CHAPTER 1

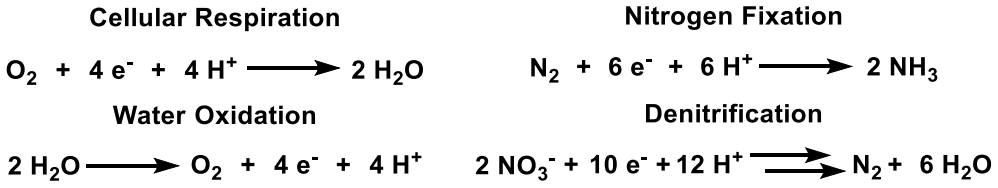
GENERAL INTRODUCTION

INTRODUCTION

Multi-Electron Transformations Relevant to Global Biogeochemical Cycles of Oxygen and Nitrogen. The chemical basis of every living organism is centralized around a handful of elements C, H, N, O, P, and S; the transformation of molecules containing these elements occurs ubiquitously and on massive scales, directly affecting life around Earth. The global chemistry of oxygen and nitrogen are particularly relevant to human life. For example, the beginning of production of molecular oxygen eons ago, and its build up in the planet's atmosphere, has been linked to the development of complex eukaryotic organisms.[1] A constant production of O_2 is required to support aerobic life, and is intimately involved in biochemical processes related to the oxidation of other biological elements. Similarly, ammonium (NH_4^+) serves as the crucial building block to all biological nitrogen-containing molecules, which includes amino acids and nucleotides, and the combination of manmade and natural ammonium synthesis is directly tied to the production of sufficient supplies of food for humans and other animals.[2]

The global cycles of oxygen and nitrogen revolve around electron transfer; the formal oxidation state of oxygen commonly varies from -2 (i.e. H_2O) to 0 (O_2), and nitrogen can vary from -3 (NH_4^+) to +5 (NO_3^-).[1] The four-electron reduction of dioxygen by cellular respiration constitutes a key component of the global oxygen cycle. The consumed dioxygen is replaced by photosynthesis through the analogous four-electron oxidation of water (Scheme 1). In a similar way, multi-electron redox processes of nitrogen provide access to its various biologically relevant forms (i.e. N_2, NH_4^+, NO, N_2O) and processes to regenerate them. The global redox transformations of oxygen and nitrogen typically occur through multi-electron pathways. This is because, often, the partially reduced/oxidized molecule is less thermodynamically stable (Figure 1).

Scheme 1. Multi-Electron Redox Processes of Oxygen and Nitrogen Relevant to Their Global Biogeochemical Cycles.



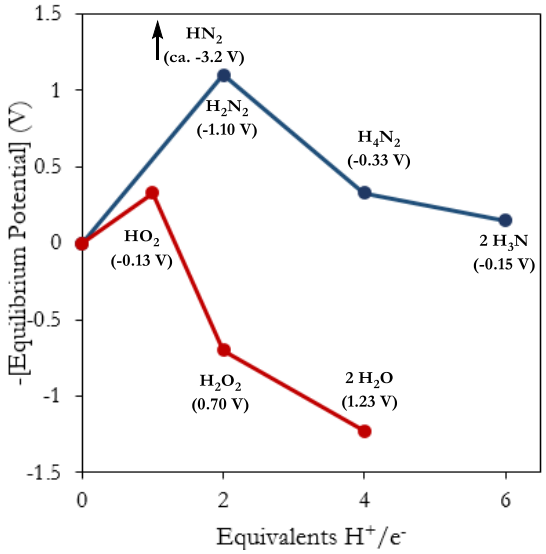


Figure 1. Equilibrium standard potentials of forms of oxygen (red) and nitrogen (blue).[3]

Biological Catalysts for Multi-Electron Redox Transformations of Oxygen and Nitrogen. Nature contains a variety of catalysts which constitute major steps in the global cycles of oxygen and nitrogen. For example, yearly, 300 tetragrams of N_2 are reduced to NH_4^+ by nitrogen-fixing microbes through a metalloenzyme called nitrogenase; this process is considered to be responsible for more than half the amount of bio-available nitrogen in the environment.[2] These enzymes display remarkable efficiency towards their native reactions and are capable of effecting complex, challenging multi-electron transformations under relatively mild, and sustainable, conditions. For this reason, researchers have examined these biological systems to gain insight into the features of these enzymes that promote efficient catalysis.

Biological enzymes responsible for redox transformations of oxygen and nitrogen are all metalloenzymes, containing either Fe, Mn, and/or Cu ions in their active site.[4] Within this set of enzymes, many of the active sites contain complex transition metal clusters (Figure 2). The structural diversity of these multinuclear active sites is notable, displaying various transition metals, nuclearities, bridging ligands, and geometric arrangements. Detailed mechanistic study of these globally relevant metalloenzymes has been a culmination of efforts from biochemists, crystallographers, spectroscopists, and theoreticians, which remain at the frontier of bioinorganic research, leading to developments in enzymology, metal ion spectroscopy, and computational techniques.

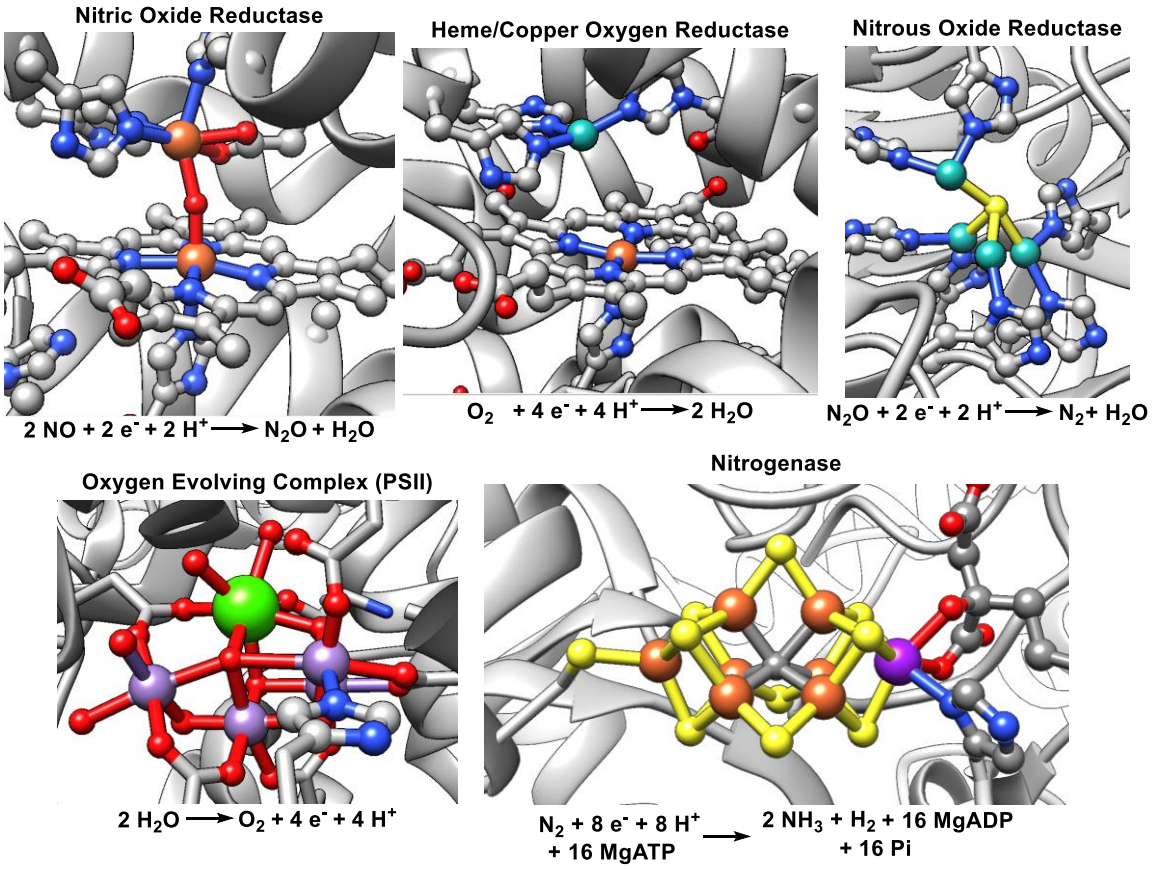


Figure 2. Active site structures of metalloenzymes competent for multi-electron redox transformations of oxygen and nitrogen: nitric oxide reductase (PDB code: 3O0R),

heme/Cu oxygen reductase (1V54), nitrous oxide reductase (1FWX), photosystem II (3WU2), and nitrogenase (1M1N). Fe (light brown), Cu (cyan), Mn (light purple), Mo (purple), Ca (green), S (yellow), O (red), N (blue).

Case Study: Mechanistic Insight to the Oxygen-Ovolving Complex (OEC) in Photosystem II. Photosynthetic organisms use the energy in sunlight to drive the reduction of plastoquinol and produce energy in the form of adenosine triphosphate (ATP). The reducing equivalents obtained are derived from water, one of the most abundant sources of electrons in the environment.[4b, 5] In a separate process, the energy and reducing equivalents are used for reduction of CO_2 and production of carbohydrates in the Calvin cycle. Water oxidation occurs in a protein assembly known as photosystem II (PSII), at a multinuclear active site composed of a heterometallic [Mn_4CaO_5] cluster, called the OEC.[6] Incoming photons induce a charge separated state at by a nearby heme center (P_{680}), which transfers a single electron through a series of mediators until reaching plastoquinone. P_{680} is regenerated by reduction from a nearby tyrosine residue (Tyr_Z). This resulting organic radical oxidizes the OEC, which translates four separate electron transfer events by P_{680} to one catalytic turnover, producing dioxygen from two molecules of water.[4b] The molecular model of OEC turnover is considered within the framework of the Kok cycle, which describes five distinct so-called S-states (S_0, S_1, …, S_4) of the OEC (Figure 3).[4c, 7] Electrons, and protons from coordinated H_xO ligands, are removed from the OEC during each S-state transition; states S_0 through S_3 have been observed spectroscopically (principally through advanced EPR and X-ray absorption techniques), and in some cases structurally characterized by X-ray diffraction. These investigations have established a number of important characteristics of the OEC catalytic cycle, including (i) the lowest oxidation state of the OEC during turnover is [$Mn^{III}_3Mn^{IV}$] and

this cluster undergoes four subsequent oxidations to reach a formal [$Mn^{VI}_3Mn^{V}$] redox state, responsible for O–O bond formation (S_4);[4b, 8] (ii) EPR spectroscopy of the OEC has shown a dynamic structure within the cluster, where at least one of the bridging oxygen atoms is exchangeable (and implicated as one of the substrate oxygen atoms);[9] (iii) recent X-ray techniques have captured structural snapshots of the OEC in the S_3 state, which displays coordination of the second substrate H_xO molecule to one of the cubane Mn centers;[10] and (iv) extensive computational studies based on experimental structural and spectroscopic parameters for the OEC suggest a high-valent terminal Mn-oxo is the key O–O bond forming intermediate in the unobserved S_4 state.[11]

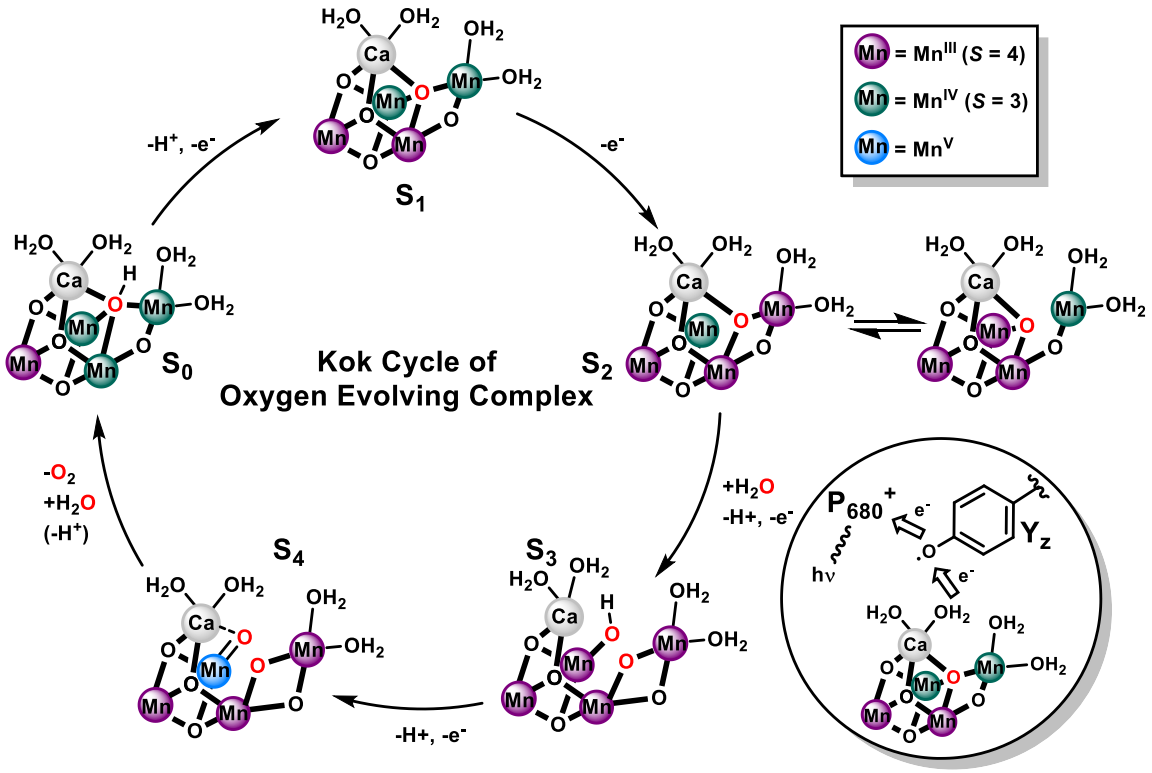


Figure 3. Contemporary proposed structures of the OEC in each stage of the Kok cycle.

These studies provide a remarkably detailed picture of water oxidation by the OEC; however, due to the inherent complexity of studying this massive protein (700 kDa), with its

multiple subunits and cofactors, there are a number of challenges to realizing a complete mechanistic understanding of the OEC. For example, the precise protonation state of the OEC, and its neighboring protein environment, is not well-understood for any S-state.[11b] Also, since PSII is necessarily studied in aqueous conditions, the OEC is always present in a large excess of substrate, which complicates isolation and characterization of the oxidized states of the cluster.

Our mechanistic understanding of the OEC exemplifies a number of possible functional roles for multiple transition metals arranged within a multinuclear active site. The presence of many redox active transition metals allows for the storage of multiple oxidizing equivalents, without requiring a large buildup of charge at a single site. This has been implicated as a cause for the high selectivity of the OEC, which produces little to no partially oxidized forms of oxygen, i.e. H_2O_2 or O_2^-, which would be detrimental to the organism. One can also envision how the relatively unique dangling cubane geometry of the metal centers in the OEC promotes reactivity between specific metal-bound oxygen atoms. Furthermore, the coupling of unpaired spins in the Mn centers may be crucial for efficient release of dioxygen, avoiding the production of reactive singlet oxygen. In general, a number of functions for neighboring metal centers in various catalytic systems can be proposed: (i) storage of redox equivalents, (ii) structurally directing reactive moieties, and (iii) tuning the electronic characteristics of a reactive metal or coordinated ligand.

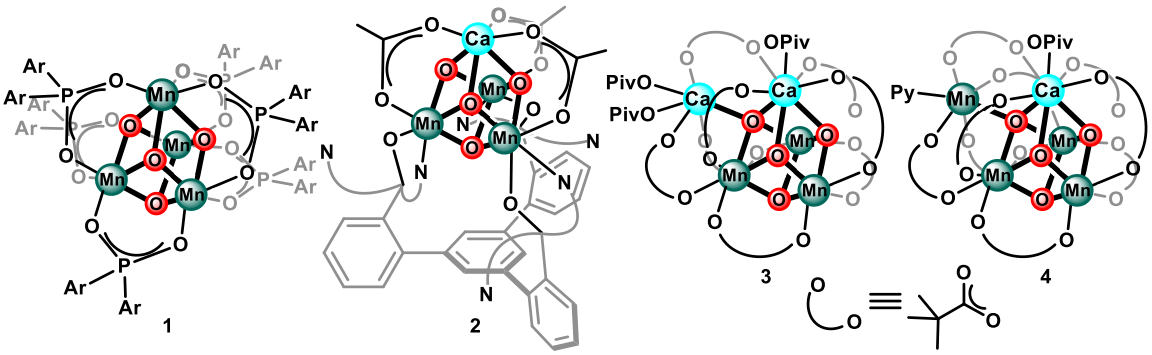
Biologically Inspired Transition Metal Complexes Relevant to Small Molecule Chemistry of Oxygen and Nitrogen. Due to the potential complexity and constraints of studying metalloenzymes directly, the complementary development of well-defined small molecule transition metal complexes has provided significant chemical insight related to these biological processes. The synthetic inorganic chemistry of Fe, Mn, and Cu complexes has been

studied extensively, including chemistry related to the global oxygen and nitrogen cycles through reactions involving relevant O- and N-containing small molecules such as H_2O, O_2, N_2, and NO. A majority of these studies are performed with mononuclear, or binuclear, transition metal complexes; the development of multinuclear systems with greater complexity that bear closer resemblance to biological active sites remains a challenge for synthetic chemists. The following survey of relevant synthetic metal complexes is by no means exhaustive, but its discussion will place this work within the wider context of previously reported synthetic transition metal complexes that are relevant to biological transformations of oxygen and nitrogen.

Synthetic Inorganic Chemistry Related to Water Oxidation by the OEC. Efforts towards a full structural model of the OEC were undertaken with the goal of elucidating the structure-property relationships of a well-defined [Mn_4CaO_5] cluster with spectroscopic and/or functional relevance to the native metalloenzyme. A variety of di- and tetramanganese oxo clusters have been reported with relevance to the OEC; notable early achievements within this field include the isolation of high-valent [Mn_4O_4] cubane clusters from the groups of Dismukes (**1**) and Christou, and the incorporation of Ca into high nuclearity Mn-oxo clusters by Christou and co-workers.[12] In 2011, Agapie and co-workers reported a [Mn_3CaO_4] cluster analogous to the cubane subunit of the OEC, **3**.[13] Since then, a more complete structural model of the OEC, with the dangler Mn has been reported by Zhang, Dong, Dau, and co-workers (**4**).[14] With these complexes, a great deal of insight has been obtained, towards understanding the electronic interactions between Mn centers in cubane clusters, and the influence of the redox-inactive Ca ion on redox and reactivity properties.[15] Most structural models of the OEC display coordinatively saturated Mn centers (Scheme 2), precluding extensive reactivity studies with

exogenous H_2O; however, these and related systems have been used to study the reactivity of the bridging oxo ligands of high valent Mn complexes with relevance to the OEC.[16]

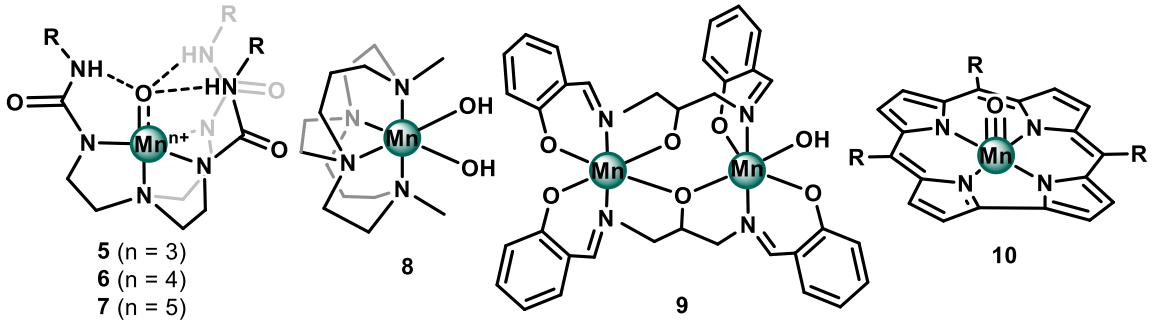
Scheme 2. Selected Structural Models of the OEC.[12b, 13-14, 15b]



Numerous lower nuclearity metal complexes have been examined for their relevance to the OEC (Scheme 3). For example, Borovik and co-workers have studied the reactivity and electronic structure of mononuclear Mn-OH, Mn-oxo, and Mn–(OH)–Ca motifs, which are related to possible intermediates of the OEC; a unique ligand capable of hydrogen bonding to the Mn–OH_x moiety facilitated characterization of a series of Mn^{III}-, Mn^{IV}-, and Mn^{V}-oxo complexes (**5** – **7**).[17] Particularly relevant to the biological system are understanding aspects that affect the homolytic bond dissociation enthalpy (BDE) of Mn–OH_x motifs; proton coupled electron transfer (PCET) has been implicated as a crucial aspect of OEC catalysis, as it avoids charge built up at the active site, promoting progression to the fully oxidized state of the cluster.[18] Along these lines, other groups have examined the PCET reactivity of mononuclear Mn–OH and -oxo complexes to understand the influence of Mn oxidation state, ligand, field, and protonation state on reactivity.[19] Examples of these types of studies with multinuclear Mn complexes are less common;[16b, 16c, 20] a binuclear Mn system has been reported by Pecoraro and co-workers (**9**), which is able to support –aquo and –hydroxide ligands in multiple Mn oxidation states, and access a reactive terminal Mn-oxo.[21]

O–O bond formation of synthetic Mn-oxo complexes has also been examined, predominantly with porphyrin ligands. Nucleophilic attack of Mn^{V}-oxo (**10**) by hydroxide produces peroxo- intermediates in Mn-corrole systems.[22] In some cases, subsequent oxidation of this intermediate releases dioxygen. The reactivity of related corrole complexes in the presence of a redox-inactive metal has shown significant perturbations to the Mn–O bonding, which could be relevant to Mn–O–Ca motifs in the OEC.[23]

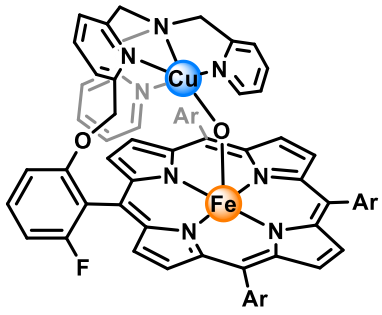
Scheme 3. Selected Examples of PCET and O–O Bond Formation with Synthetic Complexes Relevant to the OEC.



Synthetic Complexes Relevant to Heme/Cu Oxygen Reductase (HCO). Dioxygen reduction by the bimetallic active site of HCO is a key step in cellular respiration that drives transmembrane proton pumping to ultimately obtain ATP from cellular reducing equivalents.[4e] HCO enzymes are part of a wider class of metalloenzymes that reduce dioxygen; other enzymes of this class employ mono- and binuclear active sites of Fe and Cu with O_2 to oxidize organic molecules for a variety of metabolic pathways. A common element between HCO (and other Fe-containing O_2 reducing enzymes) and the OEC is the key role of a putative high-valent terminal metal-oxo intermediate. In HCO, O–O bond cleavage of a Fe–(O_2)–Cu intermediate is proposed to produce Cu^{II}–OH and Fe^{IV}-oxo, which is further reduced and protonated to afford two molecules of H_2O.

The synthetic chemistry of heme and non-heme Fe-oxo complexes has been extensively investigated, due to their relevance to members of O_2-reducing metalloenzymes.[24] Groves and co-workers have recently reviewed this topic for heme Fe-oxo complexes.[25] Close structural mimics of the bimetallic HCO active site have also been prepared (i.e. **11**); a survey of these, and related synthetic Fe/Cu systems, has been reviewed recently by Karlin and co-workers.[26] Biosynthetic approaches to study the mechanism of O_2-reduction by binuclear heme/non-heme active sites has provided great insight to structure-property relationships in HCO; Lu and co-workers have used protein scaffolds to produce close structural models of the HCO active site, through mutagenesis studies of a simpler heme protein. With a single protein scaffold, they were able to introduce binding sites for various non-heme metals (Zn, Fe, Cu) and investigate their effect on the activity and selectivity for HCO-like activity.[27]

Scheme 4. Synthetic HCO Model Complex[26b]



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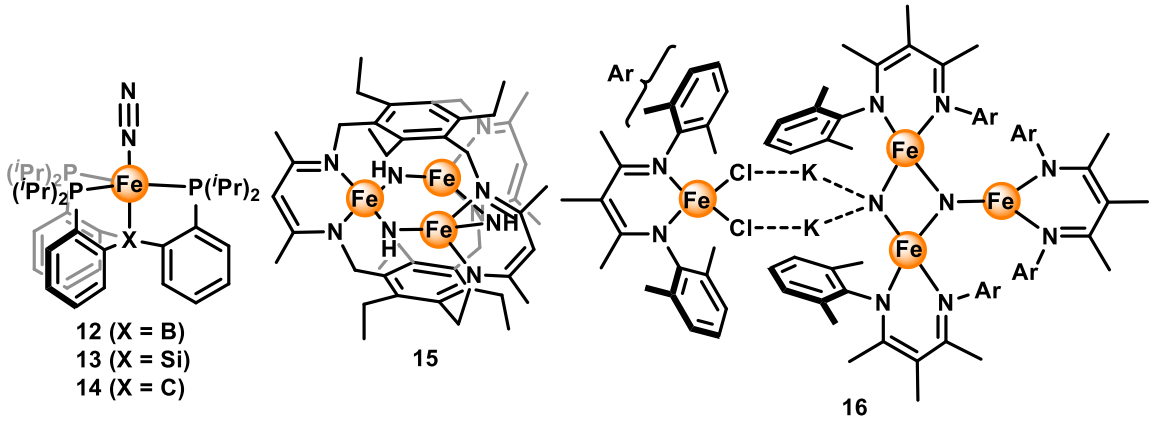
Synthetic Complexes Related to the Fe-Mo Cofactor (FeMoCo) of Nitrogenase. The FeMoco cluster of nitrogenase is a [MFe_7S_9C] cluster with a fused-cubane geometry (M = Mo);[28] versions of nitrogenase where the eighth metal is V, Mo, or Fe have been observed, but the Fe-Mo cofactor is the most well-studied. One of the unique structural features of this cluster is the interstitial μ_6-C ligand, which is not observed in any other biological cluster, and a rare motif in reported synthetic complexes. Extensive mechanistic investigations of nitrogenase by EPR

spectroscopy has characterized a number of reduced oxidation states of the cluster;[29] however the precise binding mode of the FeMoco substrate, N_2, has not been established. Peters and co-workers have examined a series of mononuclear Fe–N_2 complexes, bearing different *trans*-ligand donors (**12** – **14**), including an anionic carbon donor;[30] notably, **13** is the first example of an Fe-based molecular N_2-reduction catalyst.[31] The identity of the *trans*-donor had a strong influence on the Fe–N_2 bonding and reactivity. More recently, crystal structures of FeMoco with a displace μ_2-S between to Fe centers have been obtained, suggesting a possible substrate binding site.[32] This has led to the investigation of binuclear Fe complexes as models of FeMoco.[33] The N_2-activation chemistry of higher nuclearity Fe complexes have also been investigated, although well-characterized high-spin Fe clusters (of more than two Fe centers) with a bound N_2 ligand have yet to be reported in the literature. Despite this, reduction of multinuclear Fe complexes in the presence of N_2 has led to the isolation of a number of Fe-nitride and –imido clusters (**15** and **16**), with relevance to putative intermediates in FeMoco.[34]

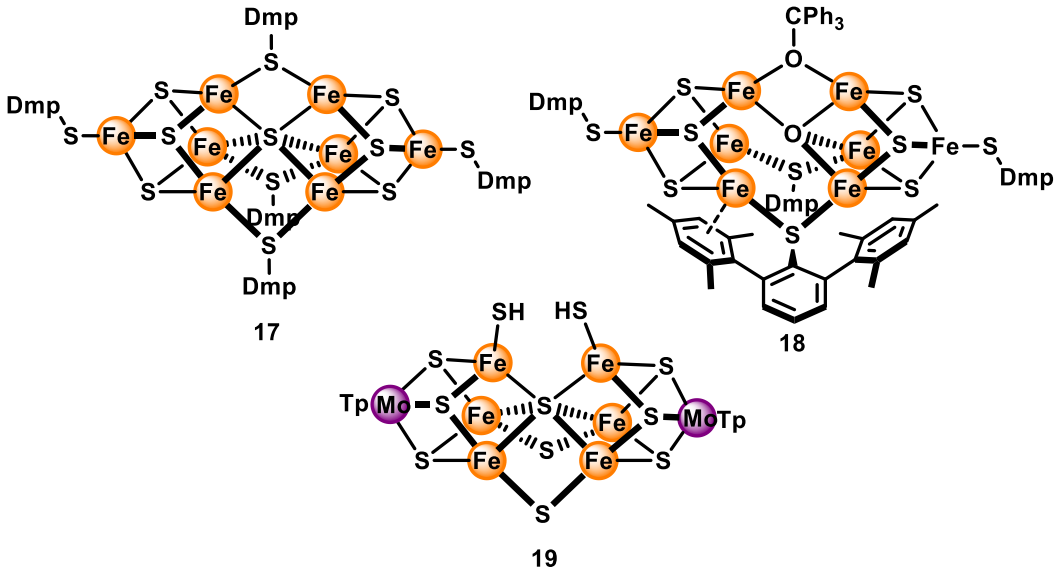
Scheme 5. Selected Functional Models of FeMoco.[30, 34]



Like the OEC, efforts to make rigorous structural mimics of FeMoco are motivated by a desire to prepare well-defined molecular models for spectroscopic and structure-property investigations. Many fused-cubane clusters reminiscent of FeMoco have been reported by the

research groups of Holm and Ohki, including a [Fe_8S_{10}] cluster bearing a μ_6-S (**17**).[35] Although the small molecule chemistry of these clusters has not been reported, related [Fe_6S_9] clusters have been combined with apo-nitrogenase proteins to produce artificial metalloproteins competent for reductive coupling of CO, $^-$CN, and C_2H_4.[36]

Scheme 6. Structural Models of FeMoco and Related Nitrogenase Clusters.[37]



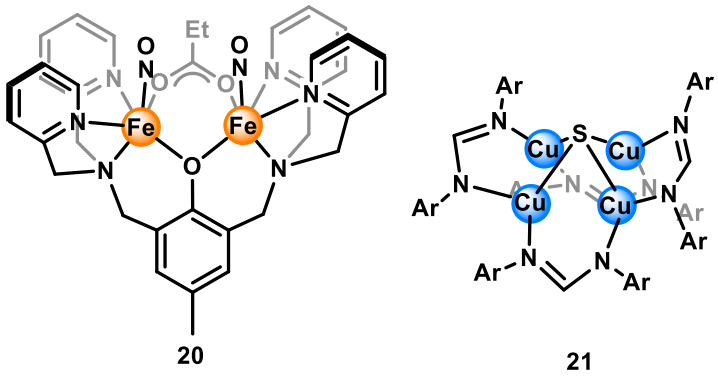
Synthetic Metal Complexes Related to Biological Denitrification. The process of denitrification is an important part of the global nitrogen cycle, nitrate (NO_3^-) is reduced to N_2 over four steps, via nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O) intermediates, as a terminal electron acceptor for an anaerobic analogue to cellular respiration.[4a] The NO and N_2O reducing steps are accomplished by multinuclear active sites of Fe and Cu, respectively.

Nitric oxide reductase (NOR) contains an active site structure similar to HCO, with a non-heme Fe center instead of Cu; similar molecular and biosynthetic systems that have been used to understand HCO have been applied to NOR, as well.[38] NO-reducing metalloenzymes with a binuclear non-heme Fe active site are also present in pathogenic bacteria for NO

detoxification. A faithful structural and functional model of this active site has recently been reported by Lehnert and co-workers (**20**).[39]

Nitrous oxide reductase (N_2O) is composed of a tetranuclear Cu active site, containing a bridging S ligand.[40] Mankad and co-workers have reported a tetranuclear Cu complex bearing a μ_4-S with a square pyramidal geometry (**21**); this complex is capable of reducing N_2O to N_2, structurally and functionally mimicking the native enzyme.[41] The proposed mechanism of N_2OR suggests N_2O is activated across two Cu centers; further mechanistic investigations of **21** or the native enzyme are required to establish the precise role of the four Cu centers in the cluster's ability to drive this transformation.

Scheme 8. Multinuclear Model Complexes of NOR and N_2OR[39, 41]



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CONSPECTUS

The oxidation and reduction of small molecules of oxygen and nitrogen occurs on a global scale and has relevance to many of the chemical processes that encompass life. In organisms across all domains of life, the metalloenzymes that catalyze the multi-electron transformations of molecules such as H_2O, O_2, N_2, and NO_3^- contain active sites with diverse, complex multinuclear transition metal structures. Mechanistic investigations of these metalloenzymes have sought to elucidate the functional purpose of these unique multinuclear arrangements; and come with a number of inherent challenges, due to difficulties in preparation and

manipulation of large protein assemblies, or the limitations of aqueous conditions. For this reason, complementary studies of synthetic transition metal complexes and their chemistry towards O- and N-based small molecules is useful for understanding mechanistic details of native biological systems. While a majority of the reported literature has focused on the synthetic chemistry of mononuclear and binuclear transition metal complexes, the synthesis and study of higher nuclearity clusters comprises an important development towards a more complete understanding of the multi-electron redox transformations of globally relevant molecules. The work detailed in this dissertation addresses the development of tetranuclear clusters of biologically relevant transition metals Fe and Mn, in particular their chemistry towards NO and H_2O, with relevance to biological multinuclear active sites responsible for multi-electron transformations of small molecules.

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