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Small Molecule Signaling Agents: The Integrated Chemistry and Biochemistry of Nitrogen Oxides, Oxides of Carbon, Dioxygen, Hydrogen Sulfide, and Their Derived Species

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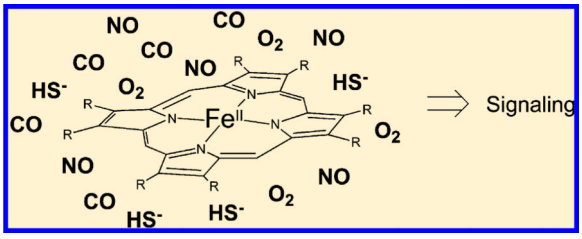
Abstract



Several small molecule species formally known primarily as toxic gases have, over the past 20 years, been shown to be endogenously generated signaling molecules. The biological signaling associated with the small molecules NO, CO, H_2S (and the nonendogenously generated O_2), and their derived species have become a topic of extreme interest. It has become increasingly clear that these small molecule signaling agents form an integrated signaling web that affects/regulates numerous physiological processes. The chemical interactions between these species and each other or biological targets is an important factor in their roles as signaling agents. Thus, a fundamental understanding of the chemistry of these molecules is essential to understanding their biological/physiological utility. This review focuses on this chemistry and attempts to establish the chemical basis for their signaling functions.

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INTRODUCTION

Since the discovery of nitric oxide (NO) as an endogenously generated signaling agent,[1] it has become increasingly evident that other endogenously generated small molecule species such as carbon monoxide (CO) and hydrogen sulfide (H_2S) as well as NO-derived species have important physiological signaling functions as well.[2–5] Interestingly, all of these species were previously well-known toxins of significant industrial and/or environmental concern. Moreover, it is well established that dioxygen (O_2) and O_2-derived species such as superoxide (O^{-}_2) and hydrogen peroxide (H_2O_2) can be vital cell signaling agents as well.[6,7] Thus, there appears to be a wide array of small molecules that are utilized in biological systems as regulators and effectors of physiological function. Indeed, the term "gasotransmitter" has been coined recently[8,9] to specifically denote this group of small molecule signaling agents. However, this term can be very misleading since all of these small molecule agents are completely soluble at the concentrations that are physiologically relevant and cannot be considered as gases in these situations. Thus, use of this term is somewhat unfortunate since it does not properly reflect the physical state of the species when they act in cell signaling. Regardless, these small molecule agents represent a relatively new and important cell signaling paradigm. It is the tenet of this review that the chemical properties and reactivity of the small molecule signaling species are of paramount importance to their biological function/utility and that Nature has evolved around these agents to form an integrated signaling web based on this chemistry.

One of the most intriguing and crucial aspects of the cell signaling associated with these small molecule species (i.e., NO, CO, H_2S, O_2, H_2O_2, O^{-}_2, etc.) is the fact that, in many cases, there is significant commonality in their biochemical targets. That is, the macromolecular and biologically relevant reactive centers for almost all of these species includes redox metals (i.e., iron and/or copper proteins) and redox active amino acids (such as cysteine thiols or tyrosine phenols) as predominant sites of action. However, the intimate chemical interactions between these small signaling species and their biological targets can be kinetically distinct, and the nature of the products can exhibit significant differences with regards to further chemistry, stability, and/or structure. Indeed, these differences at the fundamental chemical level represent the genesis and nature of the biological response. Many of these agents also participate in or affect oxidative, reductive, and/or free radical chemistry. Clearly, the fact that these agents share common reactive targets and, in some cases, react with each other is not a coincidence but, rather, represents an integrated signaling system that has evolved around this chemistry. The integrated physiological signaling associated with these species has been discussed in numerous recent reviews,[2,3,5,8,9] and it is not the intent of this review to further elaborate on this aspect of their biology. Thus, this review focuses first on the fundamental and biologically relevant chemistry of these individual small molecules, and their derivatives, followed by a brief discussion of examples of how this chemistry can integrate within the context of a biological system to form an intricate and integrated signaling system.

BIOLOGICAL CHEMISTRY OF DIOXYGEN (O_2) AND RELATED SPECIES

Dioxygen. Among all signaling agents discussed herein, O_2 has been the most extensively studied. Indeed, much of free radical biology was discovered or is derived from the study of O_2 and its reduction to reactive oxygen species (ROS) in biological systems. (At the risk of being heretical, the term “reactive oxygen species” or ROS is clearly one of the most misused and misunderstood monikers in the lexicon of biology/biochemistry. From a chemical perspective, all chemicals are potentially “reactive” depending on their environment or proximity to other reactive partners. More importantly, the inherent chemical properties/ reactivities of the ROS are very distinct, and we feel it unwise and even misleading to lump all these species together under this somewhat meaningless descriptor.) There are numerous books and reviews that discuss the chemistry, biology, physiology, and pathophysiology associated with O_2.[10–13] Thus, the focus of this review will be to discuss only the most salient and fundamental features of the chemistry associated with these species.

Dioxygen is a chemically unique and fascinating molecule. Unlike most other diatomics, its electron configuration cannot be accurately described using valence bond or Lewis structure formalisms (Figure 1a). Examination of the molecular orbital diagram for O_2 (Figure 1b) reveals that it actually has two unpaired electrons with the same spin occupying degenerate π^* antibonding orbitals. Thus, O_2 has a triplet electronic ground state.

The fact that ground state O_2 has unpaired electrons allows it to react with other species with an unpaired electron. Indeed, O_2 is often viewed as a diradical and can react accordingly, mainly reacting with other radicals. Before continuing the discussion of the chemistry of O_2 and derived species, it is worthwhile to first define the term “radical” and/or “free radical” as used herein. For future discussions, we have adopted the general definition prescribed by Halliwell and Gutteridge[13] for a free radical as being “any species capable of independent existence that contains one or more unpaired electrons”. Although the term “free” is meant to represent the property of independent existence, it is oftentimes omitted and considered inherent to some of the agents discussed herein. It needs to be stressed, however, that this definition is not rigorous. For example, many stable transition metal complexes have unpaired electrons (and are thus paramagnetic) and are not typically considered as free radicals, although they are capable of independent existence. So what distinguishes a free radical such as the hydroxyl radical (HO·, vide infra) from, for example, the paramagnetic ferric iron in hemoglobin? One distinction is that HO· is a main group (i.e., exclusion of transition elements) molecular species with an open shell configuration (i.e., there is a single unpaired electron in a valence shell orbital and therefore does not satisfy the octet rule for main group elements). Also, particularly important to the free radical designation is the implication of a certain minimum of reactivity, such as the reaction with other radical species (although this is also not a rigorously observed distinction, vide infra). Thus, “free radical” is typically used to describe atoms/molecules made up of main group elements with an unpaired electron in a valence shell orbital and that may react readily with other radicals. Therefore, designating O_2 as a free radical using these criteria is at least partially valid (although the Lewis structure for O_2 does satisfy the octet rule, it possesses unpaired electrons, and it can react with other radicals).

Historically, the term radical, when related to biological systems, was associated with extreme reactivity leading to indiscriminant and potentially deleterious chemistry. However, biologically relevant radicals exhibit many degrees of reactivity, and as we shall discuss herein, these radicals span the entire range of reactivity from virtually nonoxidizing (and even reducing) to extremely oxidizing. Moreover, it is now clear that biology utilizes many radical processes to accomplish otherwise difficult biochemical transformations.[14] Many radicals are electron poor and therefore are good oxidants. For example, the hydroxyl radical (HO·, a species to be discussed in more detail later) is a strong one-electron oxidant, as evidenced by a reduction potential of 2.31 V (vs NHE, pH 7.0). By comparison, O_2 is a poor one-electron oxidant. The reduction potential for the O_2/O^{-}_2 couple is –0.33 V (vs NHE, 1 atm O_2, pH 7.0). (It should be noted that the reduction potential for O_2 is also reported to be –0.16 V (vs NHE, pH 7.0), which represents the value if O_2 were at 1 M, rather than 1 atm.) Thus, although both HO· and O_2 possess unpaired electrons, HO· is an extremely strong one-electron oxidant, while O_2 is not.

At first glance, the fact that O_2 is a poor one-electron oxidant may appear to be contradictory to its role as the ultimate electron acceptor (oxidant) in aerobic organisms. That is, it is the energetically favorable reduction of O_2 to H_2O that serves as the basis for aerobic life. The reduction potentials for the individual steps in the overall reduction of O_2 to H_2O are shown below (reactions 1–4, all vs NHE and pH 7).[10]

$$O_2 + e^- \rightarrow O_2^- \quad \varepsilon^{\circ\prime} = -0.33\text{V} \quad (1)$$

$$O_2^- + 2H^+ + e^- \rightarrow H_2O_2 \quad \varepsilon^{\circ\prime} = +0.89\text{V} \quad (2)$$

$$H_2O_2 + H^+ + e^- \rightarrow H_2O + HO\cdot \quad \varepsilon^{\circ\prime} = +0.38\text{V} \quad (3)$$

$$HO\cdot + H^+ + e^- \rightarrow H_2O \quad \varepsilon^{\circ\prime} = +2.31\text{V} \quad (4)$$

The first one-electron reduction of O_2 to O_2^- is relatively unfavorable as evidenced by a reduction potential of –0.33 V. However, the reduction potential for the conversion of O_2 to H_2O is overall very favorable with an $\varepsilon^{\circ\prime}$ for the 4-electron reduction of +0.81 V (remember that this reduction potential represents an average “per electron” value). Thus, O_2 is a poor one-electron oxidant but a very good 4-electron oxidant. This thermodynamic barrier to the first one-electron reduction of O_2 to give O^{-}_2 serves to restrict, to a certain degree, indiscriminant and unwanted oxidations carried out by O_2.

Another way of evaluating the one-electron oxidizing potential of O_2 and related radical species is to examine their ability to perform hydrogen atom abstraction chemistry since this reaction represents the transfer of an electron (along with a proton) to the hydrogen abstracting oxidant. If O_2 and HO· were to abstract hydrogen atoms from a substrate R-H (reactions 5 and 6), the O–H bonds formed have bond dissociation energies (BDE) of 47 and 119 kcal/mol, respectively, indicating that this reaction is much more favorable for HO·.

Indeed, since the strength of most C–H or N–H bonds in biological molecules is >80 kcal/mol, this reaction is thermodynamically very unfavorable for O_2 but generally very favorable for HO·.

$$O_2 + R-H \rightarrow \cdot OO-H + R\cdot \quad O-H \quad BDE = 47 kcal/mol \quad (5)$$

$$HO\cdot + R-H \rightarrow HO-H + R\cdot \quad O-H \quad BDE = 119 kcal/mol \quad (6)$$

Thus, it is clear that O_2 is a poor one-electron oxidant in spite of the fact that it has radical character.

The fact that O_2 has a triplet electronic ground state (i.e., $^3O_2$) is significant since it kinetically restricts the types of reactions that it can participate in. For example, the reaction of O_2 with singlet organic molecules (all electrons are spin paired in bonds or as lone pairs) to generate singlet products is “spin forbidden”. That is, the reaction of triplet O_2 with singlet molecules to give singlet products would require a spin “flip” (requiring two steps and high energy intermediates) and is very slow.[15] Thus, spontaneous reactions of O_2 with organic molecules are very restricted both thermodynamically (due to the unfavorable one-electron reduction chemistry) and kinetically (due to the spin restriction). These fundamental properties of O_2 have allowed Nature to reductively harvest its tremendous thermodynamic potential without having to cope (for the most part, vide infra) with an abundance of indiscriminant and/or potentially uncontrollable processes.

Although O_2 is poor at initiating radical chemistry (vide supra), it can rapidly react with other, existing radicals. That is, if a radical center is present (for example an alkyl radical, R·), it can rapidly react with O_2 (reaction 7).

$$O_2 + R\cdot \rightarrow R-O-O\cdot \quad (7)$$

This reaction has no spin restriction and therefore can be very fast. The product of the reaction of O_2 with R· still has an unpaired electron and, therefore, maintains radical character. If the radical−radical coupling reaction occurs with a carbon-centered radical, the product is an alkylperoxyl radical (ROO·), which is a relatively strong one-electron oxidant ($\mathcal{E}^{\circ}$ for the ROO·,H^+/ROOH couple is around 1 V, pH 7.0 vs NHE).[16] In the presence of easily oxidized C−H bonds, such as those found in polyunsaturated fatty acids, the alkylperoxyl radical can react further leading to a chain reaction, where O_2 is consumed, and numerous oxidized products containing oxygen are generated. When this process occurs in lipids, it is referred to as a lipid peroxidation (Figure 2).

The bis-allylic C−H bond (the “easily oxidized” C−H shown in Figure 2) is relatively weak (approximately 76 kcal/mol).[17] Thus, initial abstraction of this hydrogen is facile as is the subsequent abstraction in the chain-carrying steps of the process (the BDE for ROO-H is approximately 87 kcal/mol).[18] The reduction potential for the bis-allylic radical has been calculated to be a relatively low 0.6 V (pH 7), also consistent with the propensity for this position to be oxidized.[16] It is important to note that this process represents a chain reaction,

meaning that a single initiating event (formation of the initial radical) can lead to the destruction of numerous lipid molecules. Also, the presence of polyunsaturated fatty acids greatly increases the susceptibility of membranes to this process since the bis-allylic motif is so easily oxidized. Finally, since O_2 is very lipid soluble and therefore will favorably partition into lipid components, membranes appear to be major sites of free radical damage. To be sure, the chemistry depicted in Figure 2 is greatly simplified as numerous products resulting from the autoxidation of lipids can be generated. In fact, some of the more prevalent products, such as 5-hydroxynonenal, appear to be important signaling molecules that are indicators of cellular oxidative stress.[19]

Singlet Oxygen ($^1O_2$)

As discussed above, dioxygen is a ground state triplet. There are two relatively low-lying excited singlet electronic states associated with $^3O_2$ designated as $^1\Sigma_g{}^+$ (representing electrons of opposite spins occupying each of the π^* orbitals, orbitals depicted in Figure 1) and $^1\Delta_g$ (paired electrons in a π^* orbital).[20,21] The $^1\Sigma_g{}^+$ is very short-lived, and it is generally considered that all reactions in biological solutions occur via the $^1\Delta_g$ species. $^1O_2$ ($^1\Delta_g$) is 22 kcal/mol higher in energy than $^3O_2$, and its lifetime in solution can range from 4−16,000 μs, depending on the solvent. Unlike $^3O_2$, reactions of $^1O_2$ do not have the spin restriction discussed above. Thus, $^1O_2$ can react spontaneously with a variety of biological nucleophiles, including molecules with unsaturations and thiols. Although $^1O_2$ has apparent signaling functions in plants[22] and has tremendous utility in medicine (e.g., as an effector species in photodynamic therapy),[23] it is currently not known to have signaling functions in mammalian systems (at least not to the extent of the other small molecule species discussed herein). Therefore, only this brief introduction to $^1O_2$ will be given.

Superoxide (O^{-}_2). One-electron reduction of O_2 generates O^{-}_2. By examining the thermodynamics of combining reaction 1 (in reverse) and reaction 2, it is clear that O^{-}_2 is unstable with respect to disproportionation (reaction 8). That is, two O^{-}_2 molecules can react, in the presence of protons, to give one molecule each of O_2 and H_2O_2.

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (8)$$

This second-order process, also referred to as a dismutation, occurs spontaneously with the fastest rate at a pH of 4.7. Since the pK_a of the conjugate acid of O_2^-, HOO·, is also 4.7, the reaction is fastest when one molecule of HOO· reacts with one molecule of O_2^- (since at pH 4.7, 50% of each is present). Importantly, this reaction is catalyzed by a class of enzymes called superoxide dismutases (SOD), which keep biological concentrations of O_2^- very low.[24]

O_2^- possesses a single unpaired electron and, therefore, is formally a radical species and capable of reacting as such. For example, O_2^- can react with other radicals such as NO leading to potentially reactive species (vide infra). The reduction potential for the O_2^-, $2H^+/H_2O_2$ couple (reaction 2) is 0.89 V (vs NHE, pH 7) indicating that O_2^- can be a decent one-electron oxidant if protons are present. Indeed, the oxidizing capabilities of O_2^- are highly proton-dependent as indicated by the changes in its reduction potential as a function

of pH (ε° = +1.44 V at pH 0, +0.89 V at pH 7, and +0.2 V at pH 14).[10] Consistent with the idea of protons being crucial to the oxidizing capability of O_2^-, the BDEs of the O−H bond of HOO-H and $^-$OO-H are 89 and 63 kcal/mol, respectively. Thus, H-atom abstraction by protonated O_2^- (reaction 9) is much more favorable than H-atom abstraction by O_2^- itself (reaction 10). Significantly, the BDE for a thiol (RS-H bond) is approximately 85−90 kcal/mol indicating HOO· will be capable of oxidizing thiols (although this is potentially a very complex process).[25]

$$HOO\cdot + \mathrm{R-H} \leftrightharpoons HOO-\mathrm{H} + \mathrm{R}\cdot \quad (9)$$

$$\mathrm{O_2}^- + \mathrm{R-H} \leftrightharpoons {}^-OO-\mathrm{H} + \mathrm{R}\cdot \quad (10)$$

It needs to be stressed, however, that the concentrations of HOO· at the physiological pH of 7 are apt to be very low since its pK_a is only 4.7, making the conjugate base, O_2^-, the predominant species present (although in acidic intracellular compartments, this chemistry can become relevant).

As indicated by the relatively unfavorable reduction potential for O_2 (reaction 1), O_2^- is also a good reductant capable of, for example, reducing redox metals (reaction 11).

$$\mathrm{O_2}^- + \mathrm{M}^{n+1} \leftrightharpoons \mathrm{O_2} + \mathrm{M}^{n} \quad (11)$$

The metal (M in reaction 11) is typically Fe^{3+} or Cu^{2+} in biological systems. O_2^- is also capable of reducing organic-type redox species such as quinones. It should be noted, however, that the reduction potentials for the same metal can vary considerably depending on the coordination or chemical environment and that quinones have widely varying reduction potentials depending on the structure and environment. Thus, O_2^- can be a one-electron reductant and in the presence of protons can be a reasonable one-electron oxidant.

Hydrogen Peroxide (H_2O_2)

Unlike O_2 and O_2^-, H_2O_2 has no unpaired electrons. Thus, it does not directly participate in radical processes of the type described above for O_2 and O_2^-. H_2O_2 is best characterized as a two-electron oxidant that reacts with nucleophilic reductants. For example, the reaction of H_2O_2 with nucleophilic thiols generates the corresponding sulfenic acid (which represents a two-electron oxidation of sulfur) (reaction 12).

$$RSH + \mathrm{H_2O_2} \rightarrow RSOH + \mathrm{H_2O} \quad (12)$$

In this reaction, H_2O_2 is an electrophile, reacting with a nucleophilic thiol resulting in a net 2-electron oxidation of the thiol. Since all the reactants are electronic singlets, these reactions are spin “allowed”, unlike the direct reactions of O_2 described above, and there are no radical intermediates. Although this reaction has been proposed extensively in the biological literature for explaining the effect of H_2O_2 on the activity of a variety of thiol proteins and peptides, it is worth noting that this reaction is generally very slow at

physiological pH in the absence of any catalytic assistance. For example, the rate constants for the noncatalyzed reaction of H_2O_2 with cysteine, glutathione, and even dithiothreitol under physiological conditions (pH 7.4, 37 °C) are all <5 M^{-1} s^{-1}.[25] The reaction of H_2O_2 with a thiol occurs primarily via the more nucleophilic ionized thiolate species, and the rate constant for this reaction is slightly greater (18−26 M^{-1} s^{-1}).[25] In a test tube, where there are no consumptive processes for H_2O_2 occurring, oxidation chemistry can be observed. However, in a biological system where numerous processes exist that degrade H_2O_2, the slow kinetics preclude many of these reactions from being relevant. Thus, oxidation by H_2O_2 should exhibit selectivity for thiols that are primarily ionized, although the low magnitude of these rate constants limits the physiological relevance of this reaction as a general process. There are, however, thiol proteins with significant rate constants for the reaction with H_2O_2 (>10^5 − 10^6 $M^{-1}s^{-1}$)[26] indicating that H_2O_2 can be a signaling molecule capable of selectively reacting with certain thiol proteins that possess catalytic entities that accelerate this reaction. It appears likely that significant increases in the rate constants for the reactions of biological nucleophiles with H_2O_2 will require acid assistance (Lewis or Lowry−Bronsted), which will enhance the electrophilicity of H_2O_2 and preclude the unfavorability of a hydroxide leaving group.

One-electron reduction of hydrogen peroxide occurs with a reduction potential of +0.38 V (H_2O_2,H^+ /H_2O,HO·, pH 7, vs NHE) (reaction 13). The products of this reduction are H_2O and HO· (the generation of extremely stable species H_2O helps drive the formation the highly reactive HO·). Thus, H_2O_2 is not a radical species but upon reduction generates a very oxidizing radical, HO·. The reduction of H_2O_2 in biological systems can occur via reaction with the reduced forms of several redox-active metals such as the ferrous ion (Fe^{2+}) or cuprous ion (Cu^{1+}) (reaction 13).

$$\mathrm{H_2O_2} + Fe^{2+}\left(Cu^{1+}\right) \rightarrow \mathrm{H_2O} + HO\cdot + Fe^{3+}\left(Cu^{2+}\right) \quad (13)$$

This reaction, referred to as the Fenton reaction, has been reported to be responsible for some of the toxicity associated with H_2O_2 since it generates a potent and indiscriminant oxidant, HO·. However, it should be realized that this form of H_2O_2toxicity is highly dependent on the presence/location of reactive forms of Fe^{2+} or Cu^{1+} ions, which can have widely variant reactivities in this regard.[27]

Hydroxyl Radical (HO·)

As discussed briefly above, HO· is a potent one-electron oxidant. This is easily seen from its reduction potential (2.31 V for the HO·,H^+/H_2O, pH 7, vs NHE) and the fact that the reduced species, H_2O, has an O−H bond dissociation energy of 119 kcal/mol. That is, one-electron reduction of HO· is highly favorable, and abstraction of a hydrogen atom by HO· generates a very strong bond. Indeed, there are very few biological molecules or functional groups that cannot be oxidized by HO·, making it a focal point in discussions of the deleterious aspects of O_2-derived species. As with all oxidizing radicals, HO· not only abstracts hydrogen atoms as a mechanism to gain an electron, but it can also add to

unsaturations, a reaction that also results in the generation of another radical (reaction 14).Because of its extreme reactivity,

(14)

HO· has a fleeting lifetime and therefore is unable to travel significant distances in a biological milieu since it quickly reacts with nearly the first molecule it encounters. The second order rate constants for the reaction of HO· with a variety of biologically relevant molecules are typically near the diffusion-controlled limit (>10^9 $M^{-1}s^{-1}$), indicating that very few collisions are required for a reaction to occur.[28] Thus, it will be expected that biological damage by HO· will be localized to its site of generation, which in many cases means the site where a catalytic metal is present (reaction 13). In fact, evidence suggests that under certain conditions “free” HO· is not generated in the Fenton reaction but that rather a metal-bound oxidant is made, and this is the ultimate oxidant.[29]

In biological systems, HO·, or the Fenton reaction product, can react with bicarbonate (HCO^-_3, a very abundant species in biological systems) to give the carbonate radical anion ($CO_3{\cdot}^-$) (reaction 15).

$$HO\cdot + HCO_3^- \rightarrow CO_{3\cdot}{}^- + H_2O \quad (15)$$

Unlike most reactions of HO· with biological molecules, which have near diffusion controlled rate constants, the rate constant for the reaction of HO· with HCO_3^- is only about 8.5×10^6 $M^{-1}s^{-1}$. The pK_a of $HCO_3\cdot$ is reported to be <0, indicating that the radical anion is the near exclusive species present at physiological pH.[30] Although less oxidizing than HO·, $CO_3{\cdot}^-$ is a strong one-electron oxidant as indicated by a reduction potential for the $CO_3{\cdot}^-, H^+/HCO_3^-$ couple of 1.78 V (vs NHE, pH 7).[31] The significance of biological $CO_3{\cdot}^-$ formation resulting from Fenton chemistry is that $CO_3{\cdot}^-$ can better diffuse from the site of HO· formation (or formation of the highly oxidizing metal oxidant) due to its lessened oxidative reactivity, possibly allowing oxidation chemistry to occur that is remote from the site of the initial HO· generation.

Coordination Chemistry of O_2

Dioxygen coordinates to a variety of metal centers in biological systems.[32,33] Examples of O_2-metal binding in biological systems include the binding and transport of O_2 using the ferrous heme moiety in hemoglobin, the biochemical reduction of O_2 that occurs during mitochondrial respiration via an O_2−metal complex in cytochrome *c* oxidase, the binding and reductive activation of O_2 via a ferrous heme prosthetic group of the metabolic enzyme cytochrome P450, and the reductive activation of O_2 using a nonheme ferrous ion of the O_2-sensing enzyme prolyl hydroxylase (vide infra). A variety of copper proteins also bind and/or activate O_2 similarly.[34] Thus, the binding to protein metal centers, especially iron and copper, represents a primary biological target/fate for O_2. For the reasons described below, O_2 binds to metals in lower oxidation states. For example, ferric (Fe^{3+}) and cupric (Cu^{2+})

proteins do not bind to O_2, whereas binding can occur with ferrous (Fe^{2+}) and cuprous (Cu^{1+}) proteins (providing there is an open coordination site, among other things). The chemistry of O_2 binding to metals is complex and typically involves at least two types of bonding interactions. Donation of a lone pair of electrons residing in an sp^{2-} hybridized orbital on O_2 into a metal d-orbital of appropriate symmetry generates a σ-bond. The spatial orientation of the lone pair sp^2 orbital predicts the bonding of O_2 to the metal to be end-on and bent (Figure 3). An antibonding π^* orbital on O_2 also has the proper symmetry to overlap with another d-orbital on the metal, leading to a bonding interaction made up of the donation of electrons from the metal to the partially filled π^* orbital on the O_2 ligand (Figure 3).

The donation of electrons from the metal into the π^* orbital of O_2 (often referred to as back-bonding) leads to a weakening of the O−O bond, and the complex is often viewed as an oxidized metal−superoxide complex (M^+-O^-_2). Depending on the metal and its coordination environment, the degree of back-donation from the metal to the ligand can vary considerably.[35] When O_2 is bound in an end-on/bent geometry (as is often the case and shown in Figure 3), there can also be a σ-type interaction between a π^* antibonding orbital (the one orthogonal to the π^* orbital depicted in Figure 3) and the d_z2 orbital on the metal. In cases where there is an unpaired electron in the d_z2 orbital, this electron can spin pair with an electron in the O_2 π^* orbital leading to a strong bonding interaction.[35]

It should be noted that O_2 can bind to metals in other ways besides that described above. For example, O_2 can bind “side-on” where both oxygen atoms ligate the metal and in cases where two coordinating metals exist in close proximity, the oxygen atoms of O_2 can bind both metal centers giving an −O-O- bridge between the metals. To be sure, these other binding modes are biologically relevant and important. However, for the purposes of illustrating how the coordination chemistry of O_2 can be compared and related to that of the other signaling species of interest, we limit our discussion to the type of coordination shown in Figure 3.

BIOLOGICAL CHEMISTRY OF NITRIC OXIDE AND RELATED SPECIES

Nitric Oxide. Unlike O_2, the Lewis structure/valence bond depictions of NO predict that it possesses an unpaired electron (Figure 4a). The existence of the unpaired electron is also confirmed by the molecular orbital diagram (Figure 4b). However, like O_2, the unpaired electron exists in a π^* orbital and is not strictly localized on the nitrogen atom (i.e., delocalized on both the N and O atoms) (Figure 4c,d). The geometries of the molecular orbitals of NO are qualitatively similar to those of O_2.

As indicated by its Lewis structure, NO does not obey the octet rule for main group elements. Thus, it may be asked why NO exists primarily as a monomer at room temperature and pressure when it can dimerize to give a species $(NO)_2$ that would appear to be more stable (or will at least satisfy the octet rule). Indeed, at low temperatures NO does dimerize, with the most stable structure being a cis-N-N bonded species. The N−N bond strength is extremely low (approximately 2 kcal/mol), only slightly greater than van der Waals forces.[36] The extremely weak (and long) N−N bond of the NO dimer is generally attributed to 1) only

partial σ N−N bond formation due to delocalization of the bonding electron over both the N and O atoms of the monomer (the π* electron)[37] and 2) repulsion between the adjacent nitrogen lone pairs in the dimer.[38]

Thus, NO is a radical without any significant tendency to dimerize under biological conditions, and this chemical property is one of the important factors in its biology. However, unlike HO· and similar to O_2, NO is not a good one-electron oxidant. In fact, the reduction potential for NO predicts that biological one-electron reduction of NO will be significantly more difficult than O (reaction 16).[39,40]

$$NO + e^- + H^+ \rightarrow HNO \quad E^{o'} = approx - 0.55\,V\,(vs \quad NHE, \quad pH7) \quad (16)$$

Consistent with the lack of one-electron oxidizing potential for NO, the H-NO bond dissociation energy is only 47 kcal/mol[41] indicating that NO will be very poor at abstracting H-atoms from biological substrates (reaction 17).

$$NO + R - H \rightarrow H - NO + R\cdot \quad (17)$$

Although NO is a poor one-electron oxidant (and therefore, will not spontaneously initiate radical chemistry), it will react with existing radicals such as O_2. NO readily reacts with O_2 in solution to give, initially, nitrogen dioxide (NO_2) (reaction 18).

$$2NO + O_2 \rightarrow 2NO_2 \quad (18)$$

Nitrogen dioxide also has an unpaired electron (and therefore a radical species) and is much more oxidizing than either precursor, NO or O_2 (vide supra). The fate of NO_2, when it is generated from the autoxidation of NO in pure water, is to react with another equivalent of NO (since both are radical species) to give dinitrogen trioxide, considered the anyhydride of nitrous acid, N_2O_3 (reaction 19).

$$NO_2 + NO \leftrightharpoons N_2O_3 \quad (19)$$

N_2O_3 then reacts with water to give two equivalents of nitrite (NO_2^-) (reaction 20).

$$N_2O_3 + H_2O \leftrightharpoons 2NO_2^- + 2H^+ \quad (20)$$

Thus, the ultimate fate of NO in a pure aerobic aqueous solution is the generation NO_2^-. It is worth noting that reactions 19 and 20 are readily reversible, indicating that an acidified and concentrated solution of NO_2^- can generate N_2O_3, NO, and NO_2. N_2O_3 can also react with other nucleophilies besides H_2O. For example, the reaction with thiols results in the formation of *S*-nitrosothiols (RSNO) (reaction 21).

$$N_2O_3 + RSH \rightarrow RS - NO + NO_2^- + H^+ \quad (21)$$

S-Nitrosothiols have been reported to be an important redox form of biological thiols and thiol proteins (vide infra). Considering their proposed regulatory function in thiol proteins, a thorough understanding of the chemistry of their formation and degradation is important to evaluate their relevance or likelihood as intermediates in signaling pathways. A discussion of this will be given later, once other reactions potentially responsible for their generation have been discussed.

A particularly important aspect of the autoxidation of NO is the fact that the rate is second order in NO ($-d[NO]/dt = 4k[NO]^2[O_2]$),[42] meaning that it will only be significant at high NO concentrations. Thus, the formation of NO_2, N_2O_3, and NO_2^- from the NO−O_2reaction will only occur when NO concentrations are very high and under aerobic conditions. In biological systems, however, NO and O_2 partition favorably into hydrophobic environments (i.e., lipid bilayers) resulting in much greater concentrations compared to the mostly aqueous compartments. This favorable partitioning into hydrophobic compartments greatly accelerates the NO/O_2 chemistry.[43]

NO will also react with other oxidizing radicals, such as those generated during lipid peroxidation (Figure 2). However, unlike O_2, the reaction of NO with a radical leads to a quenching of all radical character and a cessation of the radical chain chemistry characteristic of lipid peroxidation (reaction 22).

$$NO + \mathrm{R}\cdot \rightarrow \mathrm{R} - NO \quad (22)$$

The ability of NO to rapidly react with and quench oxidizing radicals indicates that it can be a good antioxidant.[44] Significantly, the rate constants for the reaction of NO with a variety of biologically relevant radical species are all near diffusion controlled: peroxyl (ROO·), $(1-3) \times 10^9$ $M^{-1}s^{-1}$;[45] thiyl (RS·), $(2-3) \times 10^9$ $M^{-1}s^{-1}$;[46] and tyrosyl (tyr·), $(1-2) \times 10^9$ $M^{-1}s^{-1}$.[47] Thus, one-electron oxidation chemistry occurring in the presence of NO dramatically alters the nature of the products formed, and NO generally antagonizes the radical chain chemistry.

One of the most studied of all radical reactions of NO is its reaction with O_2^-. As described above, O_2^- is a radical species, and reaction with NO also occurs with a near diffusion-controlled rate constant (6.7×10^9 $M^{-1}s^{-1}$)[48] (reaction 23).

$$NO + O_2^- \rightarrow ONOO^- \quad (23)$$

The product of reaction 23 is peroxynitrite ($ONOO^-$), and the chemistry of this species will be discussed in detail below.

As discussed throughout this review, numerous reports indicate that thiols/thiol proteins can be major targets for the biological actions of nitrogen oxides. Although several studies allude to a direct reaction between NO and thiols,[49,50] this appears to occur only under high concentrations of NO (nonphysiological) and at a very low rate (if at all). If the reaction is viewed as a nucleophilic attack of a thiol on an electrophilic NO, akin to the attack of a nucleophile on a carbonyl function, then the reaction would involve overlap between the

molecular orbital containing the nucleophilic lone pair of electrons and the π^* orbital of NO. Since the π^* orbital of NO has an electron (Figure 4b) (unlike the π^* orbital of a carbonyl function or, as discussed below, HNO, which is empty), the reaction should not occur in this way (Figure 5). Moreover, the radical product of nucleophilic addition to NO would be thermodynamically unstable. Finally, HNO has significantly greater negative charge on oxygen compared to NO, making the nitrogen atom of HNO a better electrophile. Thus, it is generally accepted that direct reactions of NO with thiols (of the type shown in Figure 5) are not physiologically relevant.

It is worth mentioning, however, that the NO dimer $(NO)_2$ is very electrophilic and has been proposed to be capable of reacting readily with nucleophiles (e.g., thiols or phosphines), leading directly to oxidized products.[51] However, as discussed above, the presence of even scant levels of NO dimers are unlikely under most biological conditions. Therefore, in the absence of any special conditions (e.g., proximal metal binding), NO dimer chemistry is likely to be inaccessible in biological systems.

Nitrogen Dioxide (NO_2). As discussed above, NO_2 can be generated during the autoxidation of NO (reaction 18), especially in hydrophobic compartments. Other possible mechanisms for NO_2 generation in biological systems exist and will be discussed later. NO_2 is a radical as shown by the valence bond depiction (Figure 6).

The reduction potential for the NO_2/NO_2^- couple is 1.04 V (vs NHE)[52] indicating that NO_2 is a reasonable one-electron oxidant. The H-ONO bond dissociation energy is calculated to be 76 kcal/mol,[53] which is consistent with the idea that NO_2 could abstract activated hydrogens (centers with a relatively weak bond to hydrogen). It should be noted that the H-NO_2 bond of hydrogen nitryl is calculated to be approximately 10 kcal/mol weaker than the H-ONO bond,[53] indicating that the O-atom of NO_2 is the hydrogen atom abstractor or oxidizing center. NO_2 has been reported to be capable of oxidizing a variety of biologically relevant functional groups. For example, phenols can be oxidized by NO_2 forming an intermediate phenoxyl radical which can further react with another NO_2 via a radical −radical combination and tautomerization reaction to generate a nitrated phenol[54] (Figure 7).

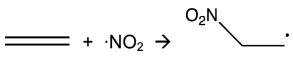
This reaction is pH dependent with increasing rates at higher pH ($k = 3.2 \times 10^6$ $M^{-1}s^{-1}$ at pH 7.5 and $k = 2 \times 10^7$ $M^{-1}s^{-1}$ at pH 11.3 for the reaction of NO_2 with tryrosine containing dipeptides)[54] indicating a faster reaction via the deprotonated phenoxide species. Thiols are also oxidized by NO_2 ($k = (2-5) \times 10^7$ $M^{-1}s^{-1}$ at pH 7.4), giving as an immediate product the thiyl radical (reaction 24).[55]

$$RSH + NO_2 \rightarrow RS \cdot NO_2^- + H^+ \quad (24)$$

Like phenol oxidation by NO_2, oxidation of thiols is also pH dependent indicating a faster reaction with the deprotonated thiolate species.

NO_2 is capable of oxidizing substrates via either H-atom abstraction[56] (reaction 25) or addition across unsaturations[57] (reaction 26). Either mechanism results in the generation of radical intermediates that can react further with other radical species.

$$\mathrm{R-H} + \cdot NO_2 \rightarrow \mathrm{R}\cdot + HONO \quad (25)$$



(26)

Most studies examining the addition of NO_2 to unsaturated bonds (reaction 26) report the addition reaction to occur via the nitrogen-centered radical species as opposed to the oxygen-centered radical (Figure 6). However, the addition of NO_2 to a nitrone spin trap has been reported to occur via the oxygen atom,[58] indicating the possibility of O-atom attack on unsaturated systems. Moreover, trapping of radicals generated via an initial NO_2 addition to an unsaturation (reaction 26) can occur via the O- or N-atom of NO_2,[59] indicating that the chemistry of either the nitrogen-centered radical species ($O_2N\cdot$) or the oxygen-centered radical (ONO·) can occur.

Dinitrogen Trioxide (N_2O_3). As described above (reaction 19), N_2O_3 can be generated from the reaction of NO_2 with NO in a facile radical−radical coupling reaction. Besides reaction with water to give NO_2^- (reaction 20), N_2O_3 can react with other nucleophiles (such as thiols or amines) leading to nitrosated (addition of "$^+$NO") products (reaction 27).

$$Nuc-\mathrm{H} + \mathrm{N_2O_3} \rightarrow Nuc-NO + {NO_2}^- + \mathrm{H}^+ \quad (27)$$

N_2O_3 can be viewed as the anhydride of nitrous acid (HONO),[60] and therefore, acidic solutions of NO_2^- can generate N_2O_3 via a simple equilibrium reaction (reverse of reaction 20). Therefore, at high concentrations of NO_2^- and under acidic conditions (note that the reverse of reaction 20 is second order in NO_2^- and is proton dependent) nitrosation reactions can occur via N_2O_3 formation.

Peroxynitrite ($ONOO^-$). Because of its possible biological relevance, $ONOO^-$ has been studied extensively.[61,62] The conjugate acid of $ONOO^-$, peroxynitrous acid (ONOOH), has a pK_a of 6.8 indicating a predominance of the anionic species at physiological pH. *cis*-Peroxynitrous acid will undergo rearrangement to give nitrate, possibly via homolytic cleavage of the O−O bond and reattachment of the radical pair (reaction 28).[63]

$$ONO-OH \rightarrow [HO\cdot + \cdot NO_2] \rightarrow {NO_3}^- + \mathrm{H}^+ \quad (28)$$

Although radical intermediates in the rearrangement of ONOOH to NO_3^- are generated, they are bound initially in a solvent cage that limits their reactivity with other species resulting in primarily reaction with each other giving the isomeric NO_3^- product. To be sure, the homolytic cleavage of the O−O bond for this rearrangement has been questioned,[64,65] and the oxidation chemistry associated with peroxynitrite (see below) proposed to occur through other species in the peroxynitrous acid-to-nitrate rearrangement.[61]

Peroxynitrite is capable of oxidizing a variety of substrates, a property that has led to hypotheses regarding its possible pathophysiological role in a variety of diseases and disorders. An important target for ONOOH/$ONOO^-$ has been proposed to be tyrosine residues. The reaction of peroxynitrite with tyrosine results in nitration, forming 3-nitrotyrosine. The mechanism for this process may proceed through an initial one-electron tyrosine oxidation (by the HO· formed in reaction 28), followed by trapping of the tyrosyl radical by NO_2 (similar to what is depicted in Figure 7).[66] Again, oxidation processes not involving HO· have been proposed as well (vide supra). Peroxynitrite also oxidizes thiols to the corresponding sulfenic acids (reaction 29) in a manner similar to that previously discussed for peroxide.

$$RSH + ONOOH \rightarrow RS-OH + NO_2^- + H^+ \quad (29)$$

As with tyrosine nitration, it is proposed that thiol oxidation can also occur via a radical pathway involving the thiyl radical.[67] Peroxynitrous acid can directly perform 2-electron oxidations (i.e., sulfide to sulfoxide) as well,[62] indicating that it can be a versatile oxidant capable of both one- and two-electron oxidations.

One of the most relevant reactions of peroxynitrite under physiological conditions is with carbon dioxide (CO_2), giving as an intermediate nitrosoperoxycarbonate ($ONOOCO_2^-$) (reaction 30).

$$ONOO^- + CO_2 \rightarrow ONOO-CO_2^- \quad (30)$$

Considering the moderately high rate constant for reaction 30 ($5.8 \times 10^4\ M^{-1}s^{-1}$) and the typically high levels of CO_2 in most tissues (>1 mM), this reaction is likely to be a primary fate of physiologically generated peroxynitrite.[68] Similar to the spontaneous decomposition of ONOOH, nitrosoperoxycarbonate also decomposes via homolytic O−O bond cleavage, generating the carbonate radical anion ($CO_3^{\cdot-}$, vide infra) and NO_2, which can recombine to give nitrocarbonate ($O_2NOCO^-_2$) (reaction 31). Hydrolysis of nitrocarbonate yields NO^-_3 and carbonate (CO_3^{2-}).

$$ONO-OCO_2^- \rightarrow \left[CO_3^{\cdot-} + \cdot NO_2\right] \rightarrow O_2NOCO_2^- \quad (31)$$

Since both $CO_3^{\cdot-}$ ($CO_3^{\cdot-}/HCO_3^-$, ε^0 = 1.78 V, vs NHE at pH 7)[31] and NO (NO/NO_2^-, ε^0 = 1.04 vs NHE) are both reasonable one-electron oxidants, the products of the reaction of CO_2 with $ONOO^-$ can still elicit oxidation chemistry.[69]

Nitrite (NO^-_2). As discussed above, NO_2^- is generated from the autoxidation of NO (reactions 18, 19, and 20). Because of the high order kinetics associated with the generation of NO_2^- from NO, this chemistry will only be physiologically relevant at high concentrations of NO.[70] Also mentioned above, NO_2^- can be a source of NO under acidic conditions and at high concentrations (due to the reversibility of reactions 20 and 19). However, more recent studies allude to other pathways for the generation of NO from NO_2^- via one-electron reduction pathways. The one electron reduction potential for NO_2^- is

highly proton dependent and, under appropriately acidic conditions, very favorable (HNO_2,H^+/NO, ε^0 = 0.98 V vs NHE)[71] yielding NO. It is reported that xanthine oxidase,[72] cytochrome *c*,[73] and hemoglobin[74,75] are capable of reducing NO_2^- to NO.

Coordination of NO_2^- to metal centers (e.g., iron hemes) can result in either an N-bound "nitro" complex or an O-bound "nitrito" complex (Figure 8).[76]

In most cases, the nitro complex is observed in both Fe(II) and Fe(III) heme proteins,[77] although the nitro complex appears to be only slightly favored over the nitrito complex.[78] However, NO_2^- binding to Fe(III)Mb gives the nitrito complex,[77] which is stabilized via a hydrogen bonding residue proximal to the bound NO_2^-.[76] Because of a great and recent interest in mammalian mechanisms of NO generation via NO_2^- reduction,[79] numerous recent studies have examined the reduction of heme-bound nitrite. Indeed, it is known that reduction of ferrousheme-bound NO_2^- can lead to the generation of NO. Significantly, computational examination of the O-bound nitrito complex of cytochrome *cd1* nitrite reductase indicates that a hydrogen bond from a protein residue (e.g., histidine) to the metal-bound oxygen allows facile NO generation with subsequent formation of a ferric hydroxo species.[78] Similarly, proton donation to an oxygen atom of the nitro complex can also result in decomposition with generation of a ferric-nitrosyl and water.[79–81] These processes are schematically depicted in Figure 9.

Consistent with the idea that proton donation to the bound nitrite is an important factor in the reduction to NO, mutation studies that eliminate the H-bonding histidine in myoglobin report a significantly decreased rate of NO_2^- reduction.[76] The physiological relevance of the NO_2^- reduction chemistry discussed above remains to be established. Moreover, other chemistries associated with the interaction of NO_2^- with hemoglobin (not discussed herein) may also be relevant to the generation of NO (or equivalent) from NO_2^-.[82]

Nitroxyl (HNO). All of the above-mentioned nitrogen oxides are oxidized with respect to NO. Recent reports allude to the possibility that reduced forms such as HNO can also be generated in mammalian systems,[83] although this has not been established. As with many nitrogen oxides, the biological chemistry of HNO likely involves reaction with thiols and metals. Indeed, HNO appears to be particularly thiolphilic.[84] It should be mentioned here that the reaction of a nucleophilic thiol with HNO is distinct from the reaction of a thiol with NO since HNO does not have a partially filled π^* orbital (Figure 4). The reaction of a thiol with HNO generates as an intermediate an *N*-hydroxysulfenamide (reaction 32, RSNHOH). This intermediate can then further react via two pathways, depending on the availability of other reactive thiols. In the presence of excess thiols (or vicinal thiols), further reaction occurs leading to the formation of the disulfide and hydroxylamine (NH_2OH) (reaction 33). Presumably, other strong nucleophiles can react similarly. In the absence of another thiol, the intermediate *N*-hydroxysulfenamide rearranges to generate a sulfinamide (reaction 34, RS(O)-NH).[85] Of particular significance with regard to HNO-mediated thiol modification is that HNO can oxidize the sulfur atom by 4 electrons in a single sequence when the sulfinamide is formed.

$$RSH + HNO \rightarrow RSNHOH \quad (32)$$

$$RSNHOH + \mathrm{R}'SH \rightarrow RSSR' + NH_2OH \quad (33)$$

$$RSNHOH \rightarrow \mathrm{R} - \mathrm{S}(\mathrm{O})NH_2 \quad (34)$$

HNO also reacts readily with metalloproteins. For example, a stable ferrous-HNO complex (N-bound) with myoglobin has been generated and characterized[86] (reaction 35), and although not unequivocally demonstrated, others have alluded to a similar reaction with the ferrous heme of the enzyme guanylate cyclase, leading to activation of the enzyme.[87] Also, the reaction of HNO with ferric heme proteins can result in the generation of the corresponding ferrous-NO complex via reaction 36.

$$Protein - Fe^{2+} + HNO \rightarrow Protein - Fe^{2+} - \mathrm{N}(\mathrm{H})\mathrm{O} \quad (35)$$

$$Protein - Fe^{3+} + HNO \rightarrow Protein - Fe^{2+} - NO + \mathrm{H}^+ \quad (36)$$

Unlike most other commonly studied nitrogen oxides, HNO cannot be stored due to a self-dimerization reaction, ultimately leading to the formation of nitrous oxide (N_2O) and H_2O (reaction 37).

$$HNO + HNO \rightarrow HON{=}NOH \rightarrow \mathrm{N_2O} + \mathrm{H_2O} \quad (37)$$

Thus, HNO is typically examined using donor compounds,[88] especially in biological systems. The HNO dimerization reaction precludes the generation of highly concentrated solutions of HNO. However, due to many other possible reactions with biological species (thiols), this reaction is likely to be scarce in biological systems due to the second order kinetics.

Although HNO reacts with O_2, it is generally thought that the relatively slow kinetics precludes this reaction from being biologically relevant.[89] Moreover, HNO does not hydrate appreciably (unlike, for example, formaldehyde) indicating that the biological activity of HNO is not likely due to the hydrated species.[84] Finally, HNO can be converted to NO via simple one electron oxidation, a process that can be performed by, for example, the cupric form of CuZn SOD[90] (reaction 38).

$$HNO + Cu^{2+} - SOD \rightarrow NO + Cu^{1+} + \mathrm{H}^+ \quad (38)$$

Coordination Chemistry of NO

Although NO can form complexes with many metals,[91] herein is discussed only its interactions with ironheme proteins since these appear to be a major target for NO in biological systems. Nitric oxide is an “amphoteric” ligand (properties of an acid and a base) as it can bind to a metal as a nitrosonium cation (NO^+) or a nitroxyl anion (NO^-). Since NO^+ is isoelectronic with CO, the binding geometry of NO^+ is linear (i.e., a linear geometry

of the metal− nitrogen−oxygen atoms; an explanation for this is given below in the discussion of CO coordination chemistry). NO^-, however, is isoelectronic with O_2 and therefore binds in a bent geometry (akin to that shown above for O_2; Figure 2). When NO binds to ferrous (Fe^{2+}) heme proteins, it binds in a bent fashion, like O_2, indicating an Fe^{3+}-NO^--like complex. However, when NO binds ferric (Fe^{3+}) proteins, it binds in a linear fashion, indicating that the Fe^{3+}−NO interaction is more appropriately represented by Fe^{2+}–(NO^+).[92] Importantly, ferric-NO complexes can undergo reductive nitrosylation leading to the formation of an oxidized nitrogen oxide (when water reacts) and a ferrous-NO complex (reactions 39, 40).

$$\left[Fe^{3+}-NO \leftrightarrow Fe^{2+}-NO^{+}\right]+\mathrm{H_2O} \rightarrow Fe^{2+}+NO_2{}^{-}+\mathrm{H}^{+} \quad (39)$$

$$Fe^{2+}+NO \rightarrow Fe^{2+}-NO \quad (40)$$

One of the most physiologically important aspects of NO coordination chemistry is its preference to form 5-coordinate complexes with ferrous heme proteins. Unlike other small molecule ligands (i.e., O_2 and CO), the coordination of NO to a ferrous heme labilizes the proximal ligand, leading to the release of the ligand and generation of a 5-coordinate, square pyramidal nitrosyl complex (Figure 10).[93]

This aspect of NO coordination chemistry is likely the reason it is such a specific activator of the ferrous heme enzyme guanylate cyclase. That is, with other ligands (CO for example) the 6-coordinate, octahedral complex is stable, and the proximal ligand is not labile. Therefore, if the release of the proximal ligand is important to protein function, as in guanylate cyclase, then activation will be fairly specific to NO.

The ability for NO to weaken the proximal ligand bond in ferrous-heme complexes (often referred to as the “trans-effect”) has been attributed to electron donation from an energetically matched and the partially filled π^* orbital of ligated NO to the $d_z{}^2$ orbital on the metal. That is, when NO binds with a “bent” geometry, the two π^* orbitals are no longer degenerate. One of the π^* orbitals remains available for back-bonding with the d_{yz} or d_{xy} orbitals on the metal, while the other is now able to form a σ-bond with the d_z2 orbital on the metal.[94] If the metal complex possesses a ligand that is proximal or trans to the NO ligand, the d_z2 is already filled with electrons from this ligand. Thus, the electron from the NO π^* orbital can only occupy the σ^* orbital associated with the σ-bond of the trans ligand. Populating this antibonding orbital of the metal−proximal ligand interaction leads to a weakening of this bond to the metal resulting in the loss of the ligand and a preference for a 5-coordinate complex[95–97] (Figure 11).

Significantly, a trans effect is weak or nonexistent with CO and O_2 due to mismatched energies of the relevant orbitals on the metal and ligand as well as other overriding effects.

S-Nitrosothiols (RS-NO). As mentioned above, S-nitrosothiol formation has been proposed to be an important event in the regulation/function of a variety of thiol proteins.[98] Therefore, it is reasonable to devote a modicum of space discussing the possible mechanisms of their

formation and degradation (or at least review and collate the chemistry already discussed). However, prior to embarking on this chemical discussion, it is worthwhile to first address the often-confusing nomenclature and terminology associated with *S*-nitrosothiols, their formation, and related species. Currently, several terms are used almost interchangeably, some of which allude to specific chemical processes, and some of which are general descriptors of structure or bonding. For example, the term “nitrosation” (i.e., *S*-nitrosation) in the strictest chemical sense refers to a process by which nitrosonium ion (NO^+) (or reactive equivalent) reacts with a nucleophile (e.g., a thiol) (reactions 21 and 27). Thus, when the term S-nitrosation is used, it is implied that an electrophilic nitrogen species with nitrosonium ion character (discussed below) has reacted with a thiol to form an S-nitrosothiol. Another term often used is “*S*-nitrosylation”. This merely implies that a bond between a sulfur atom and the nitrogen atom associated with NO has been formed and does not imply a mechanism of bond formation. Inorganic and organometallic chemists originally used “nitrosyl” to describe metal nitrosyls which are coordination complexes where the nitrogen atom of NO is bound to a metal center (M-NO, M = metal). In many cases, metal “nitrosylation” refers to processes where a direct reaction of NO with a metal center generates a metal nitrosyl, although, for the most part, the term does not imply a chemical mechanism by which this complex forms or any other chemistry associated with the complex. That is, metal nitrosyls can be made via a variety of distinct processes and can have metal-NO^+ character or metal-NO^- character;[99] the term “nitrosyl” does not distinguish between any of these. The term “nitroso” is typically used to describe a functional group whereby a bond between the nitrogen of NO and, most often, main group elements is present (e.g., C-nitroso for C-NO, S-nitroso for *S*-NO, and N-nitroso for N-NO). However, there is no strict adherence of the use of the term “nitroso” to describe the bonding in main group elements. For example, nitrosyl chloride (NOCl) and nitrosyl tetrafluoroborate ($NOBF_4$ and also referred to as nitrosonium tetrafluoroborate) are often used.

It is important to remember that NO will not react directly with thiols under biological conditions (vide supra). If a direct reaction between a thiol and NO did occur (hypothetically), the generation of an *S*-nitrosothiol would require the loss of an electron (i.e., $RSH + NO \rightarrow RSNO + e^- + H^+$), a process that will not happen without an electron acceptor present. Thus, S-nitrosothiol formation can readily occur if either the thiol or NO is first oxidized. Described above are several processes where this is the case. For example, species such as N_2O_3 (see reactions 21 and 27) or Fe^{2+}-NO^+ (see reaction 39) all represent nitrogen oxide species whereby the NO moiety is electron poor (and can be viewed as partially oxidized) and, therefore, capable of nitrosating a thiol. However, a one-electron oxidized thiol species, a thiyl radical (RS·), will directly react with NO to give an S-nitrosothiol (akin to reaction 22). Thus, NO can be oxidized by O_2 to generate a thiol nitrosating species, N_2O_3 (reactions 18 and 19−21), NO can coordinate an oxidizing metal, such as Fe^{3+}, to give it nitrosonium-like character (Fe^{2+}-NO^+, which can nitrosate a thiol (reaction 41) or a thiol can be oxidized to a thiyl radical (for example, by NO_2, reaction 24, or other oxidant) which can directly react with NO to give an *S*-nitrosothiol). Although one can envision other possible mechanisms for S-nitrosothiol formation involving other

nitrogen oxides and/or thiol redox states, those mentioned immediately above would appear to be the ones to consider first.

$$\left[Fe^{3+}-NO \leftrightarrow Fe^{2+}-NO^{+}\right]+RSH \rightarrow Fe^{2+}+RSNO+\mathrm{H}^{+} \quad (41)$$

If indeed *S*-nitrosothiol formation is an important biological regulatory pathway, the possible mechanisms of its degradation are equally important as the mechanisms of formation. There are several established chemical mechanisms by which an *S*-nitrosothiol can be degraded. One way is via a reaction with another thiol. This reaction can follow two pathways that result in two distinct products: one pathway involves an attack of the nucleophilic thiol at the sulfur atom of the RS-NO reactant and generates a disulfide and HNO (reaction 42), and the other pathway involves an attack of the nucleophilic thiol at the nitrogen atom of the RS-NO species and results in the simple transfer the NO function (or NO^{+}) from one thiol to the other (referred to as transnitrosation) (reaction 43).[85]

$$RS-NO+\mathrm{R}'SH \rightarrow RS-SR'+HNO \quad (42)$$

$$RS-NO+\mathrm{R}'SH \rightarrow RSH+\mathrm{R}'SNO \quad (43)$$

Although both reactions are well documented, the factors that govern the site of nucelophilic attack are not, as yet, known. Although reaction 43 does not lead to the overall destruction of an *S*-nitrosothiol per se, it does lead to the loss of this functionality in the original RS-NO species. It would not be surprising to find that other potent nucleophiles react similarly with *S*-nitrosothiols.

S-Nitrosothiols can also be degraded reductively. For example, cuprous ion (Cu^{1+}) can reduce an S-nitrosothiol to give a thiolate and NO (reaction 44).[100] This system is potentially catalytic since the thiolate product is capable of reducing the cupric ion (Cu^{2+}) back to cuprous ion.

$$RS-NO+Cu^{1+} \rightarrow RS^{-}+Cu^{2+}+NO \quad (44)$$

Using cyclic voltammetry, the single reduction peak potential for a series of S-nitrosothiols has been determined to be −0.8 to −1.1 V (vs Ag/AgCl).[101] These fairly negative values would indicate that RS-NO species are not easy to reduce under biological conditions.

S-Nitrosothiols can also be degraded photochemically (reaction 45).[102] Although the biological relevance of this process is questionable, this is clearly an important issue when using *S*-nitrosothiols in research. Significantly, photochemical NO release from RSNO occurs in both the UV and visible range, indicating that special care must be taken when using these compounds in the presence of light.[103]

$$RS-NO+h\nu \rightarrow RS\cdot+NO \quad (45)$$

BIOLOGICAL CHEMISTRY OF CARBON MONOXIDE

Compared to NO and O_2, the biological chemistry of CO is relatively simple. Most studies of CO biology (at least in mammalian systems) do not consider the possibility of redox processes. However, it is worth mentioning that in some bacteria CO can be used as a source of carbon and electrons. For example, the bacterial enzyme CO-dehydrogenase is capable of oxidizing CO in a reaction equivalent to the water-gas-shift reaction (reaction 46).[104]

$$CO + H_2O \leftrightharpoons CO_2 + H_2 \left(2H^+ + 2e^-\right) \quad (46)$$

Electrons derived from this process can be fed into a respiratory pathway, making this a potentially very important bacterial process. The reduction potential for the CO_2/CO couple ($\varepsilon^{o\prime} = -0.558$ V)[105] indicates that the oxidation of CO is very favorable, and therefore, it is not surprising that CO can be used as an electron source. Indeed, several studies have reported that CO/H_2O can serve as a source of electrons for the reduction of mammalian heme proteins via this chemistry.[106,107] However, the rate of reduction of, for example, cytochrome *c* oxidase, hemoglobin, or myoglobin is extremely slow (even under an atmosphere of CO) indicating that this chemistry may not be physiologically relevant in mammals.

Clearly, the most important biological aspect of CO chemistry is its ability to bind to metals. Although CO can form many types of coordination compounds, herein the focus will be on hemeprotein−CO interactions due to the known biological relevance. In general, the propensity of CO to form coordination complexes can be explained by its ability to form two types of bonds with metals (akin to O_2). Donation of a lone pair of electrons in an sp orbital on the carbon atom of CO to an empty d-orbital on the metal (typically the d_z2 orbital for heme proteins) generates a σ-bond between CO and the metal. Further bonding occurs when electrons in filled metal d-orbitals are donated "back" to the π-antibonding orbitals of CO. This bonding is analogous to that described previously for O_2 and is depicted in Figure 12.

Since the σ-bonding interaction involves an electron pair in an sp hybridized orbital on CO, the preferred geometry for CO binding to a metal is linear. As with O_2, the π-bonding (back-bonding) component of the metal−CO interaction is extremely important to the overall binding energetics. Indeed, CO and O_2 will only bind ferrous and not ferric heme proteins since the Fe^{2+} oxidation state is more electron rich and, therefore, better able to donate electrons "back" to CO. Generally speaking, the metal bonding schemes for NO, CO, and O_2 are similar in that there are both σ and π components. The degree of back-bonding differs, however. In the case of O_2, the back-bonding is so significant that an Fe^{2+}-O_2^- complex is more accurately depicted as an Fe^{3+}-O_2^- complex. This is not the case with CO, which has much less charge transfer from the metal. The degree of back-bonding for CO, NO, and O_2 is correlated to the relative energies of the p-orbitals, indicating that for X=O, the degree of charge transfer to the ligand is X = O > N > C.[108] The factors that allow heme protein discrimination between these ligands will be discussed later.

From a purely physiological perspective, the biological activity and function of endogenously generated CO is likely due to its ability to complex metal centers in mammalian systems. It is known that CO will react with, for example, HO·,[109] but this seems to be an unlikely fate/function for CO in light of the fact that HO· is such an indiscriminant oxidant and will react with almost all molecules in a cell. Thus, the biological targets for CO are likely to be metal centers (e.g., heme proteins), which may also bind O_2 and NO. Importantly, the biological activity of CO is not necessarily the result of direct actions associated with CO binding to a metal center but, rather, the ability of CO to block the actions of O_2 or NO binding. Indeed, the toxicity of CO is primarily the result of interference with O_2 transport via CO binding to hemoglobin. A comparison of the binding of these species to metal centers and the ability for proteins to discriminate between these species will be addressed below.

An important aspect of CO chemistry that sets it apart from the other signaling species (NO, O_2, and H_2S), is that it is resistant to conversion to other chemical species. As discussed above, O_2 and NO can be easily converted to numerous other species via myriad chemical/ biochemical processes, and as will be discussed below, H_2S can be converted to a variety of oxidized species as well. In mammalian systems, CO is relatively inert and appears to only serve as a metal ligand for some metalloproteins. This aspect of CO may be an important factor in its biological utility. That is, CO is resistant to degradation and can “survive” conditions that would otherwise lead to the destruction of NO, O_2, or H_2S. Thus, the signaling of CO should be more robust than, for example, NO since it is less dependent on cellular conditions and the presence of oxidizing/ reducing species. Whether this aspect of CO signaling is important remains to be determined.

BIOLOGICAL CHEMISTRY OF HYDROGEN SULFIDE AND RELATED SPECIES

The most recently proposed endogenously generated small molecule signaling agent is H_2S. Because of the paucity of current literature addressing the biologically/physiologically relevant chemistry of H_2S, a slightly more detailed discussion of this species will be given, along with brief introductions to some of its reported biochemistry. Hydrogen sulfide differs somewhat from O_2, NO, and CO in that it can be ionized in the physiological pH range. The pK_a of H_2S is 6.8, and the pK_a of HS^- is 14.1. Thus, at physiological pH, HS^- is the predominant species with vanishingly small amounts of S^{2-} present. It is important to note that the pK_a for H_2S is significantly lower than most alkylthiol species (RSH), which typically have values 1−2 pK_a units higher. Thus, there will be a greater proportion of HS^- in solution compared to RS^- under most conditions. It should be realized, however, that cysteine thiols at some protein active sites can have extremely low pK_a values (much lower than H_2S) due to protein interactions that stabilize the thiolate anion.[110] The reduction potential for the $HS\cdot/HS^-$ and $\cdot S^-,H^+/ HS^-$ couples are estimated to be approximately 0.9−1.1 V (vs NHE) similar to alkyl thiyl radicals.[52,111] This indicates that the one-electron oxidized species are biologically accessible (like thiyl radicals of cysteine peptides and proteins)[14] and are reasonable oxidants. The bond dissociation energy of the S−H bond in

H_2S is 90 kcal/mol, a value also consistent with the oxidizing potential of HS·. Anionic HS^- is also a very nucleophilic species, on par with trialkyl phosphines and alkyl thiolates.[112]

The nucleophilicity of H_2S predicts that it will readily participate in polysulfide−thiol exchange chemistry of the type shown in reaction 47.[113,114]

$$HS^- + \mathrm{R-S-S-R'} \rightarrow RSS^- + \mathrm{R'}SH \quad \text{or} \quad \mathrm{R'}SS^- + RSH \quad (47)$$

Reaction 47 depicts the reaction of HS^- with a disulfide electrophile. It is easy to envision an analogous reaction with higher polysulfides (i.e., RSSSR′) as well. A product of reaction 47 is a hydropersulfide/persulfide anion (RSSH/ RSS^-). Hydropersulfides are important biological species known primarily for their ability to donate sulfane sulfur (the terminal sulfur atom) to a variety of biochemical cofactors and prosthetic groups.[115] However, it appears likely that the biological chemistry of hydropersulfides has the potential to go beyond its ability to serve as a donor of sulfane sulfur. Indeed, literature precedence seems to indicate that the hydropersulfide oxidation state in proteins can have numerous activities (vide infra) including enhanced activity.[116,117]

As with the other small molecule signaling agents (O_2, NO, and CO), another hallmark of H_2S (or HS^-/S^{2-}) biological chemistry is metal binding. Indeed, the toxicity associated with high level H_2S exposure is thought to be due primarily to its ability to bind to cytochrome *c* oxidase, leading to the inhibition of respiration. However, unlike O_2 and CO (and for the most part NO), which bind to metals in lower oxidation states (e.g., Fe^{2+} rather than Fe^{3+}), H_2S binds metals as an anion, HS^-, and tends to bind to oxidized forms of metals (e.g., Fe^{3+} rather than Fe^{2+}). Particularly noteworthy with regards to S^{2-} metal ligation is the existence and importance of iron−sulfur (FeS) clusters in biology as electron transfer prosthetic groups and Lewis acids in enzymes.[118] Although it is likely that a portion of the biological activity of H_2S can be a result of interactions with metal-containing systems, there has yet to be identified any metallo-protein that serves as a target for its reported physiological actions (although cytochrome *c* oxidase is a potential target and has been exploited pharmacologically to induce an H_2S-mediated hibernative state).[119]

As indicated above, an eventual fate of the reaction of H_2S with oxidized thiol species can be the generation of a hydropersulfide (for example, reaction 47).[120] Thus, it is not hard to imagine that hydropersulfide generation and chemistry can be involved in the mechanism(s) of H_2S-mediated biological activity. Indeed, several reports in the literature suggest that hydropersulfides can be important mediators of enzyme function. For example, several studies from Massey's group revealed an important hydropersulfide at the active site of xanthine oxidase.[116,117] Cyanolysis of the hydropersulfide, giving the thiolate and thiocyanate (reaction 48), results in loss of enzyme activity, indicating a crucial role for the hydropersulfide in catalysis.

$$Enzyme - SS^- + {}^-CN \rightarrow Enzyme - \mathrm{S}^- + {}^-SCN \quad (48)$$

Similarly, the enzyme aldehyde oxidase was found to contain an active site hydropersulfide that was also crucial for enzyme activity.[121] When the mitochondrial enzyme malate dehydrogenase is reacted with thiosulfate and rhodanese (a system that will convert thiols to persulfides, reaction 49), a significant increase in activity was found.[122]

$$Enzyme{-}\mathrm{S}^{-}+Na_2\mathrm{S}_2\mathrm{O}_3 \rightarrow Enzyme{-}SS^{-}+Na_2SO_3 \quad (\text{catalyzed by rhodanese}) \quad (49)$$

An enzyme involved in heme biosynthesis, aminolaevulinate synthetase was also reported to be greatly activated in the presence of polysulfides (i.e., RSSSR and larger), possibly via eventual hydropersulfide formation at a protein thiol residue.[123] More recently, Kim and co-workers have reported the presence of an important hydropersulfide forming cysteine residue in a Ni carbon monoxide dehydrogenase.[124] The authors propose that this hydropersulfide may be important to the stability and generation of the active site Fe−Ni cluster and, possibly, as a redox species important for catalysis. Hydropersulfide modification of a solvent-exposed cysteine residue in the antioxidant enzyme CuZn SOD has been found and reported to have altered biophysical properties.[125] A recent and provocative study indicates that persulfide generation in glyceraldehyde-3-phosphate dehydrogenase (GAPDH) leads to an increase in activity.[126] In this study, persulfide formation was proposed to occur on the active site cysteine, indicating an increased biochemical reactivity associated with the persulfide oxidation state. However, this study did not elaborate on the mechanism or origin of the activation. Discussed later, an increase in chemical reactivity of a persulfide compared to the corresponding thiol may be predicted. To be sure, protein hydropersulfide formation has been reported to lead to enzyme inhibition as well.[127]

At this point, it seems likely that hydropersulfides are a part of the thiol redox reaction manifold that includes the more well studied and known thiols (RSH), thiyls (RS·), disulfides (RSSR), sulfenates (RSO^-), sulfinates ($RS(O)O^-$), sulfonates ($RS(O)_2O^-$), and nitrosothiols (RSNO). It should be noted that there is a strong possibility that, along with the examples listed above, many other protein hydropersulfides exist. The reason for this statement is that a likely mechanism of endogenous protein persulfide formation involves an H_2S reaction with oxidized cysteine species and that this post-translational modification has not been examined thoroughly in in-vitro preparations. This is especially true since purification of thiol proteins is typically performed under reducing conditions that will reduce the oxidized species including hydro-persulfides, precluding their isolation from natural sources. Hydropersulfides are also inherently unstable and will, among other decomposition pathways, disproportionate to sulfides (thiols) and elemental sulfur (S^0)[128] (reaction 50) making their long-term storage difficult. Thus, it is possible that hydro-persulfides are common, an inherent member of the thiol redox family, and serve specific functions as regulators of thiol protein activity.

$$\mathrm{R}-SSH \rightarrow RSH+\mathrm{S}^0 \quad (50)$$

The chemistry of hydropersulfides (especially biologically relevant hydropersulfides) is relatively unknown. Although they have been observed in numerous enzymes (vide supra)

and found to elicit both increases and decreases in activity, the chemistry associated with these effects is not established. In comparing the fundamental chemistry of a thiol to the corresponding hydropersulfide, it is known that hydro-persulfides are significanltly more acidic.[129] For example, the pK_a1 for HSSH is only 5 compared to a pK_a1 of 6.8 for H_2S. This trend holds true for alkyl hydropersulfides versus alkyl thiols as well.[130] That is, the pK_as of alkyl hydropersulfides (RSSH) are approximately 1−2 pK_a units lower than the corresponding alkyl thiols (RSH). Thus, the anionic form of a persulfide will be much more prevalent than the anionic form of the corresponding thiol. Persulfides are also better reductants than thiols, as evidenced by a significantly lower S−H bond dissociation energy (BDE) (RSS-H BDE = 70 kcal/mol, RS-H BDE = 92 kcal/mol).[131] Accordingly, persulfides should more readily donate a hydrogen atom to one-electron oxidants (reaction 51) compared to the analogous reaction with thiols.

$$RSSH + \mathrm{R}^{'}\cdot \rightarrow RSS\cdot + \mathrm{R}^{'} - \mathrm{H} \quad (51)$$

Moreover, it is likely that the persulfide anion is a better one-electron reductant compared to the corresponding thiolate as well (reaction 52) (although a reduction potential for RSS· is not reported).

$$RSS^{-} \rightarrow RSS\cdot + \mathrm{e}^{-} \quad (52)$$

Analogous to the relationship between ammonia (NH_3) and substituted derivatives hydroxylamine and hydrazine (NH_2OH and NH_2NH_2), it is expected that hydropersulfides are also better nucleophiles than the corresponding thiols. That is, putting a lone pair of electrons adjacent to the nucleophilic pair of electrons greatly increases their nucleophilic reactivity. The effect (often referred to as the “alpha-effect”) has been proposed to be due to a number of factors including ground state destabilization via lone-pair repulsion, transition state stabilization by the adjacent lone pair, and reduced solvation (and therefore destabilization) of species with adjacent lone pairs.[132]

Thus, compared to thiols, hydropersulfides possess increased acidity (indicating an increased concentration of the anionic species), greater inherent nucleophilicity (alpha-effect), and greater one-electron reducing capabilities (via H-atom donation, RSSH → RSS· + H·, or one-electron donation, RSS^- → RSS· + e^-) and are likely to be a better metal ligand (related to its increased nucleophilicity). In biological systems, thiols are known to be strong nucleophiles, metal ligands, and reducing agents. Indeed, much of the biological utility of thiols relies on these chemical properties. It is not difficult, therefore, to imagine that hydropersulfides can be hyperactivated congeners of thiols since all of the important chemical properties of thiols are seemingly enhanced in hydropersulfides.[120] Figure 13 schematically depicts the types of enhanced chemistry possibly associated with persulfide generation from thiols.

Whether the proposed enhanced reactivities of persulfides, compared to thiols, depicted in Figure 13 are relevant to the biological activity of H_2S remains to be determined.

INTEGRATED BIOLOGICAL CHEMISTRY OF O_2, NO, CO, H_2S, AND THEIR DERIVATIVES

The discussions above focused on the biological chemistry associated with, for the most part, the individual chemical species O_2, NO, CO, H_2S, and their derived species (although numerous examples of chemical interactions between these molecules have already been mentioned). As stated earlier, the tenet of this review is that all of these small molecule signaling agents have integrated physiology based on their integrated chemistry. That is, these species (or derivatives thereof) have the ability to (1) react with each other, leading to the cessation of activity of one (or both) of the individual species, (2) react with each other to (re)activate an inhibited state, (3) react with each other to generate another species with different activity, (4) compete with each other at specific biological targets leading to regulation of that target, (5) enhance or inhibit each other's actions via chemical modification at diverse sites on the same proteins or signaling system, or (6) possess similar activity but have distinct lifetimes/stabilities under certain biological states/conditions. Although other possibilities exist for meaningful interactions between these species, the focus herein will be on these.

Interactions of O_2 and Derived Species with NO, CO, and H_2S. The only small molecule species that has the potential to chemically interact with all molecules discussed herein is O_2 (and derived species). As mentioned above, O_2 reacts with NO to give a series of reactive nitrogen oxides such as NO_2, N_2O_3, and NO_2^-. The kinetics of the generation of these products indicate that this chemistry will only occur to a significant extent at elevated concentrations of NO (vide supra). The reaction of NO with O_2 leads to the formation of species with entirely different reactivity compared to the starting molecules. Neither NO or O_2 are good one-electron oxidants, whereas NO_2 is a reasonable one-electron oxidant. O_2 and NO are also not themselves reactive with nucleophiles (e.g., thiols), whereas N_2O_3 is very electrophilic and capable of modifying thiol nucleophiles (reaction 27).

O_2 will also react with H_2S. This chemistry is very complex and leads to the generation of oxidized sulfur species, including sulfite (SO_3^{2-}), thiosulfate ($S_2O_3^-$, sulfate (SO_4^{2-}), and elemental sulfur (S^0), depending on the concentrations of the reactants, ionic strength, and pH.[133,134] Many of the highly oxygenated products are a result of numerous parallel reactions occurring after an initial step that is first order in the anion HS^- and first order in O_2. This first step is thought to be a one-electron oxidation by O_2 of the anion to give, initially, a sulfhydryl radical (HS·) and superoxide (reaction 53).[133] Although the pK_a of HS· has not been accurately determined, it is generally thought to be fairly acidic (possibly as low as 3−4)[111] and therefore exists primarily as the radical anion ($S^{\cdot-}$) at pH 7.

$$HS^- + O_2 \rightarrow HS\cdot\left(S^{\cdot-}\right) + O_2 \quad (53)$$

A series of subsequent reactions can then occur, leading to the many oxidized products. For example, it is reported that HS·($S^{\cdot-}$) can react further with O_2 to give the sulfur dioxide radical anion ($SO_2^{\cdot-}$ in a very fast reaction ($k = (4-5) \times 10^9$ $M^{-1}s^{-1}$) (reaction 54).[111]

$$HS\cdot(\mathrm{S}\cdot^{-})+\mathrm{O_2}\rightarrow SO_2{}^{\cdot-}+\left(\mathrm{H}^{+}\right) \quad (54)$$

The sulfur dioxide radical anion, also known as the dithionite radical, is a good reductant (ε^0 = −0.31 V at pH 2 and above) and can react quickly with O_2 to give SO_2 and O_2^- (k = 1.5 × 10^9 $M^{-1}s^{-1}$ at pH 6.8).[135] Thus, HS·/S·⁻, which is a reasonable oxidant (vide infra), can react with O_2 to generate a good reductant.

S·⁻ can also react with HS^- to give HSSH·⁻/$HSS^{\cdot 2-}$ with a rate constant of 4 × 10^9 $M^{-1}s^{-1}$ at pH 7[111] and an association constant of 2.5 × 10^4 M^{-1} [136] (reaction 55) and it is likely that the reaction of S·⁻ with alkyl thiolates leads to similar products. HSSH·⁻ is acidic since it has been reported to exist primarily as the deprotonated dianion ($HSS^{\cdot 2-}$) at neutral pH. This species is also a good reductant capable of rapidly reacting with O_2 to give O_2^- with a rate constant of 4 × 10^8 $M^{-1}s^{-1}$ at pH 7[111] (reaction 56). Thus, similar to the situation described above, the reaction of the reasonable oxidant HS· with HS^- results in the generation of a reductant.

$$HS\cdot+HS^{-}\leftrightharpoons HSSH\cdot^{-}/HSS^{\cdot 2-},\mathrm{H}^{+} \quad (55)$$

$$HSS^{\cdot 2-}+\mathrm{O_2}\rightarrow HSS^{-}+\mathrm{O_2}^{-} \quad (56)$$

To be sure, the overall kinetics of H_2S autoxidation are fairly slow, and the likelihood of uncatalyzed H_2S autoxidation occurring in most biological systems appears to be low, especially considering other possible reactions. For example, the second order rate constants for the generation of either thiosulfate or sulfite from H_2S autoxidation are only around 0.1 to 0.5 $M^{-1}min^{-1}$.[134] However, this chemistry can be catalyzed by metals.[137–139] Thus, there may be circumstances that allow the generation of HS· in biological systems via oxidation by O_2, although this remains to be demonstrated.

There is no direct chemical interaction between O_2 and CO under most biological conditions. However, they can affect the actions of each other via competition at a common target (i.e., a heme protein). These interactions are described below.

Interactions of O_2^- with NO, H_2S, and CO. As mentioned above, NO and O_2^- readily react, generating peroxynitrite, a much better overall oxidant than either precursor (reaction 23). This reaction has been touted as a mechanism for the endogenous generation of a potent oxidant with significant (patho)physiological implications,[61] although this idea has been questioned.[140] Regardless, it is clear that O_2^- can remove NO quickly from solution, leading to a cessation of NO biological activity (and vice versa). The reaction of O_2^- and H_2S has been examined.[141,142] Superoxide is capable of oxidizing H_2S/HS^- by a single electron forming the corresponding thiyl radical (HS·/S⁻·) (reaction 57).

$$\mathrm{H_2S}/HS^{-}+\mathrm{O_2}^{-}\left(\mathrm{H}^{+}\right)\rightarrow HS\cdot/\mathrm{S}^{-}\cdot+\mathrm{H_2O_2}/HOO^{-} \quad (57)$$

These reports are consistent with the fact that the reported reduction potentials for O_2^-/H^+ and HS· are similar; thus it may be expected that O_2^- under acidic conditions can lead to the oxidation of H_2S (O_2^-,H^+/H_2O_2, ε^0 = 0.89 and HS·,H^+/ H_2S, ε^0 = 0.9−1 V, vs NHE, pH 7). The rate of this reaction has been determined to be only 6.5×10^4 $M^{-1}s^{-1}$ [141] (although a higher rate constant (1.5×10^6 $M^{-1}s^{-1}$) has also been reported using a less reliable assay system for superoxide).[142] There appears to be no significant chemical interactions between O_2^- and CO. Thus, to date the most prevalent reaction of O_2^- with the small molecule signaling species discussed herein appears to be the reaction with NO.

Interactions of H_2O_2 with NO, H_2S, and CO

As discussed previously, the primary reactivity of H_2O_2 in biological systems is as an oxidant and/or electrophile. Thus, its reaction partners are typically reductants and/or nucleophiles. For the most part, the nitrogen oxides discussed herein are themselves oxidants or electrophiles and, therefore, do not react directly with H_2O_2 under biological conditions. Being relatively inert, CO does not react with H_2O_2. However, as a possible reductant and nucleophile, H_2S is capable of direct interaction with H_2O_2. The reaction of H_2S with H_2O_2 is complex and ultimately leads to the formation of oxidized sulfur species such as S^0 and SO_4^{2-}, depending on reaction conditions.[129] The initial reaction is the attack of the nucelophilic sulfur of HS^- on an electrophilic oxygen atom of H_2O_2 leading to the sulfenic acid (HSOH) and H_2O (reaction 58). Further reaction with H_2S leads to the formation of higher order sulfides and, eventually, a stable form of elemental sulfur (S_8) (reactions 59 and 60). In the presence of excess H_2O_2, the sulfenic acid can further react with H_2O_2 leading to higher oxides of sulfur such as SO_4^{2-} (reactions 61 and 62).

$$H_2S + H_2O_2 \rightarrow HSOH + H_2O \quad (58)$$

$$HSOH + H_2S \rightarrow HSSH + H_2O \quad (59)$$

$$HSSH + HSOH \rightarrow\rightarrow\rightarrow S_8 + H_2S \quad (60)$$

$$HSOH + H_2O_2 \rightarrow S(OH)_2 + H_2O \quad (61)$$

$$S(OH)_2 + H_2O_2 \rightarrow SO_2 \cdot H_2O + H_2O \rightarrow\rightarrow\rightarrow SO_4^{2-} \quad (62)$$

Interactions of H_2S with Nitrogen Oxides

Most of the reactions of H_2S with nitrogen oxide species can be considered as analogous to the reactions of typical biological thiols. For example, due to the nucleophilicity of H_2S/HS^- it has the capability to react with electrophilic species. The reaction of H_2S/HS^- with electrophiles such as H_2O_2 or disulfides (RSSR) has already been discussed. Recently, it has been reported that H_2S can also react with *S*-nitrosothiols resulting in NO generation.[143] The chemistry responsible for this observation has not yet been delineated. However, the analogous reaction of an alkyl thiol with a nitrosothiol has been examined. As discussed

previously, Wong and co-workers propose that the reaction between a thiol and a nitrosothiol can result in either a trans-nitrosation reaction (the transfer nitrosonium (NO^+) from one sulfur to the other) (reaction 43) or the generation of the corresponding disulfide and HNO (reaction 42).[85] Considering that HNO can be converted to NO via a simple oxidation (vide supra), HNO intermediacy in the reaction of H_2S with nitrosothiols appears possible (although speculative at this time). It is likely that H_2S can also react with other electrophilic or oxidizing nitrogen oxides such as N_2O_3, NO_2, or ONOOH[144] since all of these species are known to react readily with biological thiols.[67,145] The products/intermediates in these reactions would be analogous to those found with biological alkyl thiols, namely, thiyl radicals, sulfenic acid, and nitrosated species. It needs to be mentioned, however, that normal cells contain very high levels of thiols besides H_2S (e.g., glutathione), and specific reactions of H_2S seem unlikely unless its relatively small size allows access to reaction sites not available to larger thiol species.

Common Targets: Interactions at Metals. All of the parent species mentioned herein have the ability to react with a variety of biological metal centers. Taking, for example, heme proteins as representative metalloprotein targets, many O_2, NO, CO, and HS^- complexes have been characterized. Indeed, the biological utility of O_2 and NO as well as the toxicology of CO and H_2S are, in part, attributed to reactions with heme proteins. The fundamental coordination chemistry of O_2, NO, and CO has already been discussed, and as mentioned above, all of these species are capable of binding to ferrous heme proteins such as deoxymyoglobin or deoxyhemoglogin. With respect to the ability of these diatomics to serve as signaling agents, one of the most important factors associated with their chemical biology is how metalloproteins achieve selectivity for one species over another (if indeed they do). That is, how can a metalloprotein discriminate among, for example, NO, O_2, and CO since all of these species have the potential to bind to ferrous heme proteins with an available coordination site? With free ferrous protoheme (no protein) the affinity of CO binding is approximately 20,000 times greater than that of O_2. Thus, in the absence of any protein interactions, CO has much stronger binding to the ferrous ion compared to that of O_2. However, the affinity of CO for the ferrous heme in myoglobin is only 25−100 times greater, indicating a significant effect by the protein to alter the relative affinities of CO versus O_2 for the ferrous heme component. As mentioned above, NO, CO, and O_2 all bind ferrous heme proteins utilizing primarily two types of bonding interactions, σ-donation via a lone pair of electrons from the diatomic ligand and π-back-donation, which is a result of the overlap of filled dorbitials on the metal with π^* antibonding orbitals on the diatomic ligand. With O_2, the electrons that form the σ-bond with the metal are in an sp^2 hybridized orbital indicating that O_2 prefers to bind in a bent fashion (that is, the metal−O-O geometry is bent, vide supra). However, the σ-bond-forming electrons in CO are in an sp hybridized orbital and, therefore, favoring CO binding with a linear geometry (that is, the metal− C-O atoms are linear). Interestingly, NO can bind in either fashion (i.e., like CO or O_2) depending on whether it binds as NO^+ (which is isoelectronic with CO and therefore binds in a linear fashion) or as NO^- (which is isoelectronic with O_2 and therefore binds in a bent fashion).[146] The binding of NO to, for example, Fe^{3+}-hemes results in a linear (or near linear) complex since the NO ligand has significant NO^+ character. In biological systems, NO appears to bind ferrous heme proteins in primarily a bent fashion (i.e., O_2-like). Thus, it is conceivable

that one way a protein can discriminate between, for example, O_2 (and NO) and CO is to place a steric restriction to one of the binding geometries (Figure 14).[147] That is, a steric restriction to the preferred linear binding mode of CO may inhibit its binding without significantly effecting the bent geometries of O_2 and NO binding.

The idea that discrimination between the simple diatomic ligands can be a result of unfavorable steric interactions is an attractive idea but recent reports indicate that it is likely that this effect plays only a minor role in the overall binding energetics.[104] A much more important effect that allows significant discrimination between O_2 and CO involves specific electrostatic interactions. As discussed above, the degree of backbonding present in the ferrous heme complexes of the diatomics discussed herein is in the following order: CO < NO < O_2. Indeed, the Fe^{2+}-O_2 complex is often depicted as a Fe^{3+}-O_2^- complex (a ferric-superoxide complex) to reflect the significant transfer of charge from the metal to the coordinated O_2. Thus, ferrous-bound O_2 is a much better hydrogen bond acceptor than CO. Using ferrous myoglobin as an example, it has been found that H-bond formation between bound O_2 and a distal histidine is an important aspect of the overall energetics of O_2 binding and is responsible for most of the discrimination between O_2 and CO. That is, the ability for myoglobin to exhibit a greater discrimination between O_2 and CO is due primarily to an increased affinity of ferrous myoglobin for O_2 (due to hydrogen bond formation) rather than a decreased affinity for CO (as the steric model might suggest) (Figure 15).

The ability of hemeproteins to discriminate between O_2 and CO can be at least partially rationalized using the arguments above. Discriminating between NO and O_2 is also an important factor in some heme proteins. As mentioned above, the affinity of CO over O_2 for free ferrous protoheme (protein free) is approximately 20,000-fold, and NO has an even greater affinity for protoheme than CO (approximately 20-fold greater than CO).[148] Thus, NO appears to have a particularly high natural affinity for ferrous hemes. However, signaling levels of NO are reported to be in the low nanomolar range (i.e., 1−10 nM)[70] while intracellular O_2 levels can be significantly higher (μM range). Thus, in spite of the higher normal affinity of NO versus O_2, there is still the need for NO-sensing proteins to select for NO over O_2. For example, a primary receptor for NO is the heme protein soluble guanylate cyclase (sGC). The ferrous form of this enzyme binds to NO resulting in an increase in enzyme activity.[149] Interestingly, sGC is capable of discriminating between NO and O_2 as it does not appear to bind O_2. Although the factors that allow sGC to discriminate between NO and O_2 have not been unequivocally established, it has been postulated that proteins analogous to sGC that bind O_2 possess a distal tyrosine residue in the heme pocket that, like the histidine in myoglobin, H-bonds to the coordinated O_2. Thus, the lack of a distal tyrosine in a protein like sGC will decrease O_2 affinity and, possibly, allow for significant discrimination between O_2 and NO.[150,151] Thus, in this case, the discrimination between NO and O_2 is due to a lack of stabilization of the O_2 adduct as opposed to a special stabilization of the NO complex. It is worth remembering that the coordination of NO to the ferrous heme is distinct from O_2 in that NO ligation typically results in a weakening of the proximal ligand bond and the generation of a 5-coordinate complex (vide supra). Whether the change in the coordination number/geometry between the NO and O_2 complexes play a role in ligand discrimination has not been established.

As mentioned earlier, H_2S (or HS^-, S^{2-}) binds heme proteins. However, unlike O_2 and CO, H_2S prefers to bind (likely as HS^-) the higher oxidation state ferric (Fe^{3+}) ion. Thus, there is not likely to be any direct competition between CO and O_2 with H_2S at a ferrous heme site. However, unlike O_2 and CO, NO will bind to ferric hemes, and therefore, there is the possibility of an interaction between NO and H_2S at a ferric heme protein, although this has not been reported.

It is clear that heme proteins have evolved to be able to select one small molecule signaling species over others. That is, proteins have managed to fine tune the relative affinities for these potential ligands in order to achieve signaling/biochemical specificity, and the competitiveness of the ligands under (patho)physiological conditions can vary from protein to protein. For example, one of the H_2S biosynthesis enzymes cystathionine β-synthase (CBS) is a heme protein capable of coordinating CO and NO. Catalysis by CBS is not directly dependent on heme chemistry; thus, the heme group is thought to be purely regulatory.[152] CBS can be inhibited by CO binding to the ferrous heme with a K_i of 5.6 ± 1.9 μM. This relatively low K_i indicates that CO can be an endogenously generated regulator of CBS activity. However, CBS binds NO with an extremely high K_i of 320 ± 60 μM and is therefore thought to be physiologically irrelevant.[153] CBS does not appear to bind O_2. However, O_2 will oxidize the ferrous heme to the ferric species resulting in an increase in activity[154] (vide infra). However, the previously mentioned enzyme sGC, which also contains a regulatory heme, is regulated by NO at low nano-molar levels and does not appear to be regulated by (or bind to) CO at presumed physiological levels.[149] These two examples illustrate how heme proteins are capable of responding differently to these signaling species by, at one level, altering the relative affinities for the ligands. However, this is not to say that significant interactions cannot exist between the various signaling agents at, for example, heme centers. It is conceivable that competition between these ligands at heme centers exists at (patho)physiological levels and is regulatory. A possible example of this is the terminal, O_2-binding component of respiration cytochrome c oxidase (CcOX). The normal substrate for CcOX is, of course, O_2. However, it is postulated that endogenous generation of CO and NO may be important regulators of mitochondrial respiration via competitive binding to CcOX[109,154] and, possibly, other chemistries.[155]

The above discussion focused on ligand binding only. However, it must be considered that some ligands can react further after initial binding while others may not. As discussed earlier, NO is capable of reducing a ferric heme to the ferrous species via reaction 39. Thus, a ferric heme protein unable to bind/respond to O_2 or CO can be converted to the ferrous species by NO, which would then be capable of binding all three. (To be sure, reduction of ferric hemes can be accomplished in many other ways in biological systems that are unrelated to NO.) However, a ferrous heme−O_2 complex (as mentioned above) has significant Fe^{3+}-O_2^- character, and a process referred to as autoxidation can occur whereby the O^-_2 dissociates, resulting in an oxidized, ferric species (reaction 63). Because of the fact that O_2 is a poor one-electron oxidant (vide supra), the formal transfer of an electron from the ferrous ion to O_2 is thermodynamically unfavorable.[156] However, the presence of a proton (acidic conditions) or a nucleophile greatly accelerates this autoxidation process.

$$Heme - Fe^{2+} - O_2 \leftrightarrows Heme - Fe^{3+} - O_2^- \rightarrow Heme - Fe^{3+} + O_2^- \quad (63)$$

Thus, O_2 has the potential to oxidize NO- and CO (and O_2-)-binding heme proteins to the corresponding ferric species, which will no longer bind CO and O_2 (and have significantly less affinity for NO). It is also worth noting that NO can also react with ferrous heme−O_2 complexes resulting in the ferric heme protein and nitrate ion (reaction 64).[157]

$$\left[Heme - Fe^{2+} - O_2 \leftrightarrows Heme - Fe^{3+} - O_2^-\right] + NO \rightarrow Heme - Fe^{3+} + O_3^- \quad (64)$$

This reaction is analogous to the previously discussed and facile reaction of NO with O_2^- (reaction 23), the only difference being that O_2^- is coordinated to the ferric ion prior to the reaction. Thus, NO can avidly bind to ferrous hemes, but in the presence of O_2, the combination of NO/O_2 can result in ferrous heme oxidation. For the most part, CO binding to ferrous hemes does not impart any further reactivity to either the metal or ligand. Thus, except for simple dissociation, the bound CO is thought to be inert. However, the binding of CO can protect ferrous species from the autoxidation associated with O_2 since CO will prevent O_2 binding (assuming autoxidation occurs via an inner-sphere mechanism, i.e. occurs as a result of O_2 coordination).

Interactions at Thiols. Hopefully, it is clear that metal centers represent a common target for all of the signaling species discussed herein. Moreover, some metal centers may interact with several of the signaling molecules (or not) depending on the protein environment around the heme center. The other obvious biological targets for these species are thiols/thiol proteins. As discussed earlier, the reactions of thiols with nitrogen oxides (i.e., NO_2, N_2O_3, HNO, and HOONO), O_2-derived species (i.e., O_2^- and H_2O_2), and H_2S are prevalent and potentially important biochemical occurrences. In general, the nitrogen oxide and O_2-derived species are thought to oxidatively modify protein thiols, oftentimes leading to an alteration of protein function. However, H_2S appears capable of reducing some oxidized thiol species and may even generate a hyperactivated persulfide species (vide supra). Significantly, most of the nitrogen oxide species capable of modifying thiols are generated via O_2-dependent chemistry (vide supra). Exceptions to this are HNO, which has not been determined to be physiologically relevant, and as discussed previously the possible oxidation of thiols (nitrosothiol formation) via a ferric-nitrosyl species (reaction 41). NO itself will not react directly with thiols under biological conditions (vide supra). O_2 itself also does not react directly with thiols and requires either reduction or reaction with NO before it generates a thiol-reactive species. Thus, NO/ O_2-derived species can oxidize thiols, and H_2S can reduce at least some of the oxidized thiol species, allowing for all of these signaling molecules to be involved in the redox regulation of thiol protein activity.

With regard to possible points of integrated signaling associated with NO, O_2, CO, and H_2S, of particular interest are metalloproteins (i.e., heme proteins) that also possess regulatory/redox active thiols. In these cases, the integrated signaling by these small molecules can be the result of actions at distinct regulatory sites on the same protein. Several proteins already

mentioned herein are known to be regulated via heme coordination by specific small molecules as well as by protein thiol redox modification. sGC, the primary receptor for NO is also regulated by protein cysteine modification[87] allowing enzyme activity to be responsive to not only the presence of NO but also the thiol redox status of the cell (which can be based on the relative levels of all of the thiol reactive species present). The H_2S-generating CBS can also be regulated (inhibited) by CO[153] as well as protein thiol modification[158] and heme oxidation state.[159] Interestingly, *S*-nitrosothiol formation on crucial cysteine residues on CBS results in an increase in enzyme activity[158] alluding to the possibility that generation of NO-derived nitrosating species increases activity. Moreover, since generation of CO decreases activity via complexation to the regulatory heme and heme oxidation, possibly by O_2-derived oxidants, can decrease activity, it may be that the levels and fluxes of all of these signaling species are important in the overall regulation of this enzyme. Finally, the possibility that H_2S can reverse the thiol oxidation (or even react with the ferric protein) alludes to the possibility that this protein is responsive to all the signaling species discussed herein. It is likely that many other examples of this type exist and that this type of interplay between these signaling species is prevalent.

REGULATION OF HIF1α AN EXAMPLE OF INTEGRATED SIGNALING

Although sGC and CBS (briefly mentioned above) can serve as examples of possible single target integrative signaling associated with the small molecule agents NO, CO, O_2, and H_2S, another primary example of this is the signaling system associated with the biological response to hypoxia. HIF1α (hypoxia inducible factor 1-α) is a transcription factor that is largely responsible for the biological response to low levels of O_2 (hypoxia). HIF1α activity leads to an increase in a variety of gene products including erythropoietin, vascular endothelial growth factor (VEGF), and other proteins involved in an adaptive response to hypoxia.[160] The way this system "senses" O_2 is via enzymes that utilize O_2 to oxidatively modify HIF1α at a proline, leading to degradation, or an asparagine, leading to an inhibition of the binding of coactivators. Thus, the prolyl (prolyl hydroxylase domain, PHD) and asparaginyl hydroxylases (factor inhibiting HIF, FIH) serve to deactivate or degrade HIF1α and, therefore, are the major O_2-dependent regulators of HIF1α activity. Under low O_2 conditions, the activity of these enzymes is decreased (due to the lack of the O_2 cosubstrate), and levels of HIF1α increase, leading to gene expression (Figure 16).

Although the presentation of the hypoxia-sensing signaling system is greatly oversimplified (as it is much more extensive and complex than that described herein, involving many other factors and proteins), the important point here is that the prolyl and asparginyl hydroxylases are proteins that represent possible targets for integrative signaling by the small molecule agents. For more extensive descriptions of the HIF1α system, many other reviews are available.[161,162]

PHD and FIH are members of a family of dioxygenase enzymes that utilize nonheme iron and α-ketoglutarate (also called 2-oxoglutarate) as a source of reducing equivalents to activate O_2 leading to the oxygenation of, in this case, proline. For the sake of brevity, the discussion herein will focus on PHD (of which there are three major isoforms) as a target for regulation by small molecule signaling agents. The K_m value for O_2 in PHDs is

approximately 7−8 μM, a range consistent with the idea that these enzymes are O sensors.[163] The general mechanism of 2-oxoglutarate- and O_2-dependent hydroxylation of substrate is schematically depicted below (Figure 17).[164,165]

Clearly with any O_2-binding protein there is the possibility of NO or CO binding as well, representing a possible site of interaction of these species with the HIF-1α system. Moreover, since the resting state of the protein is ferrous (Fe^{II}), oxidation of the iron may represent a redox regulation since the ferric species will not perform the same chemistry (but can bind, for example, H_2S). Finally, the PHDs have been reported to have redox thiols that can interact with thiol-modifying species, leading to changes in activity (vide infra). Thus, it is evident that the PHD-HIF-1α system is subject to possible regulation by all of the small-molecule signaling agents described herein (and their derived species) and represents an example of the potential integrative signaling of the small molecule agents. Below, evidence for this is presented.

As mentioned above, coordination of NO or CO to the nonheme ferrous iron of PHD would be expected to inhibit the oxygenation of proline by competing with O_2 and therefore mimicking hypoxia. Indeed, the NO-donor GSNO was found to inhibit PHD leading to HIF-1α accumulation under normoxia.[165,166] Inhibition of PHD by NO has been proposed to occur via an interaction between NO and the ferrous ion of PHD.[167,168] A similar effect has been noted with CO as well, albeit at high, nonphysiological levels of CO.[169] A mechanistically distinct pathway of possible NO-mediated regulation involves NO-dependent S-nitrosation (via NO-derived species) of HIF-1α leading to increased stabilization[170] and transcriptional activity.[171,172] Thus, NO has the ability to increase HIF-1α activity via multiple mechanisms that may be highly dependent on the levels of NO.[173]

Interestingly, both NO and CO have also been reported to decrease HIF-1α signaling as well[174] indicating a complex picture with respect to the small molecule signaling species and O_2 sensing. Indeed, numerous studies report decreased levels of HIF-1α upon exposure to NO (especially under hypoxic conditions).[168] One possible mechanism for this is proposed by Hagen and co-workers who found that NO inhibits respiration (and therefore O_2 consumption) leading to a redistribution of O_2 to nonrespiratory pathways such as the PHDs, leading to the degradation of HIF-1α.[175] This represents an indirect effect on the system (as opposed to the direct interactions described above for NO- and CO-mediated increases in HIF-1α activity). Inhibition of respiration by CO under hypoxic conditions may also have a similar effect.[176]

As shown in Figure 17, PHD requires ferrous iron for activity. Thus, small-molecule oxidants (O_2 or NO-derived species) may lead to oxidation of Fe(II) to Fe(III) and a decrease in PHD activity and subsequent increase in HIF-1α levels/activity. Indeed, O_2-derived oxidants (so-called ROS) have been reported to elicit an increase in HIF-1α activity, possibly via PHD iron oxidation.[177] The picture becomes extremely complex when NO and, for example, O_2^- are present since either can affect PHD activity, and upon reaction with each other, a decrease in levels of both occurs,[178,179] and the product $ONOO^-$ can have a distinct effect including possible utilization as an oxidizing equivalent in catalysis.[180] CO

appears to be able to affect HIF-1α levels indirectly since the exposure of macrophages to CO has been found to lead to a burst of ROS via interaction with mitochondria that leads to increased levels of HIF-1α.[181] Finally, several recent studies indicate a possible role for H_2S in controlling HIF-1α levels. In *C. elegans*, H_2S is capable of increasing HIF-1α activity.[182] Although the mechanism by which H_2S increases HIF-1α activity is not described, it remains possible that inhibition of respiration (or other electron transport system) and subsequent generation of O_2-derived oxidants[183] inactivate PHD via iron oxidation (akin to an effect of NO described above). Finally, the pro-angiogenic effect of H_2S has been reported to be a result of an increase in HIF-1α protein (and mRNA) levels in rat endothelial cells.[184] Again, the mechanism by which H_2S elicits this response is not established.

The above discussion of the interaction of various small molecule signaling agents, and derived species, with the HIF-1α/PHD system serves to illustrate how these multiple agents may interact either directly or indirectly to elicit a variety of outcomes that can be dependent on the levels of the mediator/ effector, the cell/cellular environment, and/or the presence of other factors/reactants. To be sure, this discussion is by no means comprehensive or complete, and clearly, other interactions exist that can have profound effects on hypoxic sensing. There are likely myriad signaling systems that are also subject to similar integrated interactions with these small molecule agents.

CONCLUSIONS

As increasing evidence mounts for important and diverse signaling functions associated with O_2, NO, CO, and H_2S (and species derived from these agents), it is becoming increasingly important to understand the chemical biology of these agents and how this chemistry is integrated and regulated by Nature. As mentioned in the beginning of this review, the fundamental chemical properties of these species predict similar/overlapping biological targets, which include metals and thiol redox systems. Moreover, the striking similarities in the nature of the biological functions/targets that likely interact with most (and in some cases all) of these small molecule signaling agents strongly suggests a system by which multiple signaling agents are able to regulate fundamental signaling pathways. It was the intention of this review to begin to describe the basic chemistry of the relevant species as a prelude to an attempt to understand the intricate and integrated signaling web. Clearly, this discussion is only beginning, and other chemistries, biological targets, and signaling systems remain to be discovered and described. In any event, the chemical biology and physiology of integrated small molecule signaling agents is a growing, exciting, and important field of research endeavor that will occupy the efforts of many laboratories in many disciplines for many years to come.

ABBREVATIONS

NHE	normal hydrogen electrode
BDE	bond dissociation energy
SOD	superoxide dismutase

SOMO	singly occupied molecular orbital
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
CcOX	cytochrome *c* oxidase
HIF1*α*	hypoxia inducible factor 1-*α*
sGC	soluble guanylate cyclase
CBS	cystathionine *β*-synthase
PHD	prolyl hydroxylase domain
FIH	factor inhibiting HIF
VEGF	vascular endothelial growth factor

REFERENCES

1. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 1991; 43:109–141. [PubMed: 1852778]
2. Li L, Moore PK. An overview of the biological significance of endogenous gases: New roles for old molecules. Biochem. Soc. Trans. 2007; 35:1138–1141. [PubMed: 17956296]
3. Kashiba M, Kajimura M, Goda N, Suematsu M. From O_2 to H_2S: A landscape view of gas biology. Keio J. Med. 2002; 51:1–10. [PubMed: 11951372]
4. Pryor WA, Houk KN, Foote CS, Fukuto JM, Ignarro LJ, Squadrito GL, Davies KJA. Free radical biology and medicine, it's a gas, man. Am. J. Physiol., Regulatory, Int. Com. Physiol. 2006; 291:491–511.
5. Fukuto JM, Collins MD. Interactive endogenous small molecule (gaseous) signaling: Implications for teratogenesis. Curr. Pharm. Des. 2007; 13:2952–2978. [PubMed: 17979740]
6. Stone JR, Yang S. Hydrogen Peroxide: A Signaling messenger. Antiox. Redox Signaling. 2006; 8:243–270.
7. Forman HJ, Fukuto J, Torres M. Redox signaling-chemistry defines which reactive oxygen and nitrogen species can act as second messengers. Am. J. Physiol. Cell Physiol. 2004; 287:C246–C256. [PubMed: 15238356]
8. Mustafa AK, Gadalla MM, Snyder SH. Signaling by gasotransmitters. Sci. Signaling. 2009; 2:1–8.
9. Olsen KR, Donald JA. Nervous control of circulation: The role of gasotransmitters, NO, CO and H_2S. Acta Histochem. 2009; 111:244–256. [PubMed: 19128825]
10. Sawyer, DT. Oxygen Chemistry. Oxford University Press; New York: 1991.
11. Foote, CS.; Valentine, JS.; Greenberg, A.; Liebman, JF. Active Oxygen in Chemistry, Search Series. Vol. 2. Blackie Academic & Professional; London: 1995.
12. Valentine, JS.; Foote, CS.; Greenberg, A.; Liebman, JF. Active Oxygen in Biochemistry, Search Series. Vol. 3. Blackie Academic and Professional; London: 1995.
13. Halliwell, B.; Gutteridge, JMC. Free Radicals in Biology and Medicine. 4th ed.. Oxford University Press; Oxford: 2007.
14. Stubbe J, van de Donk WA. Protein radicals in enzyme catalysis. Chem. Rev. 1998; 98:705–762. [PubMed: 11848913]
15. Ho, RYN.; Liebman, JF.; Valentine, J. Biological Reactions of Dioxygen: An Introduction, in Active Oxygen in Biochemistry. In: Valentine, JS.; Foote, CS.; Greenberg, A.; Liebman, JF., editors. Search Series. Vol. 3. Blackie Academic and Professional; London: 1995. p. 1-36.
16. Koppenol WH. Oxyradical reactions: from bond-dissociation energies to reduction potentials. FEBS. 1990; 264:165–167.

17. McMillen DF, Golden DM. Hydrocarbon bond-dissociation energies. Annu. Rev. Phys. Chem. 1982; 33:493–532.
18. Johnson M. Thermochemical properties of peroxides and peroxyl radicals. J. Phys. Chem. 1996; 100:6814–6818.
19. Forman HJ, Fukuto JM, Miller T, Zhang H, Rinna A, Levey S. The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. Arch. Biochem. Biophys. 2008; 477:183–195. [PubMed: 18602883]
20. Foote, CS.; Clennan, EL. Properties and Reactions of Singlet Dioxygen, in Active Oxygen in Chemistry. Foote, CS.; Valentine, JS.; Greenberg, A.; Liebman, JL., editors. Blackie Academic and Professional; London: 1995.
21. Ogilby PR. Singlet oxygen: There is indeed something new under the sun. Chem. Soc. Rev. 2010; 39:3181–3209. [PubMed: 20571680]
22. Triantaphylides C, Havaux M. Singlet oxygen in plants: Production, detoxification and signaling. Trends Pant Sci. 2009; 14:219–228.
23. Robertson CA, Hawkins D, Abrahamse EH. Photodynamic therapy (PDT): A short review on cellular mechanisms and cancer research applications for PDT. J. Photochem. Photobiol. B. 2009; 96:1–8.
24. Fridovich I. Superoxide radical and superoxide dismutases. Annu. Rev. Biochem. 1995; 64:97–112. [PubMed: 7574505]
25. Winterbourn CC, Metodiewa D. Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. Free Radical Biol. Med. 1999; 27:322–328. [PubMed: 10468205]
26. Stone JR. An assessment of proposed mechanisms for sensing hydrogen peroxide in mammalian systems. Arch. Biochem. Biophys. 2004; 422:119–124. [PubMed: 14759598]
27. Miller DM, Buettner GR, Aust SD. Transition metals as catalysts of “autoxidation” reactions. Free Radical Biol. Med. 1990; 8:95–108. [PubMed: 2182396]
28. Louit G, Hanedanian M, Taran F, Coffigny H, Renault JP, Pin S. Determination of hydroxyl rate constants by a high-throughput fluorimetric assay: towards a unified reactivity scale for antioxidants. Analyst. 2009; 134:250–255. [PubMed: 19173045]
29. Wink DA, Wink CB, Nims RW, Ford PC. Oxidizing intermediates generated in the Fenton reagent: Kinetic arguments against the intermediacy of the hydroxyl radical. Env. Health Perspect. 1994; 102:11–15. [PubMed: 7843082]
30. Czapski G, Lymar SV, Schwartz HA. Acidity of the carbonate radical. J. Phys. Chem. A. 1999; 103:3447–3450.
31. Bonini MG, Augusto O. Carbon dioxide stimulates the production of thiyl, sulfinyl and disulfide radical anion from thiol oxidation of peroxynitrite. J. Biol. Chem. 2001; 276:9749–9754. [PubMed: 11134018]
32. Jameson, GB.; Ibers, JA. Dioxygen Carriers. In: Bertini, I.; Gray, HB.; Stiefel, EI.; Valentine, JS., editors. Biological Inorganic Chemistry, Structure and Reactivity. University Science Books; Sausalito, CA.: 2007. p. 354-388.Chapter XI.4
33. Que, L,, Jr.. Dioxyen Activating Enzymes. In: Bertini, I.; Gray, HB.; Stiefel, EI.; Valentine, JS., editors. Biological Inorganic Chemistry, Structure and Reactivity. University Science Books; Sausalito, CA.: 2007. p. 388-413.Chapter XI.5
34. Lewis EA, Tolman WB. Reactivity of dioxygen-copper systems. Chem. Rev. 2004; 104:1047–1076. [PubMed: 14871149]
35. Tovrog BS, Kitko DJ, Drago RS. Nature of the bound O_2 in a series of cobalt dioxygen adducts. J. Am. Chem. Soc. 1976; 98:5144–5153.
36. Wade EA, Cline JI, Lorenz KT, Hayden C, Chandler DW. Direct measurement of the binding energy of the NO dimer. J. Chem. Phys. 2002; 116:4755–4757.
37. Ritchie JP. Comments ion the cis, trans and cyclic dimers of NO. J. Phys. Chem. 1983; 87:2466–2468.
38. Harcourt RD. The origin of the long N-N bond in N_2O_2: An ab initio valence bond study. J. Mol. Struct. (Theochem.). 1990; 206:253–264.

39. Bartberger MD, Liu W, Ford E, Miranda K, Switzer C, Fukuto JM, Farmer PJ, Wink DA, Houk KN. The reduction potential of nitric oxide (NO) and its importance to NO biochemistry. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:10958–10963. [PubMed: 12177417]
40. Shafirovich V, Lymar SV. Nitroxyl and its anion in aqueous solutions: Spin states, protic equilibria, and reactivities toward oxygen and nitric oxide. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:7340–7345. [PubMed: 12032284]
41. Dixon RN. Heats of formation of HNO and DNO. J. Chem. Phys. 1996; 104:6905–6906.
42. Ford PC, Wink DA, Stanbury DM. Autoxidation kinetics of aqueous nitric oxide. FEBS. 1993; 326:1–3.
43. Liu X, Miller MJS, Joshi MS, Thomas DD, Lancaster JR Jr. Accelerated reaction of nitric oxide with O_2 within the hydrophobic interior of biological membranes. Proc. Natl. Acad. Sci. U.S.A. 1998; 95:2175–2179. [PubMed: 9482858]
44. Rubbo H, Parthsarathy S, Barnes S, Kirk M, Kalyanaraman B, Freeman BA. Nitric oxide inhibition of lipoxygenase-dependent liposome and low-density oxidation: Termination of radical chain propagation reactions and formation of nitrogen-containing oxidized lipid derivatives. Arch. Biochem. Biophys. 1995; 324:15–25. [PubMed: 7503550]
45. Padmaja S, Huie RE. The reaction of nitric oxide with organic peroxyl radicals. Biochem. Biophys. Res. Commun. 1993; 195:539–544. [PubMed: 8373394]
46. Madej E, Folkes LK, Wardman P, Czapski G, Goldstein S. Thiyl radicals react with nitric oxide to form S-nitrosothiols with rate constants near the diffusion-controlled limit. Free Radical Biol. Med. 2008; 44:2013–2018. [PubMed: 18381080]
47. Eiserich JP, Butler J, Van Der Vliet A, Cross CE, Halliwell B. Nitric oxide rapidly scavenges tyrosine and tryptophan radicals. Biochem. J. 1995; 310:745–749. [PubMed: 7575405]
48. Huie RE, Padmaja S. The reaction of NO with superoxide. Free Radical Res. Commun. 1993; 18:195–199. [PubMed: 8396550]
49. Pryor WA, Church DF, Govindan CK, Crank G. Oxidation of thiols by nitric oxide and nitrogen dioxide: Synthetic utility and toxicological implications. J. Org. Chem. 1982; 47:159–161.
50. DeMaster EG, Quast BJ, Redfern B, Nagasawa HT. Reaction of nitric oxide with the free sulfhydryl group of human serum albumin yields a sulfenic acid and nitrous oxide. Biochemistry. 1995; 34:11494–11499. [PubMed: 7547878]
51. Zhao Y-L, Bartberger MD, Goto K, Shimada K, Kawashima T, Houk KN. Theoretical evidence for enhanced dimerization in aromatic hosts: Implications for the role of the electrophile $(NO)_2$ in nitric oxide chemistry. J. Am. Chem. Soc. 2005; 127:7964–7965. [PubMed: 15926791]
52. Stanbury DM. Reduction potentials involving inorganic free radicals in aqueous solution. Adv. Inorg. Chem. 1989; 33:69–138.
53. Jursic BS. Complete basis set and Gaussian computational study of bond dissociation energies, enthalpy of formation and rearrangement barriers for the XNO nitric oxide derivatives. J. Mol. Struct. (Theochem.). 1999; 492:35–43.
54. Prutz WA, Monig H, Butler J, Land EJ. Reaction of nitrogen dioxide in aqueous model systems: Oxidation of tyrosine units in peptides and proteins. Arch. Biochem. Biophys. 1985; 243:125–134. [PubMed: 4062299]
55. Ford E, Hughes MN, Wardman P. Kinetics of the reactions of nitrogen dioxide with glutathione, cysteine and uric acid at physiological pH. Free Radical Biol. Med. 2002; 32:1314–1323. [PubMed: 12057769]
56. Lv CL, Liu YD, Zhong R. Theoretical investigation of nitration and nitrosation of dimthylamine by N_2O_4. J. Phys. Chem. 2008; 112:7098–7105.
57. Chatterjee J, Coombes RG, Barnes JR, Fildes MJ. Mechanism of reaction of nitrogen dioxide with alkenes in solution. J. Chem. Soc., Perkin Trans. 1995; 2:1031–1032.
58. Astolfi P, Greci L, Panagiotaki M. Spin trapping of nitrogen dioxide and radicals generated from nitrous acid. Free Radical Res. 2005; 39:137–144. [PubMed: 15763961]
59. Claridge RP, Deeming AJ, Paul N, Tocher DA, Ridd JH. The reactions of nitrogen dioxide with dienes. J. Chem. Soc., Perkin Trans. 1998; I:3523–3528.
60. Williams DLH. Nitrosation Mechanisms. Adv. Phys. Org. Chem. 1983; 19:381–429.

61. Beckman JS, Koppenol WH. Nitric oxide, superoxide and peroxynitrite: The good, the bad, and the ugly. Am. J. Physiol. (Cell Physiol.). 1996; 271:C1424–C1437.
62. Pryor WA, Squadrito GL. The chemistry of peroxynitrite: a product from the reaction of nitric oxide and superoxide. Am. J. Physiol. (Lung Cell. Mol. Physiol.). 1995; 268:L699–L722.
63. Gunaydin H, Houk KN. Molecular dynamics simulation of the HOONO decomposition and HO·/NO2· caged radical pair in water. J. Am. Chem. Soc. 2008; 130:10036–10037. [PubMed: 18613683]
64. Koppenol WH, Kissner R. Can O=NOOH undergo hemolysis? Chem. Res. Toxicol. 1998; 11:87–90. [PubMed: 9511898]
65. Koppenol WH. Thermodynamic reactions involving nitrogen-oxygen compounds. Methods Enzymol. 1996; 268:7–12. [PubMed: 8782569]
66. Gunaydin H, Houk KN. Mechanisms of peroxynitrite-mediated nitration of tyrosine. Chem. Res. Toxicol. 2009; 22:894–898. [PubMed: 19374346]
67. Carballal S, Radi R, Kirk MC, Barnes S, Freeman BA, Alvarez B. Sufenic acid formation in human serum albumin by hydrogen peroxide and peroxynitrite. Biochemistry. 2003; 42:9906–9914. [PubMed: 12924939]
68. Ferrer-Sueta G, Radi R. Chemical biology of peroxynitrite: Kinetics, diffusion, and radicals. ACS Chem. Biol. 2009; 4:161–177. [PubMed: 19267456]
69. Augusto O, Bonini MG, Amanso AM, Linares E, Santos CCX, De Menezes SL. Nitrogen dioxide and carbonate radical anion: Two emerging radicals in biology. Free Radical Biol. Med. 2002; 32:841–859. [PubMed: 11978486]
70. Thomas DD, Ridnour LA, Isenberg JS, Wilmarie F-S, Switzer CH, Donzelli S, Hussain P, Vecoli C, Paolocci N, Ambs S, Colton CA, Harris CC, Roberts DD, Wink DA. The chemical biology of nitric oxide: Implications in cellular signaling. Free Radical Biol. Med. 2008; 45:18–31. [PubMed: 18439435]
71. Bratsch SG. Standard electrode potentials and temperature coefficients in water at 298.15 K. J. Phys. Chem. Ref. Data. 1989; 18:1–21.
72. Godber BLJ, Doel JJ, Sapkota GP, Blake DR, Stevens CR, Eisenthal R, Harrison R. Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase. J. Biol. Chem. 2000; 275:7757–7763. [PubMed: 10713088]
73. Basu S, Azarova NA, Font MD, King SB, Hogg N, Gladwin MT, Shiva S, Kim-Shapiro DB. Nitrite reductase activity of cytochrome c. J. Biol. Chem. 2008; 283:32590–32597. [PubMed: 18820338]
74. Kim-Shapiro DB, Gladwin MT, Patel RP, Hogg N. The reaction between nitrite and hemoglobin: the role of nitrite in hemoglobin-mediated hypoxic vasodilation. J. Inorg. Biochem. 2005; 99:237–246. [PubMed: 15598504]
75. Cosby K, Partovi KS, Crawford JH, Patel R, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AXN, Cannon R,O III, Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nature Med. 2003; 9:1498–1505. [PubMed: 14595407]
76. Yi J, Heinecke J, Tan H, Ford PC, Richter-Addo GB. The distal pocket histidine residue in horse heart myoglobin directs the)-binding mode of nitrite to the heme iron. J. Am. Chem. Soc. 2009; 131:18119–18128. [PubMed: 19924902]
77. Copeland DM, Soares AS, West AH, Richter-Addo GB. Crystal structures of the nitrite and nitric oxide complexes of horse heart myoglobin. J. Inorg. Biochem. 2006; 100:1413–1425. [PubMed: 16777231]
78. Silaghi-Dumitrescu R. Linkage isomerism in nitrite reduction by cytochrome cd1 nitrite reductase. Inorg. Chem. 2004; 43:3715–3718. [PubMed: 15180427]
79. Gladwin MT, Schechter AN, Kim-Shapiro DB, Patel RP, Hogg N, Shiva S, Cannon RO III, Kelm M, Wink DA, Espey MG, Oldfield EH, Pluta RM, Freeman JR, Lancaster JR Jr. Feelisch M, Lundberg JO. The emerging biology of nitrite anion. Nat. Chem. Biol. 2005; 1:308–314. [PubMed: 16408064]

80. Perissinotti LL, Marti MA, Doctorovich F, Luque FJ, Estrin DA. A microscopic study of the deoxyhemoglobincatalyzed generation of nitric oxide from nitrite anion. Biochemistry. 2008; 47:9793–9802. [PubMed: 18717599]

81. Salgado MT, Nagababu E, Rifkind JM. Quantification of intermediates formed during the reduction of nitrite by deoxyhemoglobin. J. Biol. Chem. 2009; 284:12710–12718. [PubMed: 19270306]

82. Gladwin MT, Grubina R, Doyle MP. The new chemical biology of nitrite reactions with hemoglobin: R-State catalysis, oxidative denitrosylation, and nitrite reductase/anhydrase. Acc. Chem. Res. 2010; 42:157–167. [PubMed: 18783254]

83. Paolocci N, Jackson MI, Lopez BE, Miranda K, Tocchetti CG, Wink DA, Hobbs AJ, Fukuto JM. The pharmacology of nitroxyl (HNO) and its therapeutic potential: Not just the janus face of NO. Pharmacol. Ther. 2007; 113:442–458. [PubMed: 17222913]

84. Bartberger MD, Fukuto JM, Houk KN. On the acidity and reactivity of HNO in aqueous solution and biological systems. Proc. Natl. Acad. Sci. U.S.A. 2001; 98:2194–2198. [PubMed: 11226215]

85. Wong PSY, Hyun J, Fukuto JM, Shirota FN, DeMaster EG, Shoeman DW, Nagasawa HT. Reaction between S-nitrosothiols and thiols: Generation of nitroxyl (HNO) and subsequent chemistry. Biochemistry. 1998; 37:5362–5371. [PubMed: 9548918]

86. Sulc F, Immoos CE, Pervitsky D, Farmer PJ. efficient trapping of HNO by deoxymyoglobin. J. Am. Chem. Soc. 2004; 126:1096–1101. [PubMed: 14746478]

87. Miller TW, Cherney ME, Lee AJ, Francoleon N, Farmer PJ, King SB, Hobbs AJ, Miranda K, Burstyn JN, Fukuto JM. The effects of nitroxyl (HNO) on soluble guanylate cyclase activity: interactions at ferrous heme and cysteine thiols. J. Biol. Chem. 2009; 284:21788–21796. [PubMed: 19531488]

88. Miranda KM, Nagasawa HT, Toscano JP. Donors of HNO. Curr. Top. Med. Chem. 2005; 5:649–664. [PubMed: 16101426]

89. Jackson MI, Han TH, Dutton A, Ford E, Miranda KM, Houk KN, Wink DA, Fukuto JM. Kinetic feasibility of nitroxyl (HNO) reduction by physiological reductants and biological implications. Free Radical Biol. Med. 2009; 47:1130–1139. [PubMed: 19577638]

90. Murphy ME, Sies H. Reversible conversion of nitroxyl anion to nitric oxide by superoxide dismutase. Proc. Natl. Acad. Sci. U.S.A. 1991; 88:10860–10864. [PubMed: 1961756]

91. Enemark JH, Feltham RD. Principles of structure, bonding, and reactivity for metal nitrosyl complexes. Coord. Chem. Rev. 1974; 13:339–406.

92. Cooper CE. Nitric oxide and iron proteins. Biochim. Biophys. Acta. 1999; 1411:290–309. [PubMed: 10320664]

93. Traylor TG, Sharma VS. Why NO? Biochemistry. 1992; 31:2847–2849. [PubMed: 1348002]

94. Wayland BB, Minkiewicz JV, Abd-Elmageed ME. Spectroscopic studies of tetraphenylporphyrincobalt(II) complexes of CO, NO, O_2, RNC, and $(RO)_3P$, and a bonding model for complexes of CO, NO, and O_2 with cobalt (II) and Iron(II) porphyrins. J. Am. Chem. Soc. 1974; 96:2795–2801.

95. Mingos DMP. A general bonding model for linear and bent transition metal-nitrosyl complexes. Inorg. Chem. 1973; 12:1209–1211.

96. Perutz MF, Kilmartin JV, Kyoshi N, Szabo A, Simon SR. Influence of globin structures on the state of the heme. Ferrous low spin derivatives. Biochemistry. 1976; 15:378–387. [PubMed: 1247524]

97. Goodrich LE, Paulat F, Praneeth VKK, Lehnert N. Electronic structure of heme-nitrosyls and its significance for nitric oxide reactivity, sensing, transport, and toxicity in biological systems. Inorg. Chem. 2010; 49:6293–6316. [PubMed: 20666388]

98. Hess DT, Matsumoto A, Kim S-O, Marshall HE, Stamler JS. Protrein S-nitrosylation: Purview and parameters. Nature Rev. 2005; 6:150–166.

99. Richter-Addo, GB.; Legzdins, P. Metal Nitrosyls. Oxford University Press; New York: 1992.

100. Williams DLH. S-Nitrosothiols and role of metal ions in decomposition to nitric oxide. Methods Enzymol. 1996; 268:299–308. [PubMed: 8782596]

101. Yongchun H, Wang J, Arias F, Echegoyen L, Wang PG. Electrochemical studies of S-nitrosothiols. Bioorg. Med. Chem. Lett. 1998; 8:3065–3070. [PubMed: 9873677]

102. Barrett J, Fitzgibbons LJ, Glauser J, Still RH, Young PNW. Photochemistry of the S-nitroso derivatives of hexane-1-thiol and hexane-1,6-dithiol. Nature. 1966; 211:848. [PubMed: 5968756]
103. Sexton DJ, Muruganandam A, McKenney DJ, Mutus B. Visible light photochemical release of nitric oxide from S-nitrosoglutathione: Potential photochemotherapeutic applications. Photochem. Photobiol. 1994; 59:463–467. [PubMed: 8022889]
104. Oelgeschlager E, Rother M. Carbon monoxide-dependent energy metabolism in anaerobic bacteria and archea. Arch. Microbiol. 2008; 190:257–269. [PubMed: 18575848]
105. Grahame DA, DeMoll E. Substrate and accessory protein requirements and thermodynamics of acetyl-CoA synthesis and cleavage in Methanosarcina barkeri. Biochemistry. 1995; 34:4617–4624. [PubMed: 7718564]
106. Bickar D, Bonaventura C, Bonaventura J. Carbon monoxide-driven reduction of ferric heme and heme proteins. J. Biol. Chem. 1984; 259:10777–10783. [PubMed: 6088517]
107. Bonaventura C, Godette G, Tesh S, Holm DE, Bonaventura J, Crumbliss AL, Pearce LL, Peterson J. Internal electron transfer between hemes and Cu(II) bound at cysteine β93 promotes methemoglobin reduction by carbon monoxide. J. Biol. Chem. 1999; 274:5499–5507. [PubMed: 10026163]
108. Spiro TG, Jarzecki AA. Heme-based sensors: Theoretical modeling of heme-ligand-protein interactions. Curr. Opin. Chem. Biol. 2001; 5:715–723. [PubMed: 11738184]
109. Piantadosi CA. Biological chemistry of carbon monoxide. Antiox. Redox Signaling. 2002; 4:259–270.
110. Kortemme T, Darby NJ, Creighton TE. Electrostatic interactions in the active site of the N-terminal thioredoxin-like domain of protein disulfide isomerase. Biochemistry. 1996; 35:14503–14511. [PubMed: 8931546]
111. Das TN, Huie RE, Neta P, Padmaja S. Reduction potential of the sulfhydryl radical: Pulse radiolysis and laser flash photolysis studies of the formation and reactions of ·SH and $HSSH^{\cdot-}$ in aqueous solutions. J. Phys. Chem. A. 1999; 103:5221–5226.
112. Pearson RG, Sobel H, Songstad J. Nucleophilic reactivity constants toward methyl iodide and trans-$[Pt(py)_2Cl_2]$. J. Am. Chem. Soc. 1968; 90:319–326.
113. Rao GS, Gorin G. Reaction of cystine with sodium sulfide in sodium hydroxide solution. J. Org. Chem. 1959; 24:749–753.
114. Cavallini D, Fererici G, Barboni E. Interactions of proteins with sulfide. Eur. J. Biochem. 1970; 14:169–174. [PubMed: 5447431]
115. Mueller EG. Trafficking in persulfides: Delivering sulfur in biosynthetic pathways. Nature Chem. Biol. 2006; 2:185–194. [PubMed: 16547481]
116. Massey V, Edmondson D. On the mechanism of inactivation of xanthine oxidase by cyanide. J. Biol. Chem. 1970; 245:6595–6598. [PubMed: 5536559]
117. Edmondson D, Massey V, Palmer G, Beacham LM III, Elion GB. The resolution of active and inactive xanthine oxidase by affinity chromatography. J. Biol. Chem. 1972; 247:1597–1604. [PubMed: 4335003]
118. Beinert H. Iron-sulfur proteins: Ancient structures, still full of surprises. J. Biol. Inorg. Chem. 2000; 5:2–15. [PubMed: 10766431]
119. Blackstone E, Morrison M, Roth MB. H_2S Induces a suspended animation-like state in mice. Science. 2005; 308:518. [PubMed: 15845845]
120. Francoleon NE, Carrington SJ, Fukuto JM. The reaction of H_2S with oxidized thiols: Generation of persulfides and implications to H_2S biology. Arch. Biochem. Biophys. 2011; 516:146–153. [PubMed: 22001739]
121. Branzoli U, Massey V. Evidence for an active site persulfide residue in rabbit liver aldehyde oxidase. J. Biol. Chem. 1974; 249:4346–4349. [PubMed: 4276457]
122. Agro AF, Mavelli I, Cannella C, Federici G. Activation of porcine heart mitochondrial malate dehydrogenase by zero valence sulfur and rhodanese. Biochem. Biophys. Res. Commun. 1976; 68:553–560. [PubMed: 1252245]
123. Sandy JD, Davies RC, Neuberger A. Control of 5-aminolaevulinate synthetase activity in Rhodopseudomonas spheroids. Biochem. J. 1975; 150:245–257. [PubMed: 1080999]

124. Kim EJ, Feng J, Bramlett MR, Lindahl PA. Evidence for a proton transfer network and a required persulfide-bond-forming cysteine residue in Ni-containing carbon monoxide dehydrogenase. Biochemistry. 2004; 43:5728–5734. [PubMed: 15134447]
125. deBeus MD, Chung J, Colon W. Modification of cysteine 111 in Cu/Zn superoxide dismutase results in altered spectroscopic and biophysical properties. Protein Sci. 2007; 13:1347–1355.
126. Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, Snyder SS. H_2S signals through protein S-sulfhydration. Sci. Signaling. 2009; 2:1–8.
127. Valentine WN, Toohey JI, Paglia DE, Nakatani M, Brockway RA. Modification of erythrocyte enzyme activities by persulfides and methanethiol: Possible regulatory role. Proc. Natl. Acad. Sci. U.S.A. 1987; 84:1394–1398. [PubMed: 3469673]
128. Wood JL. Sulfane sulfur. Methods Enzymol. 1987; 143:25–29. [PubMed: 3657542]
129. Hoffmann MR. Kinetics and mechanism of oxidation of hydrogen sulfide by hydrogen peroxide in acidic solution. Environ. Sci. Technol. 1977; 11:61–66.
130. Everett SA, Wardman P. Perthiols as antioxidants: Radical-scavenging and prooxidative mechanisms. Methods Enzymol. 1995; 251:55–69. [PubMed: 7651231]
131. Benson SW. Thermochemistry and kinetics of sulfur-containing molecules and radicals. Chem. Rev. 1978; 78:23–35.
132. March, J. In Advanced Organic Chemistry. 4th ed.. Wiley; New York: 1992. p. 351-352.
133. Chen KY, Morris JC. Kinetics of oxidation of aqueous sulfide by O_2. Environ. Sci. Technol. 1972; 6:529–537.
134. O'Brien DJ, Birkner FB. Kinetics of oxygenation of reduced sulfur species in aqueous solution. Environ. Sci. Technol. 1977; 11:1114–1120.
135. Neta P, Huie RE, Ross AB. Rate constants for reactions of inorganic radicals in aqueous solutions. J. Phys. Chem. Ref. Data. 1988; 17:1027–1284.
136. Mills G, Schmidt KH, Matheson MS, Meisel D. Thermal and photochemical reactions of sulfhydryl radicals. Implication for colloid photocorrosion. J. Phys. Chem. 1987; 91:1590–1596.
137. Hoffmann MR, Lim BC. Kinetics and mechanism of the oxidation of sulfide by oxygen: Catalysis by homogenous metal-phthalocyanine complexes. Environ. Sci. Technol. 1979; 13:1406–1414.
138. Hong AP, Boyce SD, Hoffmann MR. Catalytic autoxidation of chemical contaminants by hybrid complexes of cobalt(II) phthlaocyanine. Environ. Sci. Technol. 1989; 23:533–540.
139. Kotronarou A, Hoffmann MR. Catalytic aitoxidation of hydrogen sulfide in wastewater. Environ. Sci. Technol. 1991; 25:1153–1160.
140. Fukuto JM, Ignarro LJ. In vivo aspects of nitric oxide (NO) chemistry: Does peroxynitrite ($^-$OONO) play a major role in cytotoxicity? Acc. Chem. Res. 1997; 30:149–152.
141. Searcy DG, Whitehead JP, Maroney MJ. Interaction of Cu,Zn superoxide dismutase with hydrogen sulfide. Arch. Biochem. Biophys. 1995; 318:251–263. [PubMed: 7733652]
142. Asada K, Kanematsu S. Reactivity of thiols with superoxide radicals. Agric. Biol. Chem. 1976; 40:1891–1892.
143. Ondrias K, Stasko A, Cacanyiova S, Sulova Z, Krizanova O, Kristek F, Malekova L, Knezl V, Breier A. H2S and HS- donor NaHS releases nitric oxide from nitrosothiols, metal nitrosyl complex, brain homogenate and murine L1210 leukaemia cells. Pflugers Arch. Eur. J. Physiol. 2008; 457:271–279. [PubMed: 18458940]
144. Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong B-S, Cheung NS, Halliwell B, Moore PK. The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite ‘scavenger’? J. Neurochem. 2004; 90:765–768. [PubMed: 15255956]
145. Jourd'heuil D, Jourd'heuil FL, Feelisch M. Oxidation and nitrosation of thiols at low micromolar exposure to nitric oxide. J. Biol. Chem. 2003; 278:15720–15726. [PubMed: 12595536]
146. Hoshino M, Laverman L, Ford PC. Nitric oxide complexes of metalloporphyrins: An overview of some mechanistic studies. Coord. Chem. Rev. 1999; 187:75–102.
147. Collman JP, Brauman JL, Halbert TR, Suslick KS. Nature of O_2 and CO binding to metalloporphyrins and heme proteins. Proc. Natl. Acad. Sci. U.S.A. 1976; 73:3333–3337. [PubMed: 1068445]

148. Gullotti M, Santagostini L, Monzani E, Casella L. effect of strain in the proximal ligand on the binding of nitric oxide and carbon monoxide to chelated protoheme. Inorg. Chem. 2007; 46:8971–8975. [PubMed: 17845031]

149. Denninger JW, Marletta MA. Guanylate cyclase and the NO/cGMP signaling pathway. Biochim. Biophys. Acta. 1999; 1411:334–350. [PubMed: 10320667]

150. Boon EM, Marletta MA. Ligand discrimination in soluble guanylate cyclase and the H-NOX family of heme sensor proteins. Curr. Opin. Chem. Biol. 2005; 9:1–6.

151. Cary SPL, Winger JA, Derbyshire ER, Marletta MA. Nitric oxide signaling: No longer simply on or off. Trends Biochem. Sci. 2006; 31:231–239. [PubMed: 16530415]

152. Banerjee R, Zou C-G. Redox regulation and mechanism of human cystathionine β-synthase: a PLP-dependent hemesensor protein. Arch. Biochem. Biophys. 2005; 433:144–156. [PubMed: 15581573]

153. Taoka S, Banerjee R. Characterization of NO binding to human cystathionine β-synthase: Possible implications of the effects of CO and NO binding to the human enzyme. J. Inorg. Biochem. 2001; 87:245–251. [PubMed: 11744062]

154. Giulivi C, Kato K, Cooper CE. Nitric oxide regulation of mitochondrial oxygen consumption I: cellular physiology. Am. J. Physiol. Cell Physiol. 2006; 291:C1225–1231. [PubMed: 16885394]

155. Taylor CT, Moncada S. Nitric oxide, cytochrome c oxidase, and the cellular response to hypoxia. Arterioscler., Thromb., Vasc. Biol. 2010; 30:643–647. [PubMed: 19713530]

156. Shikama K. The molecular mechanism of autoxidation for myoglobin and hemoglobin: A venerable puzzle. Chem. Rev. 1998; 98:1357–1373. [PubMed: 11848936]

157. Yukl ET, de Vries S, Moenne-Loccoz P. The millisecond intermediate in the reaction of nitric oxide with oxymyoglobin is an iron(III)-nitrato complex, not a peroxynitrite. J. Am. Chem. Soc. 2009; 131:7234–7235. [PubMed: 19469573]

158. Eto K, Kimura H. A novel enhancing mechanism for hydrogen sulfide-producing activity of cystathionine β-synthase. J. Biol. Chem. 2002; 45:42680–42685. [PubMed: 12213817]

159. Taoka S, West M, Banerjee R. Characterization of the heme and pyridoxal phosphate cofactros of human cystathionine β-synthase reveals nonequivalent active sites. Biochemistry. 1999; 38:2738–2744. [PubMed: 10052944]

160. Semenza GL. Regulation of oxygen homeostasis by hypoxia-inducible factor 1. Physiology. 2009; 24:97–106. [PubMed: 19364912]

161. Semenza GL. Hydroxylation of HIF-1: Oxygen sensing at the molecular level. Physiology. 2004; 19:176–182. [PubMed: 15304631]

162. Metzen E, Ratcliffe PJ. HIF hydroxylation and cellular oxygen sensing. Biol. Chem. 2004; 385:223–230. [PubMed: 15134335]

163. Hirsila M, Koivunen P, Gunzler V, Kivirikko KI, Myllyharju J. Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. J. Biol. Chem. 2003; 278:30772–30780. [PubMed: 12788921]

164. Costas M, Mehn MP, Jensen MP, Que L Jr. Dioxygen activation at mononuclear nonheme iron active sites: enzymes, models and intermediates. Chem. Rev. 2004; 104:939–986. [PubMed: 14871146]

165. Clifton IJ, Hsueh L-C, Baldwin JE, Harlos K, Schofield CJ. Structure of proline-3-hydroxylase, evolution of the family of 2-oxoglutarate dependent oxygenases. Eur. J. Biochem. 2001; 268:6625–6636. [PubMed: 11737217]

166. Metzen E, Zhou J, Jelkmann W, Fandrey J, Brune B. Nitric oxide impairs normoxic degradation of HIF-1α by inhibition of prolyl hydroxylases. Mol. Biol. Cell. 2003; 14:3470–3481. [PubMed: 12925778]

167. Berchner-Pfannschmidt U, Yamac H, Trinidad B, Fandrey J. Nitric oxide modulates oxygen sensing by hypoxiainducible factor 1-dependent induction of prolyl hydroxylase 2. J. Biol. Chem. 2007; 282:1788–1796. [PubMed: 17060326]

168. Sumbayev VV, Yasinska IM. Mechanims of hypoxic signal transduction regulated by reactive nitrogen species. Scan. J. Immunol. 2007; 65:399–406.

169. Lahiri S, Roy A, Baby SM, Hoshi T, Semenza GL, Prabhakar NR. Oxygen sensing in the body. Prog. Biophys. Mol. Biol. 2006; 91:249–286. [PubMed: 16137743]

170. Sumbayev V,V, Budde A, Zhou J, Brune B. HIF-1α protein as a target for S-nitrosation. FEBS Lett. 2003; 535:106–112. [PubMed: 12560087]

171. Yasinska IM, Sumbayev VV. S-Nitrosation of Cys-800 of HIF-1α protein activates its interactionwith p300 and stimulates its transcriptional activity. FEBS Lett. 2003; 549:105–109. [PubMed: 12914934]

172. Li F, Sonveaux P, Rabbani ZN, Liu S, Yan B, Huang Q, Vujaskovic Z, Dewhirst MW, Li C-Y. Regulation of HIF-1α stability through S-nitrosylation. Mol. Cell. 2007; 26:63–74. [PubMed: 17434127]

173. Thomas DD, Espey MG, Ridnour LA, Hofseth LJ, Mancardi D, Harris CC, Wink DA. Hypoxic inducible factor 1α, extracellular signal-regulated kinase, and P53 are regulated by distinct treshol concentrations of nitric oxide. Proc. Natl. Acad. Sci. U.S.A. 2004; 101:8894–8899. [PubMed: 15178764]

174. Huang LE, Willmore WG, Gu J, Goldberg MA, Bunn HF. Inhibition of hypoxia-inducible factor 1 activation by carbon monoxide and nitric oxide. J. Biol. Chem. 1999; 274:9038–9044. [PubMed: 10085152]

175. Hagen T, Taylor CT, Lam F, Moncada S. Redistribution of intracellular oxygen n hypoxia by nitric oxide: Effect on HIF1α. Science. 2003; 302:1975–1978. [PubMed: 14671307]

176. D'Amico G, Lam F, Hagen T, Moncada S. Inhibition of cellular respiration by endogenously produced carbon monoxide. J. Cell Sci. 2006; 119:2291–2298. [PubMed: 16723735]

177. Siddiq A, Aminova LR, Ratan RR. Hypoxia inducible factor prolyl 4-hydroxylase enzymes: Center stage in the battle against hypoxia, metabolic compromise and oxidative stress. Neurochem. Res. 2007; 32:931–946. [PubMed: 17342411]

178. Kohl R, Zhou J, Brune B. Reactive oxygen species attenuate nitric oxide-mediated hypoxia-inducible factor-1α stabilization. Free Radical Biol. Med. 2006; 40:1430–1442. [PubMed: 16631533]

179. Brune B, Zhou J. Nitric oxide and superoxide: Interface with hypoxic signaling. Cardiovasc. Res. 2007; 75:275–282. [PubMed: 17412315]

180. Sumbayev VV, Yasinska IM. Peroxynitrite as an alternative donor of oxygen in HIF-1α proline hydroxylation under low oxygen availability. Free Radical Res. 2006; 40:631–635. [PubMed: 16753841]

181. Chin BY, Jiang G, Wegiel B, Wang HJ, MacDonald T, Zhang XC, Gallo D, Cszimadia E, Bach FH, Lee PJ, Otterbein LE. Hypoxia-inducible factor 1α stabilization by carbon monoxide results in cytoprotective preconditioning. Proc. Natl. Acad. Sci. U.S.A. 2007; 104:5109–5114. [PubMed: 17360382]

182. Budde MW, Roth MB. Hydrogen sulfide increases hypoxia-inducible factor-1 activity independently of von Hippel-Lindau tumot suppressor-1 in C. elegans. Mol. Biol. Cell. 2010; 21:212–217. [PubMed: 19889840]

183. Truong DH, Eghbal MA, Hindmarsh W. Molecular mechanisms of hydrogen sulfide toxicity. Drug Metab. Rev. 2006; 38:733–744. [PubMed: 17145698]

184. Liu X, Pan L, Zhuo Y, Gong Q, Rose P, Zhu Y. Hypoxia-Inducible factor-1α is involved in the pro-angiogenic effect of hydrogen sulfide under hypoxic stress. Biol. Pharm. Bull. 2010; 33:1550–1554. [PubMed: 20823573]

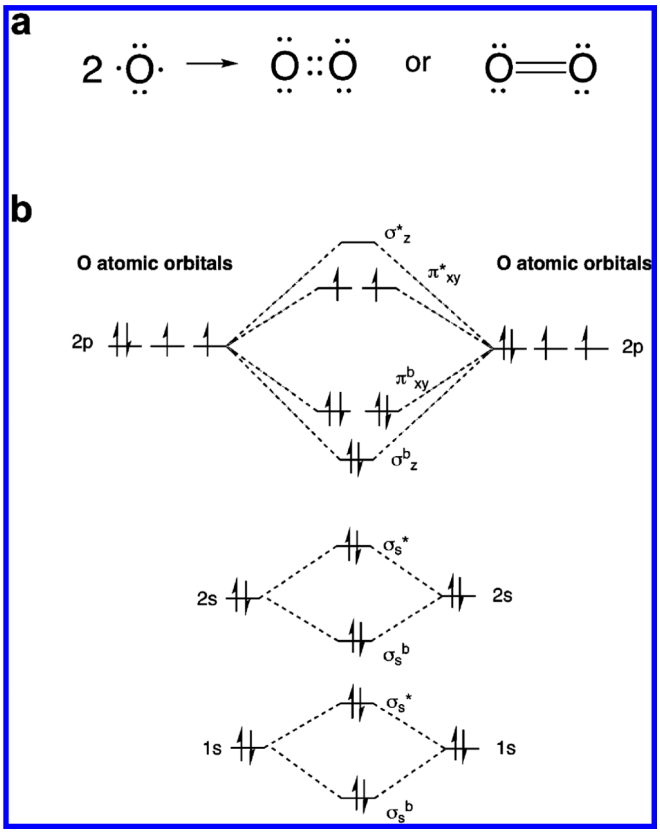


Figure 1.
(a) Lewis and valence bond depiction of O_2. (b) Molecular orbital diagram for O_2.

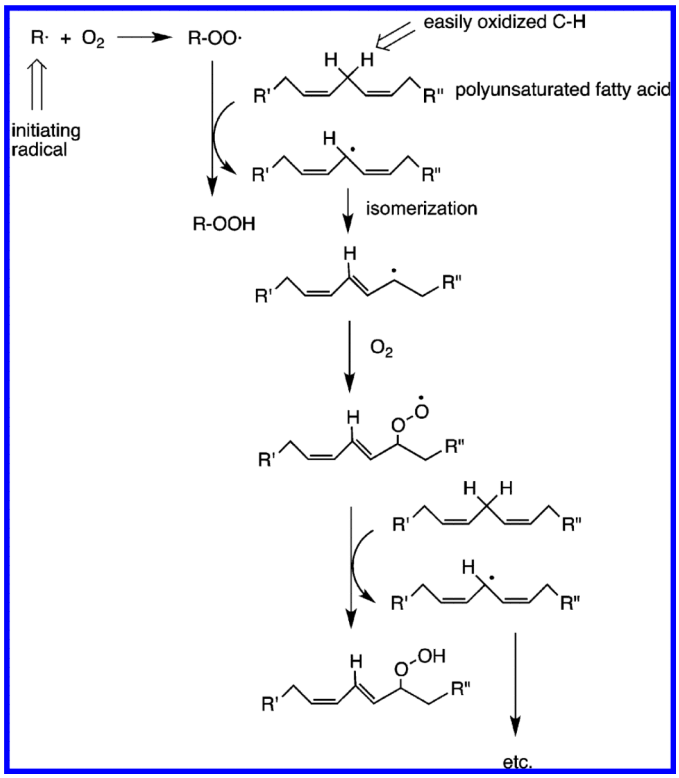


Figure 2.
Lipid peroxidation. Numerous oxidized products can be generated. Only the simplest alkylperoxide product is shown.

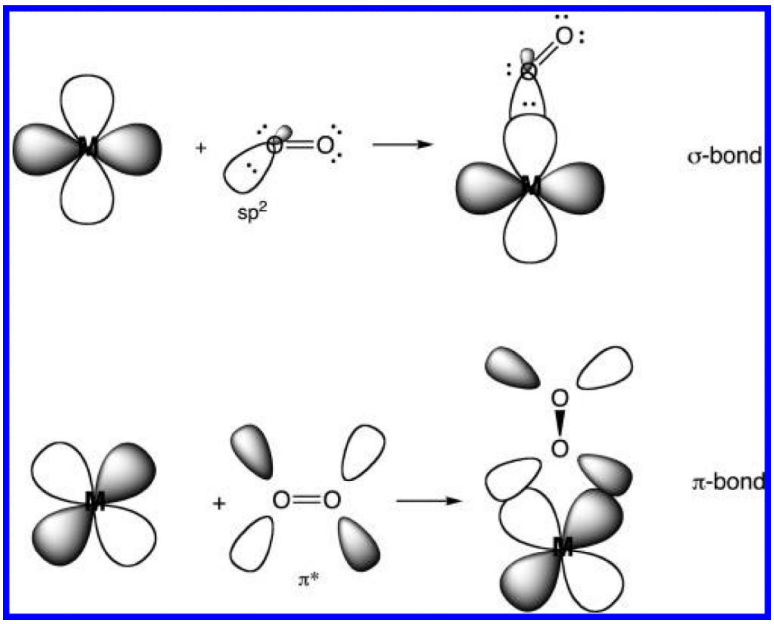


Figure 3.
Bonding schemes for end-on O_2 binding to metals.

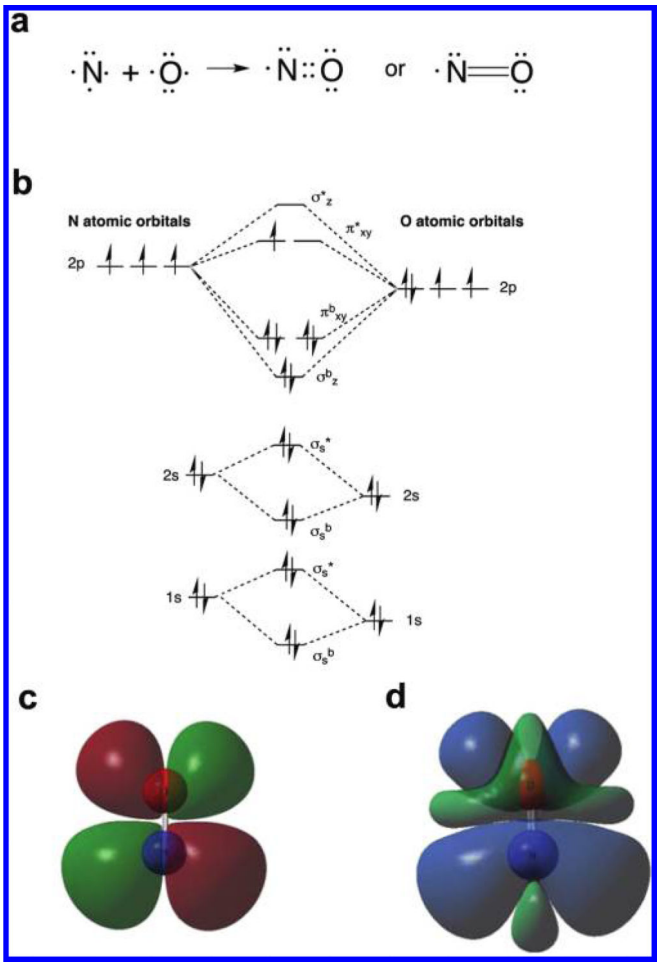


Figure 4.

(a) Lewis structure/valence bond depiction of NO, (b) molecular orbital diagram for NO, (c) singly occupied molecular orbital (SOMO) of NO, and (d) NO spin density. Note: panels c and d were calculated at the CCSD(T)/6-311++G(3df,3pd)//MP2/ 6-311++G(3df,3pd) level (isovalues of 0.0004 and 0.02, respectively).

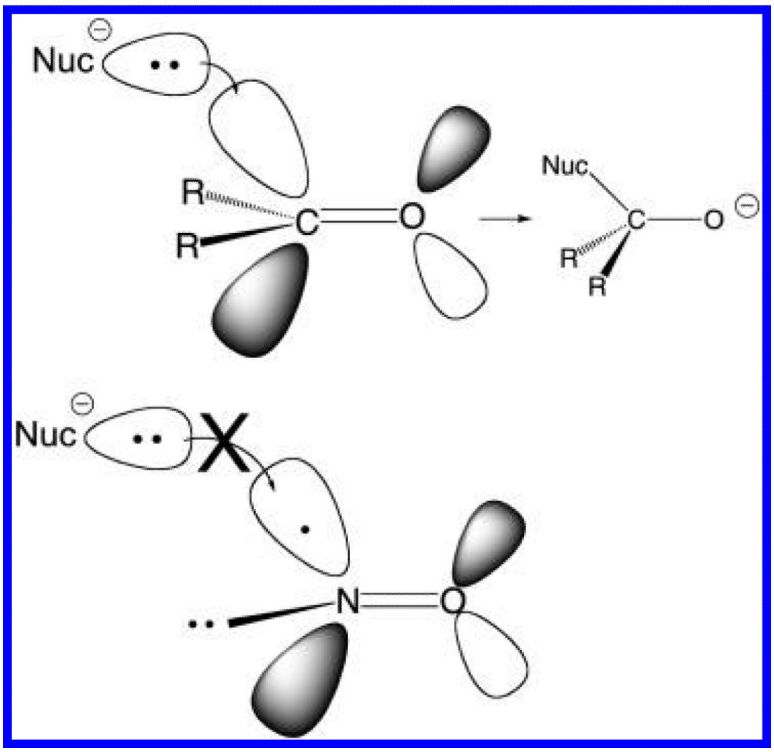


Figure 5.

Attack of a nucleophile on a carbonyl versus attack on NO (note: electron in the NO π^* orbital is not localized in the lobe shown but is distributed throughout the π^* orbitals).

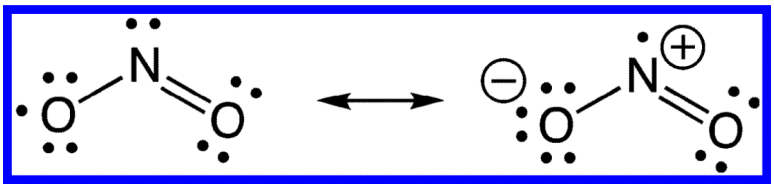


Figure 6.
Valence bond depiction of NO_2 (note: only two of several resonance forms are shown).

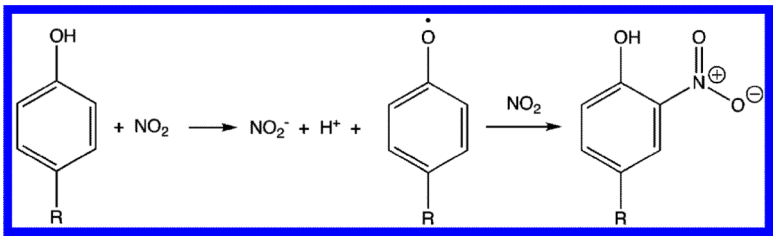


Figure 7.
Oxidation of substituted phenol by NO_2.

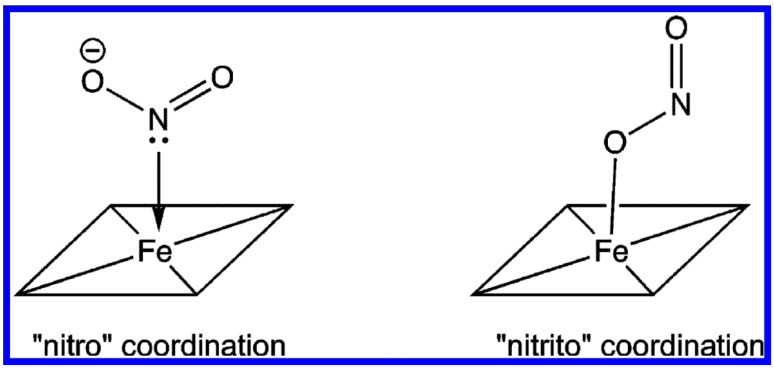


Figure 8.
Nitro and nitrito coordination of NO_2^- to the iron center.

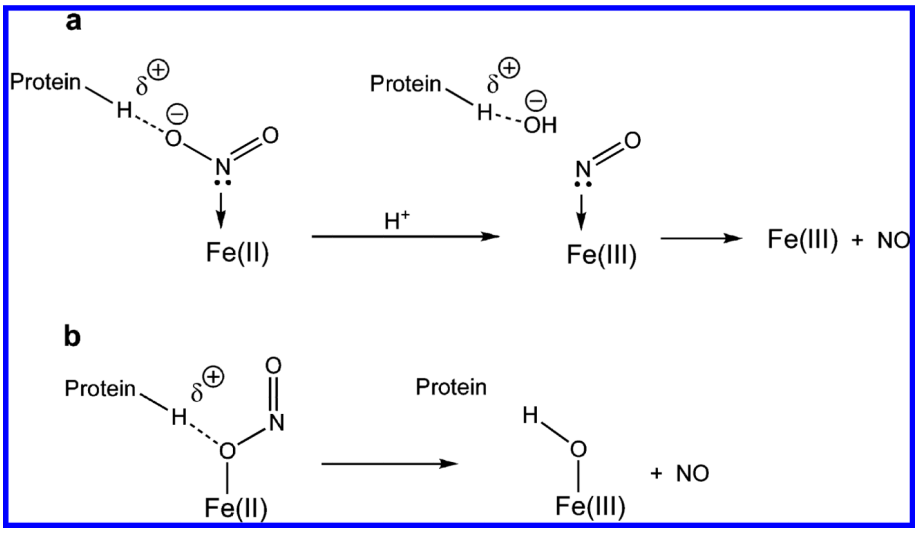


Figure 9.

Possible mechanisms for the generation of NO via the reduction of ferrous-heme-bound nitrite. (a) Ferrous ion reduction of the nitro complex and (b) reduction of the nitrito complex.

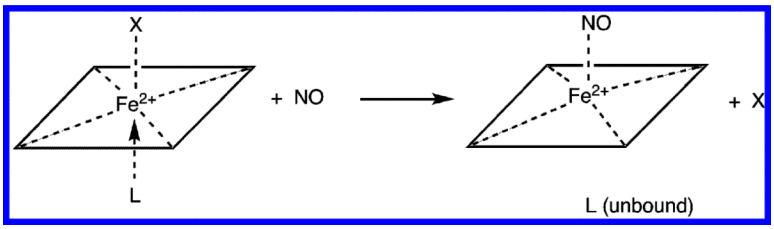


Figure 10.
Proximal ligand release via the coordination of NO to a ferrous heme.

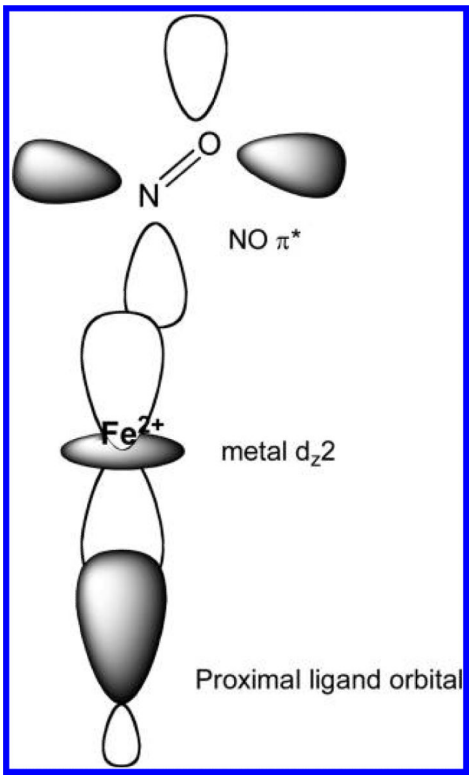


Figure 11.
Donation of electrons to the metal from the NO π^* orbital in the bent geometry leading to a weakening of the trans-ligand bond (note: other bonding interactions are not shown).

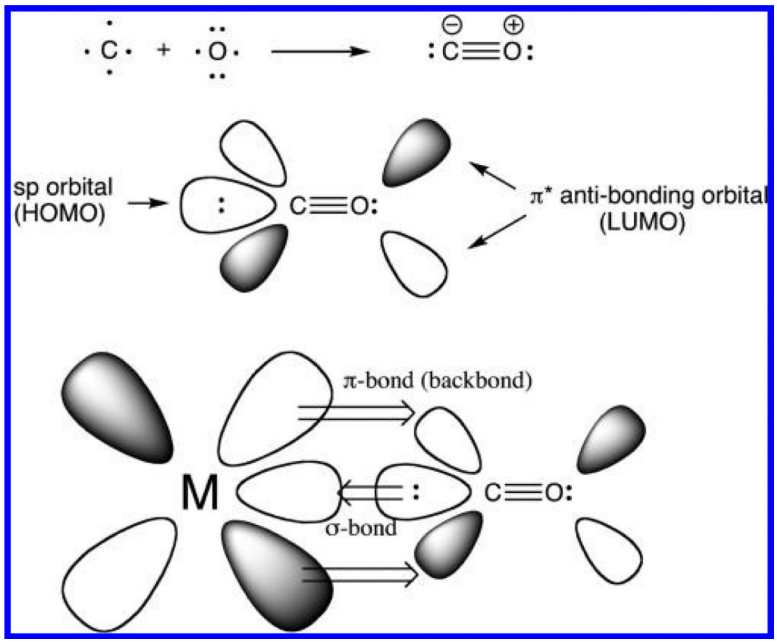


Figure 12.
Bonding in CO and CO−metal complexes.

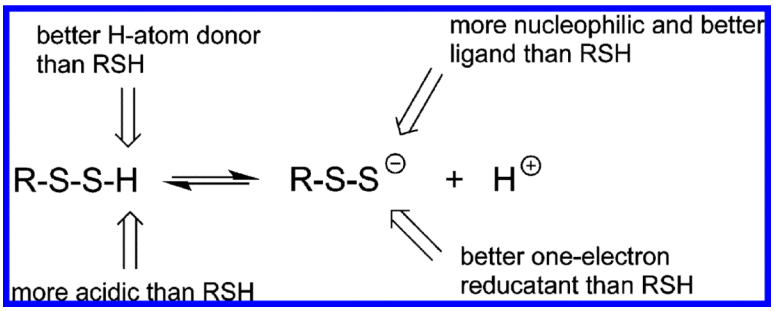


Figure 13.
Enhanced chemical properties of persulfides compared to thiols.

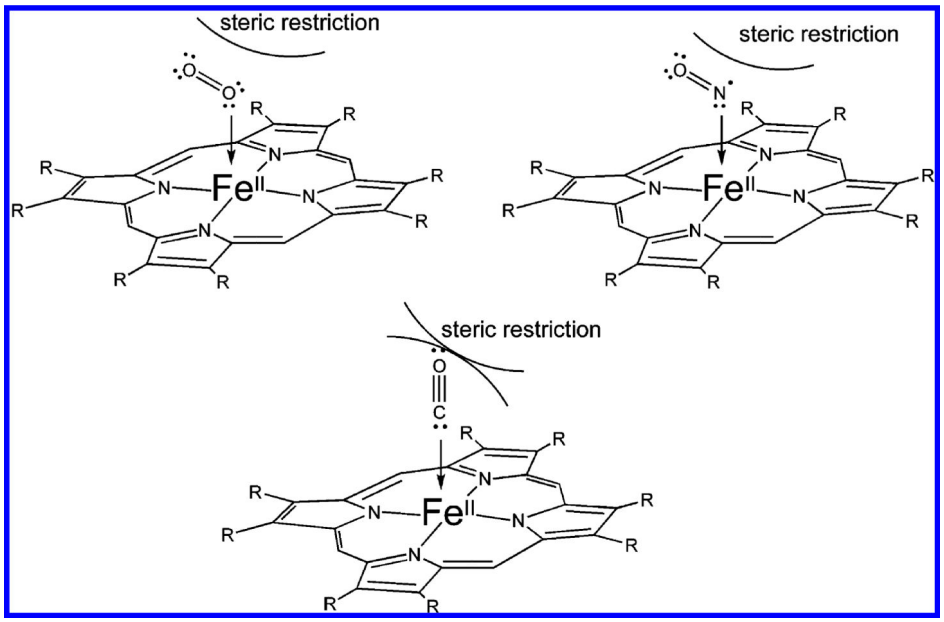


Figure 14.

Binding geometries of O_2, NO, and CO to a ferrous heme protein. Preferred linear binding of CO causes steric crowding that is thought to inhibit binding.

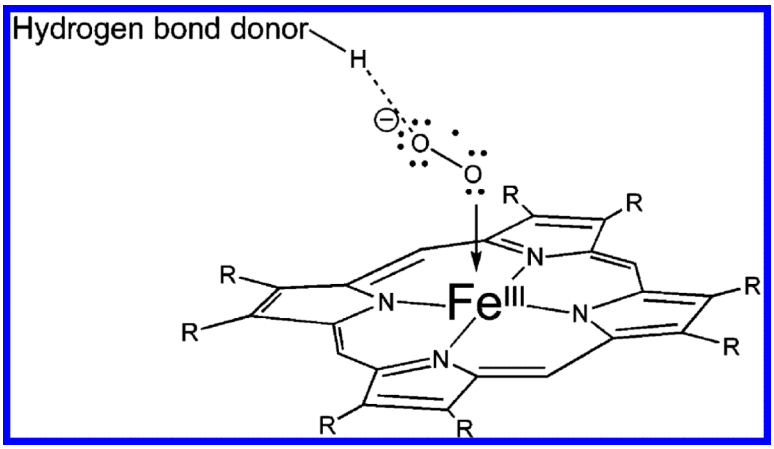


Figure 15.
Hydrogen bond stabilization of the heme Fe^{3+}-O_2^- complex.

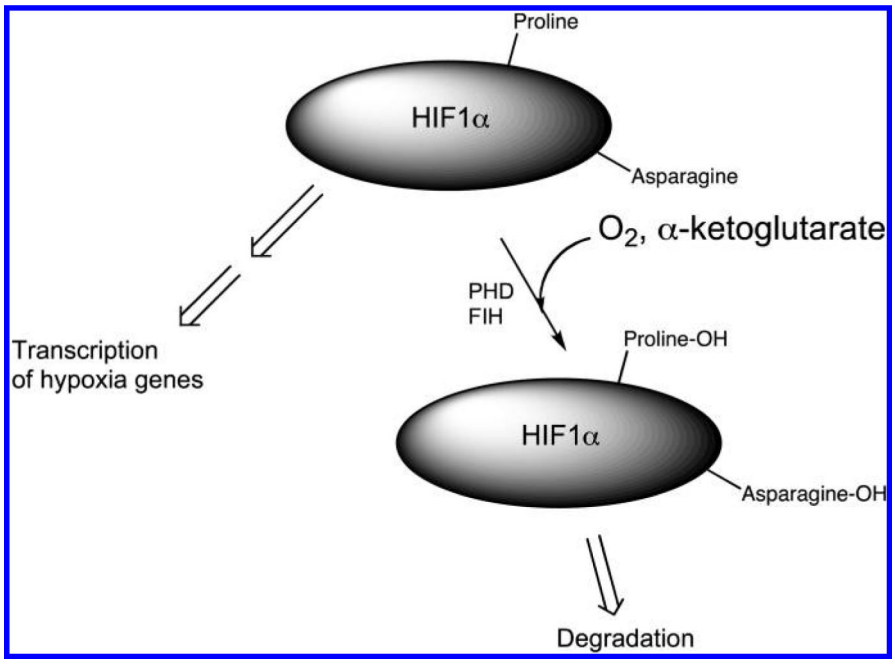


Figure 16.
Simplistic scheme for HIF1a regulation by O_2 via PHD and FIH.

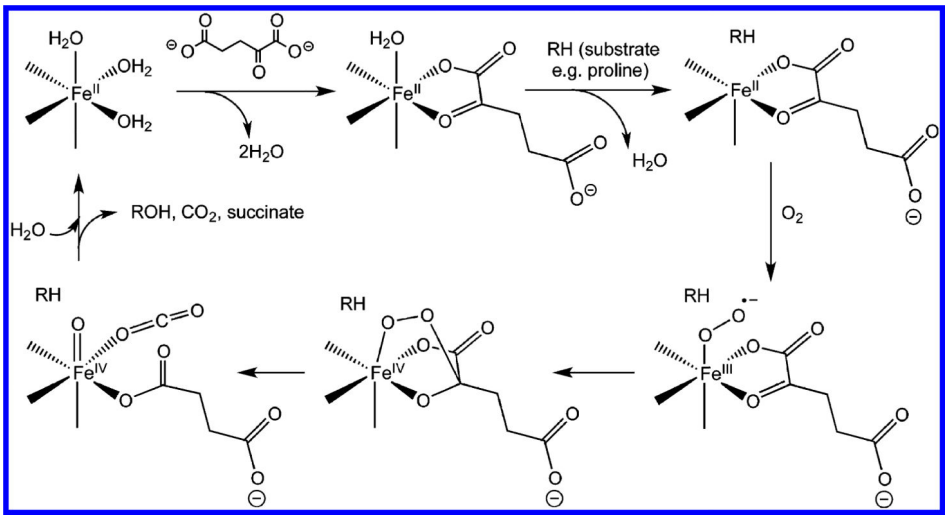


Figure 17.
General mechanism for 2-oxoglutarate-dependent, nonheme iron hydroxylases.