (*61*). Detailed mechanistic studies (*55, 62*) revealed that a Cu_2-O_2 intermediate **8** (with suggested structure shown) forms reversibly upon the reaction of **7** with O_2. As in an enzyme active site, the peroxo group in **8** is located in a highly favorable proximity to the xylyl ligand substrate, and facile hydroxylation occurs by electrophilic attack on the arene substrate π system. An "NIH shift" mechanism is involved, like that previously established for Fe hydroxylases (*62*). This xylyl hydroxylation model system serves as a functional mimic for Cu hydroxylases, revealing how a Cu_2 center can activate O_2 for hydrocarbon oxidation under mild conditions.

Polymetal Sites in Peptide or Phosphate Ester Hydrolysis

Hydrolysis reactions of peptide amides or nucleotide phosphate esters are important enzymatically catalyzed processes, which are particularly sluggish transformations from a chemical perspective. In inorganic biochemistry, the best-studied systems are those with single Zn active sites (*63*) such as carboxypeptidase (*64*), thermolysin (*65*), and carbonic anhydrase (*66*), the last catalyzing the hydration of CO_2. One feature thought to contribute significantly to activity is the low acidity constant (pK_a) of the active-site, metal-bound water (for example, $pK_a \sim 7.5$ in carbonic anydrase). Model systems for mononuclear hydrolytic enzymes are numerous (*6, 67*), and in one developed by Kimura and co-workers (*68*), the macrocycle 1,5,9-triazacyclododecane gives a four-coordinate Zn complex with a near-physiological pK_a of coordinated water.

An emerging new structural-functional motif in bioinorganic chemistry is the presence of di- or trinuclear metal-cluster centers involved in amide (such as peptide), inorganic pyrophosphate, or phosphate ester hydrolysis functions (Table 2) (*69*). A surprising range of metal ions has been observed to provide activity to many of the enzymes listed, including those that are redox-active. The identity of the in vivo metal ion is not known with certainty in many cases.

As an example, the active-site structure of the tri-zinc structure of phospholipase C

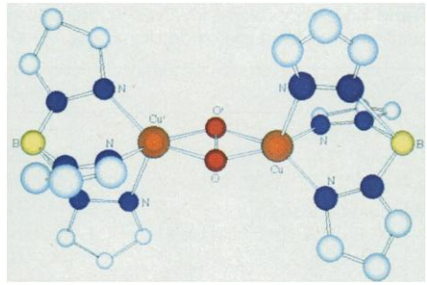


Fig. 3. Chemical three-dimensional representation of the Cu_2-O_2 coordination in Kitajima's model complex **6** and that observed in oxy-hemocyanin. The ring 3,5-isopropyl groups are not shown.

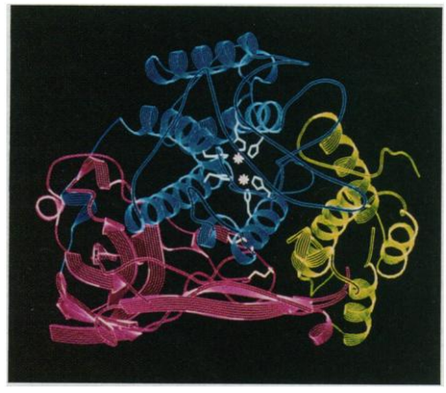
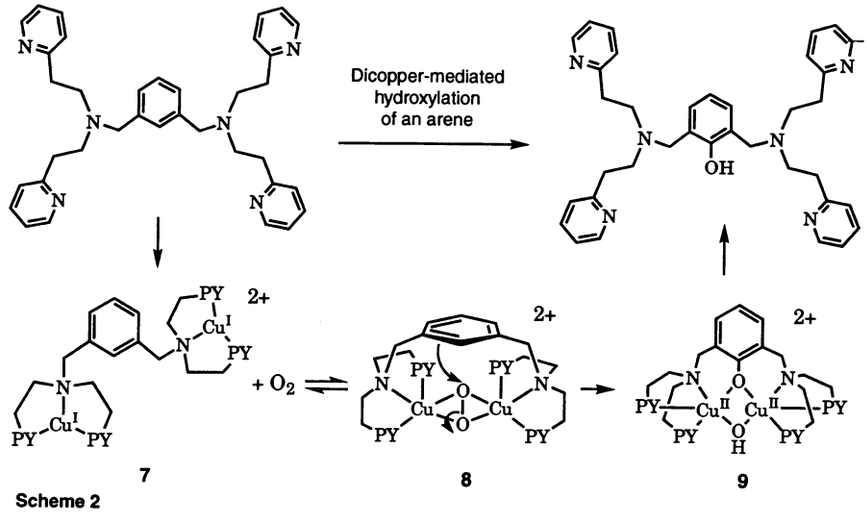
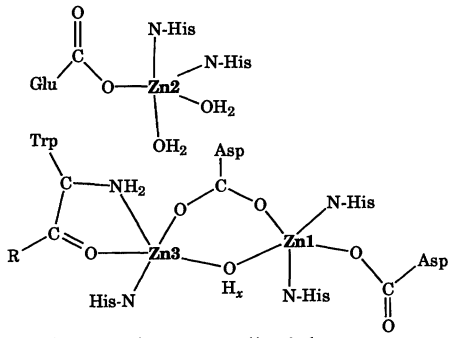


Fig. 4. Subunit structure of oxy-hemocyanin.



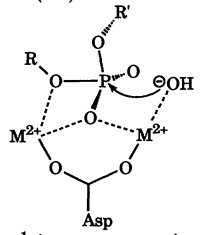
Scheme 2

is depicted below. Prominent features include extensive His and Asp or Glu (carboxylate) ligation, pentacoordinate zinc(II), and a bridging carboxylate and H_2O (or hydroxide) for the dinuclear pair, Zn1 and Zn3. These two are 3.3 Å apart, and the third ion, Zn2, is close enough that inorganic phosphate, PO_4^{3-}, can simultaneously bridge all three metal ions. This active-site structure is similar to that found in alkaline phosphatase and P1 nuclease.



The metal ions in all of these enzymes are not bound tightly and can be exchanged for others in solution media, which may partly explain the observed activity with a range of metals such as Mg^{2+}, Mn^{2+}, Co^{2+}, and Zn^{2+}. The particular affinity for a given site varies, and as for DNA polymerase I (*70*), the cooperative binding of metal ions occurs so that a third metal ion also binds near the dinuclear center. This affinity may facilitate trinuclear cluster formation or be involved in the catalysis.

Detailed catalytic mechanisms for these enzymes (Table 2) have not been elucidated, but two-metal ion models for phosphate ester hydrolysis have been suggested (*29, 71, 72*). One Lewis acidic metal ion serves to activate a coordinated H_2O molecule, lowering its pK_a and directing the attack of the resulting hydroxide (or Ser hydroxyl, for alkaline phosphatase) on the substrate phosphate ester. The second metal ion could facilitate departure of the alcohol (that is, the 3′ oxygen), whereas the dinuclear metal pair binds, orients, and activates the substrate and stabilizes the pentacoordinate phosphorus transition state. A similar mechanism can be envisioned for peptidases involving dinuclear metal centers (*73*).

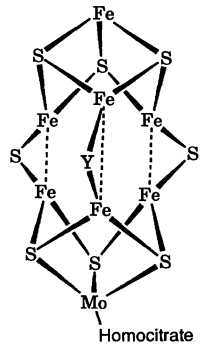


The metal-ion centers in proteins in Table 2 have extensive carboxylate anion coordinations that provide effective binding sites for a variety of cationic metals but would lower the effective charge on the complex, making formation of a hydroxide nucleophile less likely. The catalytic power may be provided by the presence of proximate substrate and metal-bound water, perhaps with the activation by hydrogen-bonding interactions by means of protein side chain residues. Alternatively, a H_2O or OH^- molecule bridging two metals [that is, M–(OH^-)–M] should have a considerably lowered pK_a. Perhaps this group acts as the attacking nucleophile while the substrate is bound to a di- or trinuclear metal core. Few examples of hydrolysis reactions effected by dinuclear coordination complexes exist (*74*), but further protein and inorganic model studies should elucidate the mechanisms of such metal-cluster–mediated reactions.

Iron-Sulfur Clusters

Metal-ion clusters occur widely in biological systems (*69, 75*), with the iron-sulfur tetranuclear Fe_4S_4 core as perhaps the most pervasive prosthetic group (*76–78*). Cubane-shaped clusters consist of Fe(II) and Fe(III) ions tetrahedrally coordinated to inorganic sulfide (S^{2-}) and protein cysteinyl thiolate donors. These species act as single electron–transfer agents, usually operating as a $[Fe_4S_4(SR_{Cys})]^{2-,3-}$ redox couple. Alterations in cluster redox potential and charge are modulated by the protein environment, for example, through the N–H···S hydrogen bonding of neighboring amino acid residues with iron-ligated S atoms (*79*). The structures and electronic properties of these Fe_4S_4 cube clusters are well understood, with many contributions coming from Holm's synthetic analog investigations (*1, 76*). Yet, exciting developments in the last few years have considerably expanded the dimensions of the known structure and reactivity of clusters containing Fe-S. Clusters of Fe_4S_4 are not the only type found, and such centers are not necessarily merely electron transfer agents.

1) Bacterial nitrogenases play key roles in the global nitrogen cycle, catalyzing the reduction of molecular nitrogen to ammonia. The results of x-ray crystallographic studies on a molybdenum-containing form have provided dramatic insights into the active-site structure of this important enzyme (*80*). Aside from Fe-S "P clusters" with two tightly coupled (Fe_4S_4) cubes that transfer two electrons each, a Mo-Fe cofactor with composition $MoFe_7S_8$· (homocitrate) is present (a schematic of the current picture of this basic structure is given below). The merged, adjacent double-cubane structure contains a homocitrate-bound molybdenum atom at the periphery. In particular, the detailing of the interface of the cubes requires further resolution (for example, the identification of Y), and speculation centers on N_2 binding at a coordinatively unsaturated Fe atom or atoms in the middle. A protein structure generates an exciting phase of research activity, providing a fresh focus for biochemical and inorganic modeling studies and, it is hoped, elucidating the relations among structure, activity, and mechanism.



2) Bacterial Fe hydrogenases are Fe-S proteins that catalyze the reversible reduction of protons to hydrogen gas

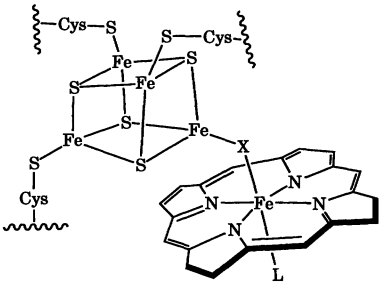
$$2H^+ + 2e^- \rightleftarrows H_2$$

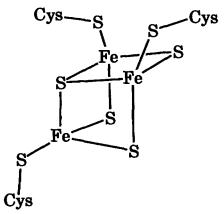
These proteins have been proposed to contain "H clusters" that have six irons, effecting the catalytic reactions of substrate (*77*). Six-iron clusters should be considered as a newly discovered structural motif because such an entity has been definitively identified for at least one other protein (*81*).

3) An Fe_4S_4 electron-transfer moiety is covalently bound through a S or S_{Cys} X group to a catalytic (siro)heme-Fe site in *E. coli* sulfite reductase (shown below) (*82*). Such a close association of different redox centers probably occurs in nickel proteins such as hydrogenase (*83*), carbon monoxide dehydrogenase, and acetyl CoA synthase (*84*). The intimate contact with an adjacent substrate binding site (for example, SO_3^{2-} or CO) would allow the efficient transfer of reducing equivalents to facilitate the redox and atom-transfer reactions required.

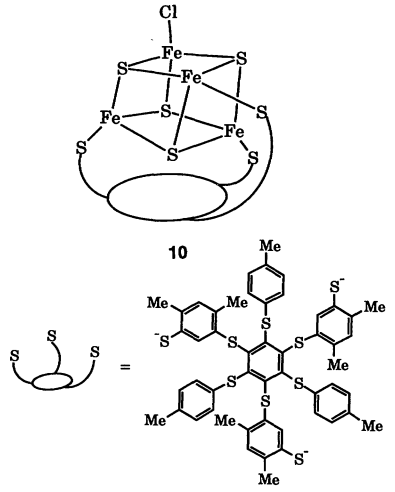
4) Clusters of Fe_4S_4 and Fe_2S_2 have been implicated in the catalysis of important non-redox reactions. Endonuclease III, an Fe_4S_4 DNA repair enzyme, possesses both glycolase and lyase activities (*85*). Aconitase (*86, 87*) catalyzes the stereospecific hydration-rehydration of citrate to isocitrate through important steps in the Krebs cycle and is the best understood member of this class. A three-iron $[Fe_3S_4(S_{Cys})_3]$ enzyme form (shown below) can occur, and clusters of this nuclearity have been known for some time (*88*). The three-iron form is inactive, and regeneration of the Fe_4S_4 cluster by reconstitution with solution iron(II) appears to be critically involved in iron regulatory processes (*89*). Thus, full activity of aconitase requires an $[Fe_4S_4(S_{Cys})_3]$ cluster with one

corner Fe atom having a H_2O or OH^- coordinated labile site instead of Cys; this cluster binds substrate and effects the rearrangement reaction.





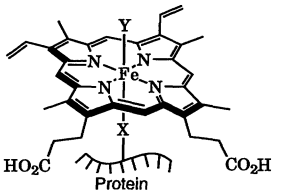
There have been corresponding advances in syntheses of Fe-S containing cluster compounds, aimed at defining and characterizing new speculative enzyme structural or functional models (*76*, *77*, *90*). A powerful new approach to the study of clusters has been developed by Holm involving Fe-S "subsite-differentiated" compounds, or those in which the chemistry can be restricted to one corner Fe atom of an Fe_4S_4 cube (*76*). As in complex **10**, a trithiolate tridentate ligand spans and protects three terminal Fe coordination sites, allowing specific chemistry at the one Fe corner. Numerous and varied ligand groups can be attached to the subsite Fe, and this metal can even be replaced by others (*76*). Future elaborations are likely to lead to structural and functional models for Fe-S containing enzymes such as those described above.



10

Nitric Oxide and NO Synthase

Heme proteins contain the iron-protoporphyrin IX prosthetic group and are the most significant historically because they include hemoglobin, the protein that transports mammalian blood O_2 (*91*). In addition to their numerous functions in O_2 metabolism (Table 1), hemoproteins also participate in critical electron-transfer roles (*92*). The various chemical functions that are supported by the same basic structural unit can be ascribed to differences in the way that the individual protein classes interact with (i) the heme (that is, buried or heme edge exposed) or (ii) potential substrates (*14*, *93*). Electron transport proteins have two strong protein-derived axial ligands *X* and *Y* (such as His and Met) and do not bind peroxides or O_2. All others have one accessible coordination site (*Y*) for the binding of small ligands, substrates, or both. Metalloporphyrin model chemistry is a highly developed area, and studies of synthetic analogs have critically contributed to an understanding of the properties of hemes (*1*), especially physical characteristics and reversible O_2 binding requirements (*94*, *95*), and of the mechanisms and applications of cytochrome P-450 monooxygenase chemistry (*94*, *96*).



Nitric oxide and CO are traditionally viewed as toxic pollutants, with well-studied metal-ion chemistries, and as useful metalloenzyme spectroscopic probes. However, recent advances have associated these with other heme enzymes. Nitric oxide has been identified as a key chemical messenger, stimulating the relaxation of blood vessels and initiating other beneficial physiological reactions (*97*); CO may serve in a similar role (*98*). Both may regulate guanosine 3′,5′-monophosphate (cGMP) concentrations by the activation of the heme protein guanylate cyclase, which converts guanosine triphosphate (GTP) to cGMP. Nitric oxide is produced biologically by the enzyme NO synthase, a cytochrome P-450 hydroxylase–type hemoprotein (*99*). This enzyme converts the amino acid L-Arg to citrulline and NO in a five-electron oxidation process that also requires O_2, reduced nicotinamide adenine dinucleotide phosphate, tetrahydrobiopterin, and flavin adenine dinucleotide and flavin mononucleotide coenzymes. A chemical system described by Traylor and co-workers (*100*) addresses the possible mechanism of NO-mediated activation of guanylate cyclase. The binding of NO to the heme triggers the release of the proximal His ligand, allowing the free imidazole to act as a general or nucleophilic catalyst; the model study supports this possibility.

The discovery of this NO (and CO) bioinorganic chemistry will undoubtedly revitalize interest in these small molecules, their prosthetic group chemistry and biochemistry, new heme chemistry, and the development of reagents or drugs to deliver NO or control its concentrations (*101*). Nitric oxide is extremely reactive toward redox-active metals and metalloproteins and can oxidize to other species like nitrite, NO_2^-. The production of NO as a chemical

Table 2. Peptidase and phosphatase enzymes with polymetal active sites. Reference sources are in parentheses.

Metal and active site	Enzyme and function
Zinc	
2 Zn	Leucine aminopeptidase (*73*)*
3 Zn (2 Zn, 1 Mg)	Alkaline phosphatase (phosphomonoesterase) (*71*)*
3 Zn	P1 nuclease (endonuclease) (*107*)*
3 Zn	Phospholipase C, *Bacillus cereus* (*108*)*
Iron	
2 Fe (or Fe, Zn)	Purple acid phosphatases (*29*)
Cobalt	
2 Co	Methionine aminopeptidase (*E. coli*) (*109*)*
Manganese	
2 Mn	Arginase (*l*-Arg) → *l*-ornithine + urea) (*110*)
2 Mn (Mg)	Enolase (hydratase) (*111*)
2 Mn, 2 M^{2+}	Ribonuclease H domain of human immunodeficiency virus 1 (*112*); reverse transcriptase (phosphodiesterase)
3 Mn	*Saccharomyces cerevisiae* inorganic pyrophosphatase (*113*)*
Nickel	
2 Ni	Urease (urea → carbonic acid + ammonia) (*114*)
Other	
2 Mg^{2+}, 2 Mn^{2+}, or 2 Zn^{2+}	*E. coli* DNA polymerase I (Klenow fragment) (3′,5′-exonuclease activity) (*72*)*

*Protein x-ray structure available.

messenger might also be mediated by other enzymes such as heme iron or copper nitrite reductases, providing an alternative means to control the free NO concentration.

Conclusions and Future Challenges

With the already recognized multifarious metal-catalyzed processes that use a diverse array of metal-containing structures, one should expect many discoveries of novel metalloenzymes and chemistry. These findings may include more examples of (i) di-iron or di-manganese centers involved in unprecedented functions; (ii) metal-ion centers capable of redox activity used in non-redox roles; (iii) Fe-S or other electron-transfer centers juxtaposed and electronically linked to another metal-ion–containing prosthetic group; (iv) the use of a radical-induced redox or rearrangement chemistry, generated at or on a protein residue near a metal active site (*102*); (v) metal centers functioning in conjunction with adjacent organic cofactors; and (vi) critical interactions of NO or CO with other metalloenzymes, such as nonheme Fe or Cu.

Many of the challenges in inorganic modeling outlined by Ibers and Holm (*1*) remain, and others have emerged. The next few years are likely to see considerable advances, with increased activity and successes in functional modeling leading not only to structural but detailed mechanistic insights.

1) Metal-dioxygen reactivity mechanisms are still not well understood. Nonheme iron monooxygenase (such as MMO) and dioxygenase model chemistry (*103*) should help to elucidate peroxide-Fe structures along with O–O cleavage and substrate reaction mechanisms. Dioxygen reduction, activation, or both at mono- and trinuclear Cu centers will attract increased attention because many enzymes such as Cu-MMO, laccase and ascorbate oxidase, and dopamine β-hydroxylase (Table 1) operate with such metal cores.

2) Recent investigations of bacterial systems have considerably advanced the understanding of cytochrome c oxidases, which reduce O_2 to H_2O at a heme-iron–Cu bimetallic center. Model studies should also help to detail the O_2 binding, reduction, O–O cleavage, and accompanying proton transfer chemistry (*104*).

3) The reverse process, the formation of an O–O bond in the H_2O oxidation that is catalyzed by photosystem II, is effected by a tetra-manganese cluster that also requires Ca^{2+} and Cl^- (*18, 20*). Further developments in Mn cluster synthesis are desirable because they will lead to advanced structural, spectroscopic, and functional insights.

4) Future progress in Fe-S cluster chemistry is likely, such as in Fe-S linked to nickel complex or heme chromophores. The recent nitrogenase structures may also spur the generation of new merged double-cube–type structures such as P clusters or Fe-Mo cofactor models. The goal is to generate further insights into the reduction chemistry of N_2 and H^+ (to H_2).

5) Molybdenum enzymes with a pervasive Mo-oxo group and accompanying pterin cofactor play key roles in biological cycles involving N, S, C, and As. Recent advances in biochemical and inorganic modeling will likely elaborate the role of the pterin and the oxo-transfer function (*105*).

6) Discrete active-site cofactors are also involved in a number of metalloenzymes that help to process O_2. For example, such cofactors occur in pterin-dependent Fe hydroxylases, α-ketoglutarate–dependent Fe dioxygenases, and the redox-active constituents involved with the copper oxidase, galactose oxidase, and amine oxidases. More examples are likely to be found.

7) Atom and group transfer functions are also effected by Co vitamin B-12 (*106*) and nickel enzymes, involving organometallic (M-R) intermediates. Nickel (sometimes with Fe-S clusters) is found in nickel- or sulfur-containing ligand environments, effecting chemistry including CH_3, H, or O transfer reactions (*84*). There has been considerably increased activity in related Ni complex chemistry, and functional models will provide insights into structure and reactivity.

8) Until recently, the modeling of hydrolysis reactions of amides or phosphate esters by metal centers has been a more often pursued by organic and bioorganic chemists. With the more complex structural questions regarding hydrolytic enzymes that use di- or trinuclear metal centers, inorganic contributions can be considerable. New efforts in this area will typify the inevitable trend toward the increased merging of traditional approaches, in which organic and inorganic synthetic methods are used along with structural, spectroscopic, and mechanistic investigations.

This brief survey illustrates the considerable scope and nature of research approaches toward the elucidation of metalloenzyme structures, spectroscopy, and reaction mechanisms. Applications to industry, biotechnology, and medicine are likely. Many important metals and their bioinorganic chemistries have not been discussed here. In any case, division by metal ion is not necessarily a desirable classification because there is more than one means (enzyme type, metal, or prosthetic group) by which to effect a given reaction type, and researchers should look beyond any one system to focus on structural or functional themes such as O_2 binding and activation, hydrolysis, electron transfer, cluster chemistry, metal center–protein radical chemistry, and atom or group transfer. Although the discipline is rapidly expanding, the questions and goals seem nevertheless more clear.

REFERENCES AND NOTES

1. J. A. Ibers and R. H. Holm, *Science* **209**, 223 (1980).
2. W. H. Armstrong, in *Metal Clusters in Proteins*, L. Que Jr., Ed. (American Chemical Society, Washington, DC, 1988), pp. 1–27; J. A. Tainer, V. A. Roberts, E. D. Getzoff, *Curr. Opin. Biotechnol.* **3**, 378 (1992); W. A. Hendrickson and K. Wüthrich, Eds., *Macromolecular Structures, 1991* (Current Biology, London, 1992).
3. H. A. O. Hill, *Chem. Br.* **1976**, 119 (1976); S. J. Lippard, *Prog. Inorg. Chem.* **18**, v (1973).
4. G. L. Eichhorn and L. G. Marzilli, Eds., *Models in Inorganic Biochemistry* (Prentice-Hall, Englewood Cliffs, NJ, 1993), vol. 9.
5. M. R. Ermácora, J. M. Delfino, B. Cuenoud, A. Schepartz, R. O. Fox, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6383 (1992).
6. J. Chin, *Acc. Chem. Res.* **24**, 145 (1991).
7. T. M. Rana and C. F. Meares, *J. Am. Chem. Soc.* **113**, 1859 (1991); I. E. Burgeson and N. M. Kosti'c, *Inorg. Chem.* **30**, 4299 (1991).
8. T. Stephens, *J. NIH Res.* **3**, 21 (1991).
9. M. H. Thiemens and W. C. Trogler, *Science* **251**, 932 (1991).
10. R. Breslow, *ibid.* **218**, 532 (1982).
11. D. L. Oxender and C. F. Fox, Eds., *Protein Engineering* (Liss, New York, 1989); W. F. DeGrado, D. P. Raleigh, T. Handel, *Curr. Opin. Struct. Biol.* **1**, 984 (1991); A. Pessi *et al.*, *Nature* **362**, 367 (1993); F. A. Quiocho, *ibid.*, p. 293.
12. P. G. Schultz, R. A. Lerner, S. J. Benkovic, *Chem. Eng. News* **68**, 26 (May 1990).
13. T. C. Bruice, in *Biomimetic Chemistry*, D. Dolphin, C. McKenna, Y. Murakami, I. Tabushi, Eds. (American Chemical Society, Washington, DC, 1980), vol. 191, pp. 89–118; S. Ghisla and V. Massey, *Eur. J. Biochem.* **181**, 1 (1989); F. S. Mathews, *Curr. Opin. Struct. Biol.* **1**, 954 (1991); L. I. Ingraham and D. L. Meyer, *Biochemistry of Dioxygen*, vol. 4 of *Biochemistry of the Elements*, E. Frieden, Ed. (Plenum, New York, 1985), pp. 157–171.
14. J. H. Dawson, *Science* **240**, 433 (1988).
15. J. B. Howard and D. C. Rees, *Adv. Protein Chem.* **42**, 199 (1991).
16. K. D. Karlin and Z. Tyeklár, Eds., *Bioinorganic Chemistry of Copper* (Chapman & Hall, New York, 1993).
17. E. I. Solomon and M. D. Lowery, *Science* **259**, 1575 (1993); E. I. Solomon, M. J. Baldwin, M. D. Lowery, *Chem. Rev.* **92**, 521 (1992).
18. V. L. Pecoraro, Ed., *Manganese Redox Enzymes* (VCH, New York, 1992).
19. J. Reedijk, Ed., *Bioinorganic Catalysis* (Dekker, New York, 1993).
20. G. C. Dismukes, in *ibid.*, pp. 317–346.
21. A. Butler, in *ibid.*, pp. 425–445.
22. L. Que, Jr., in *ibid.*, pp. 347–393.
23. J. Stubbe, *Curr. Opin. Struct. Biol.* **1**, 788 (1991).
24. L. Que, Jr., and A. E. True, *Prog. Inorg. Chem.* **38**, 97 (1990).
25. J. B. Vincent, G. L. Olivier-Lilley, B. A. Averill, *Chem. Rev.* **90**, 1447 (1990).
26. D. M. Kurtz, Jr., *ibid.*, p. 585.
27. S. J. Lippard, *Angew. Chem. Int. Ed. Engl.* **27**, 344 (1988).
28. J. LeGall *et al.*, *Biochemistry* **27**, 1636 (1988).
29. J. B. Vincent, M. W. Crowder, B. A. Averill, *Trends Biochem. Sci.* **17**, 105 (1992).
30. K. L. Taft, G. C. Papaefthymiou, S. J. Lippard, *Science* **259**, 1302 (1993); E. C. Theil, *Adv. Enzymol. Relat. Areas Mol. Biol.* **63**, 421 (1989); R. R. Crichton and R. J. Ward, *Biochemistry* **31**, 11255 (1992); P. M. Harrison *et al.*, *Adv. Inorg. Chem.* **36**, 449 (1991).

31. W. H. Armstrong and S. J. Lippard, *J. Am. Chem. Soc.* **105**, 4837 (1983).
32. K. Wieghardt, K. Pohl, W. Gebert, *Angew. Chem. Intl. Ed. Engl.* **22**, 727 (1983).
33. J. Sanders-Loehr, W. D. Wheeler, A. K. Shiemke, B. A. Averill, T. M. Loehr, *J. Am. Chem. Soc.* **111**, 8084 (1989).
34. E. I. Solomon and Y. Zhang, *Acc. Chem. Res.* **25**, 343 (1992).
35. M. A. Holmes, I. L. Trong, S. Turley, L. C. Sieker, R. E. Stenkamp, *J. Mol. Biol.* **218**, 583 (1991).
36. P. Chaudhuri, K. Wieghardt, B. Nuber, J. Weiss, *Angew. Chem. Int. Ed. Engl.* **24**, 778 (1985).
37. Y. Dong *et al.*, *J. Am. Chem. Soc.* **115**, 1851 (1993).
38. N. Kitajima, H. Fukui, Y. Moro-oka, *ibid.* **112**, 6402 (1990).
39. Y. Hayashi, M. Suzuki, A. Uehara, Y. Mizutani, T. Kitagawa, *Chem. Lett.* **1992**, 91 (1992).
40. P. Reichard, *Science* **260**, 1773 (1993); M. Fontecave, P. Nordlund, H. Eklund, P. Reichard, *Adv. Enzymol.* **65**, 147 (1992); J. Stubbe, *J. Biol. Chem.* **265**, 5329 (1990).
41. P. Nordlund, H. Eklund, B. M. Sjöberg, *Nature* **345**, 593 (1990).
42. M. Atta, P. Nordlund, A. Aberg, H. Eklund, M. Fontecave, *J. Biol. Chem.* **267**, 20682 (1992).
43. R. L. Rardin, W. B. Tolman, S. J. Lippard, *New J. Chem.* **15**, 417 (1991).
44. J. M. Bollinger, Jr., *et al.*, *Science* **253**, 292 (1991); J. M. Bollinger, Jr., J. Stubbe, B.-H. Huynh, D. E. Edmondson, *J. Am. Chem. Soc.* **113**, 6289 (1991).
45. D. P. Goldberg, S. P. Watton, A. Masschelein, L. Wimmer, S. J. Lippard, *J. Am. Chem. Soc.* **115**, 5346 (1993).
46. B. G. Fox and J. D. Lipscomb, in *Biological Oxidation Systems*, C. Reddy and G. A. Hamilton, Eds. (Academic Press, New York, 1990), vol. 1, pp. 367–388.
47. K. E. Liu, C. C. Johnson, M. Newcomb, S. J. Lippard, *J. Am. Chem. Soc.* **115**, 939 (1993).
48. P. Nordlund, H. Dalton, H. Eklund, *FEBS Lett.* **307**, 257 (1992).
49. J. G. DeWitt *et al.*, *J. Am. Chem. Soc.* **113**, 9219 (1991).
50. D. H. R. Barton and D. Dollar, *Acc. Chem. Res.* **25**, 504 (1992).
51. R. A. Leising, B. A. Brennan, L. Que, Jr., B. G. Fox, E. Münck, *J. Am. Chem. Soc.* **113**, 3988 (1991).
52. L. C. Stewart and J. P. Klinman, *Annu. Rev. Biochem.* **57**, 551 (1988).
53. J. C. Freeman, J. J. Villafranca, D. J. Merkler, *J. Am. Chem. Soc.* **115**, 4923 (1993).
54. S. I. Chan, H.-H. T. Nguyen, A. K. Shiemke, M. E. Lidstrom, in (*16*), pp. 184–195.
55. K. D. Karlin, Z. Tyeklár, A. D. Zuberbühler, in (*19*), pp. 261–315.
56. N. Kitajima, *Adv. Inorg. Chem.* **39**, 1 (1992).
57. R. R. Jacobson, Z. Tyeklár, K. D. Karlin, S. Liu, J. Zubieta, *J. Am. Chem. Soc.* **110**, 3690 (1988); Z. Tyeklár *et al.*, *ibid.* **115**, 2677 (1993).
58. N. J. Blackburn, R. W. Strange, A. Farooq, M. S. Haka, K. D. Karlin, *ibid.* **110**, 4263 (1988).
59. N. Kitajima, K. Fujisawa, Y. Moro-oka, *ibid.* **111**, 8975 (1989); N. Kitajima *et al.*, *ibid.* **114**, 1277 (1992).
60. K. A. Magnus, H. Ton-That, J. E. Carpenter, in (*16*), pp. 143–150.
61. K. D. Karlin *et al.*, *J. Am. Chem. Soc.* **106**, 2121 (1984).
62. M. S. Nasir, B. I. Cohen, K. D. Karlin, *ibid.* **114**, 2482 (1992).
63. B. L. Vallee and D. S. Auld, *Biochemistry* **29**, 5647 (1990); J. E. Coleman, *Annu. Rev. Biochem.* **61**, 897 (1992); D. W. Christianson, *Adv. Protein Chem.* **42**, 281 (1991).
64. D. W. Christianson and W. N. Lipscomb, *Acc. Chem. Res.* **22**, 62 (1989).
65. B. W. Matthews, *ibid.* **21**, 333 (1988).
66. D. N. Silverman and S. Lindskog, *ibid.*, p. 30.
67. J. Suh, *ibid.* **25**, 273 (1992); T. H. Fife, *Perspect. Bioinorg. Chem.* **1**, 43 (1991).
68. E. Kimura, T. Shiota, T. Koike, M. Shiro, M. Kodama, *J. Am. Chem. Soc.* **112**, 5805 (1990); E. Kimura, *Prog. Inorg. Chem.* **41**, 443 (1993).
69. B. L. Vallee and D. S. Auld, *Biochemistry* **32**, 6493 (1993); D. E. Fenton and H. Okawa, *J. Chem. Soc. Dalton Trans.*, p. 1349.
70. H. Han, J. M. Rifkind, A. S. Mildvan, *Biochemistry* **30**, 11104 (1991).
71. E. E. Kim and H. W. Wyckoff, *J. Mol. Biol.* **218**, 449 (1991).
72. L. S. Beese and T. A. Steitz, *EMBO J.* **10**, 25 (1991).
73. S. K. Burley, P. R. David, A. Taylor, W. N. Lipscomb, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6878 (1990); S. K. Burley, P. R. David, W. N. Lipscomb, *ibid.* **88**, 6916 (1991).
74. P. Hendry and A. M. Sargeson, *Prog. Inorg. Chem.* **38**, 201 (1990); N. N. Murthy, M. Mahroof-Tahir, K. D. Karlin, unpublished results.
75. L. Que Jr., Ed., *Metal Clusters in Proteins*, vol. 372 of the *ACS Symposium Series* (American Chemical Society, Washington, DC, 1988).
76. R. H. Holm, *Adv. Inorg. Chem.* **38**, 1 (1992); _____, S. Ciurli, J. A. Weigel, *Prog. Inorg. Chem.* **38**, 1 (1990).
77. P. A. Lindahl and J. A. Kovacs, *J. Cluster Sci.* **1**, 29 (1990).
78. R. Cammack, *Adv. Inorg. Chem.* **38**, 281 (1992); H. Beinert, *FASEB J.* **4**, 2483 (1990).
79. G. Backes *et al.*, *J. Am. Chem. Soc.* **113**, 2055 (1991).
80. M. K. Chan, J. Kim, D. C. Rees, *Science* **260**, 792 (1993); J. T. Bolin, A. E. Ronco, T. V. Morgan, L. E. Mortenson, N.-H. Xuong, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1078 (1993); J. Kim and D. C. Rees, *Nature* **360**, 553 (1992); W. H. Orme-Johnson, *Science* **257**, 1639 (1992); M. M. Georgiadis *et al.*, *ibid.*, p. 1653.
81. I. Moura *et al.*, *J. Biol. Chem.* **267**, 4489 (1992).
82. D. E. McRee, D. C. Richardson, J. S. Richardson, L. M. Siegel, *ibid.* **261**, 10277 (1986).
83. M. J. Maroney, G. J. Colpas, C. Bagyinka, N. Baidya, P. K. Mascharak, *J. Am. Chem. Soc.* **113**, 3962 (1991).
84. S. W. Ragsdale, *CRC Crit. Rev. Biochem. Mol. Biol.* **26**, 261 (1991); R. Cammack, in (*19*), pp. 189–225; J. A. Kovacs, in (*4*), pp. 173–218; A. F. Kolodziej, *Prog. Inorg. Chem.* **41**, 493 (1993).
85. C.-F. Kuo *et al.*, *Science* **258**, 434 (1992).
86. M. H. Emptage, in (*75*), pp. 343–371.
87. M. C. Kennedy and C. D. Stout, *Adv. Inorg. Chem.* **38**, 323 (1992).
88. H. Beinert and A. J. Thomson, *Arch. Biochem. Biophys.* **222**, 333 (1983).
89. T. V. O'Halloran, *Science* **261**, 715 (1993); M. D. Kennedy, L. Mende-Mueller, G. A. Blondin, H. Beinert, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 11730 (1992); D. J. Haile, T. A. Rouault, C. K. Tang, J. B. Harford, R. D. Klausner, *ibid.*, p. 7546.
90. D. Coucouvanis, *Acc. Chem. Res.* **24**, 1 (1991); D. J. Evans, R. A. Henderson, B. E. Smith, in (*19*), pp. 89–130.
91. M. F. Perutz, *Annu. Rev. Biochem.* **48**, 327 (1979); R. E. Dickerson and I. Geis, *Hemoglobin* (Benjamin/Cunnings, Menlo Park, CA, 1983).
92. G. McLendon *et al.*, *J. Am. Chem. Soc.* **115**, 3665 (1993); H. Pelletier and J. Kraut, *Science* **258**, 1748 (1992); C. C. Moser, J. M. Keske, K. Warncke, R. S. Farid, P. L. Dutton, *Nature* **355**, 796 (1992); D. N. Beratan, J. N. Onuchic, J. R. Winkler, H. B. Gray, *Science* **258**, 1740 (1992); B. M. Hoffman, M. J. Natan, J. M. Nocek, S. A. Walin, *Struct. Bonding* (*Berlin*) **75**, 85 (1991); G. McLendon and R. Hake, *Chem. Rev.* **92**, 481 (1992).
93. P. R. Ortiz de Montellano, *Acc. Chem. Res.* **20**, 289 (1987).
94. T. G. Traylor, *Pure Appl. Chem.* **63**, 265 (1991).
95. G. E. Wuenschell, C. Tetreau, D. Lavalette, C. A. Reed, *J. Am. Chem. Soc.* **114**, 3346 (1992); J. P. Collman, T. R. Halbert, K. S. Suslick, in *Metal Ion Activation of Dioxygen*, T. G. Spiro, Ed. (Wiley, New York, 1980), pp. 1–72; K. S. Suslick and T. J. Reinert, *J. Chem. Educ.* **62**, 974 (1985).
96. T. L. Poulos and R. Raag, *FASEB J.* **6**, 674 (1992); Y. Watanabe and J. T. Groves, in *Mechanisms of Catalysis*, D. S. Sigman, Ed. (Academic Press, San Diego, 1992), vol. XX, pp. 405–452; M. J. Gunter and P. Turner, *Coord. Chem. Rev.* **108**, 115 (1991); D. Ostovic and T. C. Bruice, *Acc. Chem. Res.* **25**, 314 (1992); B. Meunier, *Chem. Rev.* **92**, 1411 (1992); R. S. Drago, *Coord. Chem. Rev.* **117**, 185 (1992).
97. M. A. Marletta, M. A. Tayeh, J. M. Hevel, *BioFactors* **2**, 219 (1990); S. H. Snyder and D. S. Bredt, *Sci. Am.* **266**, 68 (May 1992); L. J. Ignarro, *Blood Vessels* **28**, 67 (1991); J. S. Stamler, D. J. Singel, J. Loscalzo, *Science* **258**, 1898 (1992).
98. A. Verma, D. J. Hirsch, C. E. Glatt, G. V. Ronnett, S. H. Snyder, *Science* **259**, 381 (1993); M. Zhuo, S. A. Small, E. R. Kandel, R. D. Hawkins, *ibid.* **260**, 1946 (1993).
99. K. A. White and M. A. Marletta, *Biochemistry* **31**, 6627 (1992); R. C. Prince and D. E. Gunson, *Trends Biochem. Sci.* **18**, 35 (1993).
100. T. G. Traylor, A. F. Duprat, V. S. Sharma, *J. Am. Chem. Soc.* **115**, 810 (1993).
101. D. Christodoulou *et al.*, in (*16*), pp. 427–436; D. Christodoulou, C. George, L. K. Keefer, *J. Chem. Soc. Chem. Commun.* (1993), p. 937.
102. J. A. Stubbe, *Annu. Rev. Biochem.* **58**, 257 (1989); E. Mulliez, M. Fontcave, J. Gaillard, P. Reichard, *J. Biol. Chem.* **268**, 2296 (1993).
103. Y. Zang, T. E. Elgren, Y. Dong, L. Que, Jr., *J. Am. Chem. Soc.* **115**, 811 (1993); Y.-M. Chiou and L. Que, Jr., *ibid.* **114**, 7567 (1992).
104. J. A. Fee *et al.*, in (*16*), pp. 485–500; G. T. Babcock and M. Wikström, *Nature* **356**, 301 (1992). See also a special issue (12 articles) devoted to cytochrome oxidase: *J. Bioenerg. Biomembr.* **25** (no. 2), 69 (1993). For recent inorganic model studies, see S. C. Lee and R. H. Holm, *J. Am. Chem. Soc.* **115**, 5833 (1993); A. Nanthakumar *et al.*, *ibid.*, in press.
105. R. S. Pilato and E. I. Stiefel, in (*19*), pp. 131–188.
106. L. G. Marzilli, in *ibid.*, pp. 227–259.
107. A. Volbeda, A. Lahm, F. Sakiuama, D. Suck, *EMBO J.* **10**, 1607 (1991).
108. S. Hansen, L. K. Hansen, E. Hough, *J. Mol. Biol.* **225**, 543 (1992); E. Hough *et al.*, *Nature* **338**, 357 (1989).
109. S. L. Roderick and B. W. Matthews, *Biochemistry* **32**, 3907 (1993).
110. R. S. Reczkowski and D. E. Ash, *J. Am. Chem. Soc.* **114**, 10992 (1992).
111. R. R. Poyner and G. H. Reed, *Biochemistry* **31**, 7166 (1992).
112. J. F. Davies II, Z. Hostomska, Z. Hostomsky, S. R. Jordan, D. A. Matthews, *Science* **252**, 88 (1991).
113. B. S. Cooperman, A. A. Baykov, R. Lahti, *Trends Biochem. Sci.* **17**, 262 (1992).
114. E. P. Day, J. Peterson, M. S. Sendova, M. J. Todd, R. P. Hausinger, *Inorg. Chem.* **32**, 634 (1993).

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