PERSPECTIVE

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# The chemistry and biochemistry of the copper–radical interaction

**Wolfgang Kaim**
*Institut für Anorganische Chemie der Universität, Pfaffenwaldring 55, D-70550 Stuttgart, Germany. E-mail: kaim@iac.uni-stuttgart.de*

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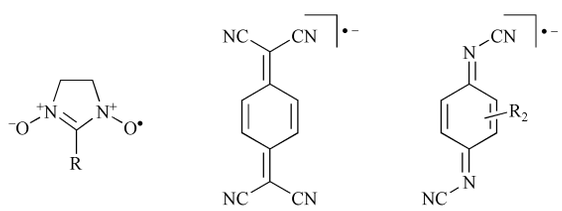
**This Perspective describes the plausible oxidation state combinations of $Cu^{n}/L^{m}$ systems where $L^{m}$ is an O- or N-based redox system with a paramagnetic intermediate such as $O_2^{\bullet -}$, $NO^{\bullet}$, phenoxyl, *o*-semiquinone or azo radical anion. The biochemical relevance is discussed using superoxide dismutase, nitrite reductase, galactose oxidase and amine oxidase enzyme examples. Particular emphasis will be given to the identification of oxidation states and to the manifestations of intramolecular electron transfer in enzymes and model compounds.**

## 1. Introduction

Copper can adopt oxidation states ranging between +I and +III in its stable coordination compounds.[1] Physiologically relevant are copper(I) and—only within the context of electron transfer reactivity (not hydrolysis!)—copper(II).[2,3] While the possible occurrence of copper(III) is discussed here in sections 2 and 4 within the context of galactose oxidase and tyrosinase enzyme action, the copper(0) attribute has sometimes been misleadingly used for copper(I) complexes of one-electron reduced non-innocent ligands (section 5).[4] When surveying the known copper enzymes and their functions[2,3] it is striking that their reactivity is typically linked to dioxygen or compounds directly synthesised from $O_2$ (nitrogen oxides, phenols, quinones) and that many reactions obviously involve radicals. It is essential for the special interaction between copper oxidation states and these O or N containing radicals (organic or inorganic) that the redox potential for the central $Cu^{II}/Cu^{I}$ couple typically lies around 0 V, *i.e.* in a similar range as the potentials of the biologically important redox couples $O_2/O_2^{\bullet -}/O_2^{2-}$, $NO^{+}/NO^{\bullet}/NO^{-}$, phenoxyl/phenolate, or *o*-quinone/*o*-semiquinone/catecholate. Obviously, this leads to a variety of interesting oxidation state combinations in the corresponding complexes, the relevance of which will be discussed in the following.

This Perspective is meant to particularly point out parallels between the results from recent biochemical discoveries and the relevant chemical reactivity found in "model systems". The interplay between radicals and copper complexes has also great significance for organic transformations (including technical catalysis)[5] and in the very active new field of molecular magnetism where spin–spin interactions between paramagnetic copper centres and radical ligands such as aminoxyls, imino nitroxides, nitronyl nitroxides ($NIT^{\bullet}$), $TCNQ^{\bullet -}$ or $DCNQI^{\bullet -}$ are being studied (Scheme 1);[6–8] however, these research areas are outside the scope of this article.




**Scheme 1**

The distinction in chemistry between inorganic and organic species, although often useful, is not inevitably justified from a target-oriented approach to chemical reactivity. Thus, it is now obvious that organisms make use of inorganic elements in addition to "organic" material.[2] Moreover, it is often just the functional cooperativity between organic *and* inorganic components in complex proteins that allows the performance of sophisticated catalysis or the fabrication of composite materials. A long established case in point is the heme group with its $O_2$ transport and widely variegated enzymatic functions where the substrate-binding "inorganic" iron centre ($Fe^{II}$, $Fe^{III}$, $Fe^{IV}$) and the non-innocent "organic" porphinato ligand ($P^{\bullet 3-}$, $P^{2-}$, $P^{\bullet -}$) interact in a most intricate way to bring about very specific chemical reactivity.[2,9]

Whereas the heme structure, spectroscopy and function is fairly well established,[9] there are similar such metal–ligand cooperativities in biochemistry which have been unravelled only recently. Besides the pterin/molybdenum or pterin/tungsten interaction[10] another such metal/ligand coupled system involves copper and radical-forming amino acid derivatives in the copper-dependent oxidoreductases galactose oxidase and amine oxidase. We shall describe two such systems below (sections 4 and 5) after discussing two examples with inorganic substrate radical intermediates, *viz*., superoxide dismutase and nitrite reductase. The important role of radicals in many areas

*Wolfgang Kaim graduated in 1974 from the universities of Frankfurt/Main and Konstanz and obtained his Ph.D. degree in 1978, following work with Hans Bock in main group organometallic chemistry. After a postdoctoral year with F. A. Cotton at Texas A&M University he returned to Frankfurt in 1979 and developed research in the areas of heterocycles, coordination compounds and bioinorganic model systems. Studies of charge and electron transfer processes led to the extensive application of coupled electrochemical and spectroscopic methods (UV-vis-NIR, IR, EPR). In 1987 he moved to the University of Stuttgart to take the Chair of Inorganic Chemistry. He is coauthor of an award-winning textbook on bioinorganic chemistry and of about 400 research publications, his main current interests include charge transfer and electron transfer phenomena in transition metal compounds with electroactive ligands.*

**Wolfgang Kaim**

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of enzymatic catalysis has been recognised only rather recently,[11] based on improved techniques for the generation, isolation and physical studies of corresponding intermediates.

The aim of this article is to provide an overview from a wider perspective. While all efforts were made to refer to the most recent relevant literature, a detailed treatment of spectroscopic, magnetic or corresponding theoretical studies is outside the scope of this review. Generally accepted results are used as arguments in the discussion of the chemistry. Similarly, detailed reaction mechanisms are not presented because such hypothetical schemes can become rapidly obsolete following new experimental results. Instead, the possible roles of ligand-based radicals and their environment for enzymatic catalysis are highlighted, as is the (partial) model character of non-enzymatic analogues.

## 2. Superoxide dismutases and the $O_2/O_2^{\bullet -}/O_2^{2-}$ redox system

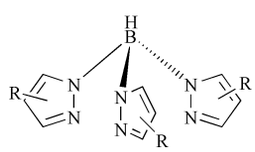
Although enzymes for "superoxide dismutation" (the disproportionation of metastable hyperoxide $O_2^{\bullet -}$) can involve iron, manganese, nickel or copper as the essential redox centre the Cu,Zn-containing superoxide dismutase (SOD) from erythrocytes has been studied most extensively with respect to structure and mechanism.[12] In the oxidised form both divalent metal ions are bridged in a unique fashion by a deprotonated imidazole ring from a histidine side chain, the other ligands of the copper centre are three "normal" histidines (type 2 copper).[2] The roles of redox-inactive $Zn^{2+}$, of protons and of organic components such as the special histidinato ligand remain to be fully established, however, there is general consensus about the function of copper along eqns. (1) and (2):[2,3,12]

$$Cu^{II} + O_2^{\bullet -} \longrightarrow Cu^{I} + O_2 \qquad (1)$$

$$Cu^{I} + O_2^{\bullet -} \longrightarrow Cu^{II} + O_2^{2-} \qquad (2)$$

where $E(O_2/O_2^{\bullet -}) = -0.33$ V and $E(O_2^{\bullet -}/H_2O_2) = +0.89$ V (pH 7, NHE).

The very rapid reaction is controlled and accelerated by the action of $H^+$ *e.g.* for adding to the peroxide product or to the histidinato ligand during the catalytic cycle. In the absence of such rate-enhancing effects the copper coordination chemistry with $O_2^{\bullet -}$ and its neighbouring oxidation states has been investigated in connection with other copper enzymes (oxidases) using $O_2$ as a substrate.[13] Within such studies only one (EPR-silent) superoxide copper(II) complex has been structurally characterised with a side-on ($\eta^2$) bonded $O_2^{\bullet -}$ ligand and a sterically shielding hydridotrispyrazolylborato (Tp) ligand (Scheme 2), rendering the metal five-coordinate.[14,15] The comparatively[2b] small O–O distance of 1.22(3) Å may reflect the unusual $\eta^2$ coordination mode.



**Scheme 2**

Generally, such systems are believed to be intermediates *en route* from adducts between $Cu^{I}$ precursors and $O_2$ to the better accessible dinuclear species involving peroxide or oxide ligands.[16] The corresponding alternatives are shown in eqn. (3) with the established cases in bold.

The reduced forms (4) have been less well documented. The poor binding between "soft" copper(I) and "hard" $O_2^{\bullet -}$ and their propensity for rapid electron transfer has prompted us to modify that state in order to obtain persistent $Cu^{I}$ radical

$$Cu^{I}(O_2)Cu^{I} \longleftrightarrow Cu^{I}(O_2^{\bullet -})Cu^{II} \longleftrightarrow \mathbf{Cu^{II}(O_2^{2-})Cu^{II}} \longleftrightarrow \mathbf{Cu^{III}(O^{2-})_2Cu^{III}} \quad (3)$$

$$-e^- \rightleftharpoons +e^-$$

$$Cu^{I}(O_2^{\bullet -})Cu^{I} \longleftrightarrow Cu^{I}(O_2^{2-})Cu^{II} \qquad (4)$$

complexes. Using the familiar chemical analogy between "isolobal" O and NR groups we have been interested for some time in the coordination chemistry of non-innocent azo ligands.[17] The formal analogy between the dioxygen and azo redox systems is given in eqns. (5) and (6):

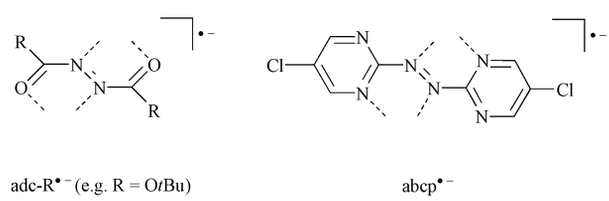
$$\mathbf{O} \quad O_2 \overset{e^-}{\rightleftharpoons} O_2^{\bullet -} \overset{e^-}{\rightleftharpoons} O_2^{2-} \qquad (5)$$

dioxygen — hyperoxide (superoxide) — peroxide

$$\mathbf{NR} \quad RN{=}NR \overset{e^-}{\rightleftharpoons} (RN\text{-}NR)^{\bullet -} \overset{e^-}{\rightleftharpoons} (RN\text{-}NR)^{2-} \qquad (6)$$

azo compound (diazene derivative) — diorganohydrazido(2–)

Despite the formal analogy the two redox systems differ significantly in terms of redox potentials and thus preferred oxidation states and radical reactivity [mainly oxidising $O_2^{\bullet -}$, predominantly reducing $(RNNR)^{\bullet -}$]. With coordinating acceptor substituents R = C(O)O$^t$Bu[18] (adc-O$^t$Bu$^{\bullet -}$) and 2-(5-chloropyrimidyl)[19] (abcp$^{\bullet -}$, Scheme 3) two kinds of



adc-R$^{\bullet -}$ (e.g. R = O$t$Bu) abcp$^{\bullet -}$

**Scheme 3**

bis-chelate complexes with such radical ligands $(RNNR)^{\bullet -}$ and diphosphinecopper(I) species were structurally characterised. The N–N bond lengths between 1.24 and 1.35 Å were found at similar values as the O–O bond distances in $O_2^{\bullet -}$ species.[1,19] EPR studies at variable frequencies also confirmed the radical formulation (7)

$$\mathbf{Cu^{I}(RNNR^{\bullet -})Cu^{I}} \leftrightarrow Cu^{I}(RNNR^{2-})Cu^{II} \qquad (7)$$

with rather little metal contribution as evident from the small $^{63,65}$Cu hyperfine coupling ($a$ < 2 mT) and little $g$ anisotropy ($g_1 - g_3 < 0.02$), Fig. 1.[18–20] Copper(II) systems typically have $a(^{63,65}Cu) > 5$ mT and $g_1 - g_3 > 0.1$ ($^{63}$Cu: 69.2% natural

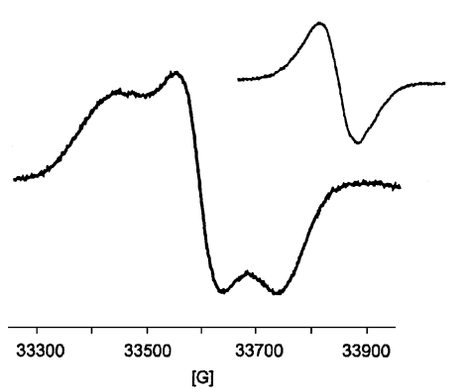


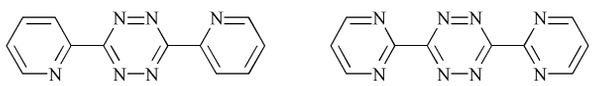

**Fig. 1** EPR spectra of {(μ-abcp)[Cu(PPh$_3$)$_2$]$_2$}(BF$_4$) at 110 K in dichloromethane at X band frequency (9.5 GHz, insert) and at W band frequency (95 GHz): $g_1 = 2.016$, $g_2 = 2.0065$, $g_3 = 1.998$.[19]

abundance, $I$ = 3/2; $^{65}$Cu: 30.8%, $I$ = 3/2; $A(^{65}Cu)/A(^{63}Cu)$ = 1.07).

Similarly stable dicopper(I) complexes could be obtained in straightforward comproportionation reactions (8) with azo-containing tetrazine ligands, followed by spectroscopic and structural identification.[21]

$$2bxtz + 8PPh_3 + 3Cu + Cu^{2+} \longrightarrow 2[(bxtz^{\bullet -})Cu^{I}_2(PPh_3)_4]^+ \quad (8)$$

where bxtz represents bptz [3,6-bis(2-pyridyl)-1,2,4,5-tetrazine] or bmtz [3,6-bis(2-pyrimidyl)-1,2,4,5-tetrazine], Scheme 4.



bptz bmtz

**Scheme 4**

## 3. Nitrite reductase and the $NO^+/NO^{\bullet}/NO^-$ redox system

Some nitrite reducing microorganisms use copper-dependent enzymes instead of heme proteins to catalyse process (9).[22]

$$2NO_2^- + 4e^- + 6H^+ \longrightarrow N_2O + 3H_2O \quad (9)$$

The structurally characterised trimeric enzyme from *Achromobacter cycloclastes*[23a] exhibits type 2[3] copper centres as the presumed active sites.[23b] The proposed nitrosyl-copper intermediates[3,23a] prompted the preparation and investigation of synthetic analogues, again stabilised as mononuclear species through heavily substituted hydridotrispyrazolylborato ligands.[24,25] Detailed EPR studies in conjunction with MO calculations[25] showed that within system (10) the NO radical-centred formulation $Cu^I$–$NO^{\bullet}$ is dominant with $g_{1,2} \approx 1.99$, $g_3 \approx 1.83$, $A_{1,2}(^{14}N) \approx 3$ mT, and a relatively small $^{63,65}$Cu hyperfine coupling. Heterogeneously adsorbed nitrosyl[25] or complexes of neither strongly reducing or oxidising $NO^{\bullet}$ with $[(NC)_5M^{II}]^{3-}$, M = Fe, Ru,[26] show similar $g$ and $A(^{14}N)$ characteristics whereas $NO^+$ as an ancillary non-radical ligand exhibits isotropic $a(^{14}N)$ values of less than 0.5 mT.[27]

$$Cu^{o}\text{-}NO^{+} \longleftrightarrow \mathbf{Cu^{I}\text{-}NO^{\bullet}} \longleftrightarrow Cu^{II}\text{-}NO^{-} \quad (10)$$

$$+e^- \rightleftharpoons -e^-$$

$$Cu^{I}\text{-}NO^{+} \longleftrightarrow Cu^{II}\text{-}NO^{\bullet} \longleftrightarrow Cu^{III}\text{-}NO^{-} \quad (11)$$

The copper(II) form in (10) seems also plausible. $NO^-$ has been postulated as ligand in an NO-bridged dicopper(II) complex[28a,b] and in $[Fe(NO)(H_2O)_5]^{2+}$.[28c] However, the lability of the N,O compounds opens up biochemically relevant possibilities such as the disproportionation (12) of the $Cu^I/NO^{\bullet}$ combination[24b] to yield the nitrous oxide product (*cf.* 9) while the oxidised alternative (11) is capable of yielding free $NO^{\bullet}$ from a dissociation reaction (13).[29]

$$LCu^{I} + 3\,NO^{\bullet} \longrightarrow LCu^{II}(NO_2) + N_2O \quad (12)$$

$$L\text{-}Cu^{I}(NO_2) + 2\,H^+ \rightleftharpoons H_2O + [L\text{-}Cu^{I}(NO)]^{2+} \quad (13)$$

$$\downarrow$$

$$[L\text{-}Cu^{II}]^{2+} + NO^{\bullet}$$

where L represents a 1,4,7-triazacyclononane derivative or the enzyme pocket.

Controlled pathways for $NO^{\bullet}$ release are of great interest in view of the wide-ranging physiological effects of this messenger molecule.

## 4. Galactose oxidase and the phenoxyl/phenolate ($Y^{\bullet}/Y^-$) redox couple

Copper–phenolate interactions are relevant not only for synthetic and technical processes (oxidation, polymerisation)[5] but have been increasingly invoked in biochemical transformations. These include the tyrosinase activity of several enzymes (oxidation to catechols)[30] and the reaction (14) catalysed by galactose oxidase,[31] the two-electron conversion of primary alcohols to aldehydes using dioxygen.

$$RCH_2OH + O_2 \longrightarrow RCHO + H_2O_2 \quad (14)$$

A related enzyme, glyoxal oxidase, has been shown to have a rather similar reactivity (15) and active site composition.[32]

$$RCHO + O_2 + H_2O \longrightarrow RCO_2H + H_2O_2 \quad (15)$$

For some time a mononuclear copper(III) intermediate had been postulated[33] to account for the two-electron catalysis before detailed spectroscopic and finally structural studies established the interaction between the $Cu^I/Cu^{II}$ couple and a special tyrosyl/tyrosinate ligand[32] (Scheme 5) according to (16–18) in the enzymatic reaction (14),[31]

$$\mathbf{Cu^{II}\text{-}Y^{\bullet}} \longleftrightarrow Cu^{III}\text{-}Y^- \quad (16)$$

$$-e^- \rightleftharpoons +e^-$$

$$Cu^{I}\text{-}Y^{\bullet} \longleftrightarrow \mathbf{Cu^{II}\text{-}Y^-} \quad (17)$$

$$-e^- \rightleftharpoons +e^-$$

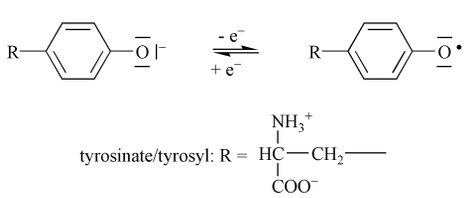
$$\mathbf{Cu^{I}\text{-}Y^-} \quad (18)$$

where YH = a phenol derivative. $E$(tyrosyl/tyrosinate) = +0.95 V. Galactose oxidase enzyme data:

$$E(Cu^{II}\text{–}Y^{\bullet}/Cu^{II}\text{–}Y^-) = +0.41\ V$$
$$E(Cu^{II}\text{–}Y^-/Cu^{I}\text{–}Y^-) = +0.16\ V\ (pH\ 7,\ NHE)$$

The fully oxidized form contains a copper(II) centre magnetically coupled with a tyrosyl radical in one of the equatorial positions of a tetragonal pyramidal arrangement which is completed by two histidines and a substrate docking position in the equatorial plane and a regular tyrosinate in the apical position. The special tyrosyl (Y272) is stabilised through covalent linking with a cysteine residue in the *ortho* position and through π–π stacking with the heterocycle from a tryptophane (Fig. 2).[3,31]

The occurence of copper(III) in a biochemical connection remains sketchy, the postulate of synthetically established dinuclear $Cu^{III}(\mu\text{-}O)_2Cu^{III}$ (Scheme 6) as an alternative (3) to



**Scheme 5**

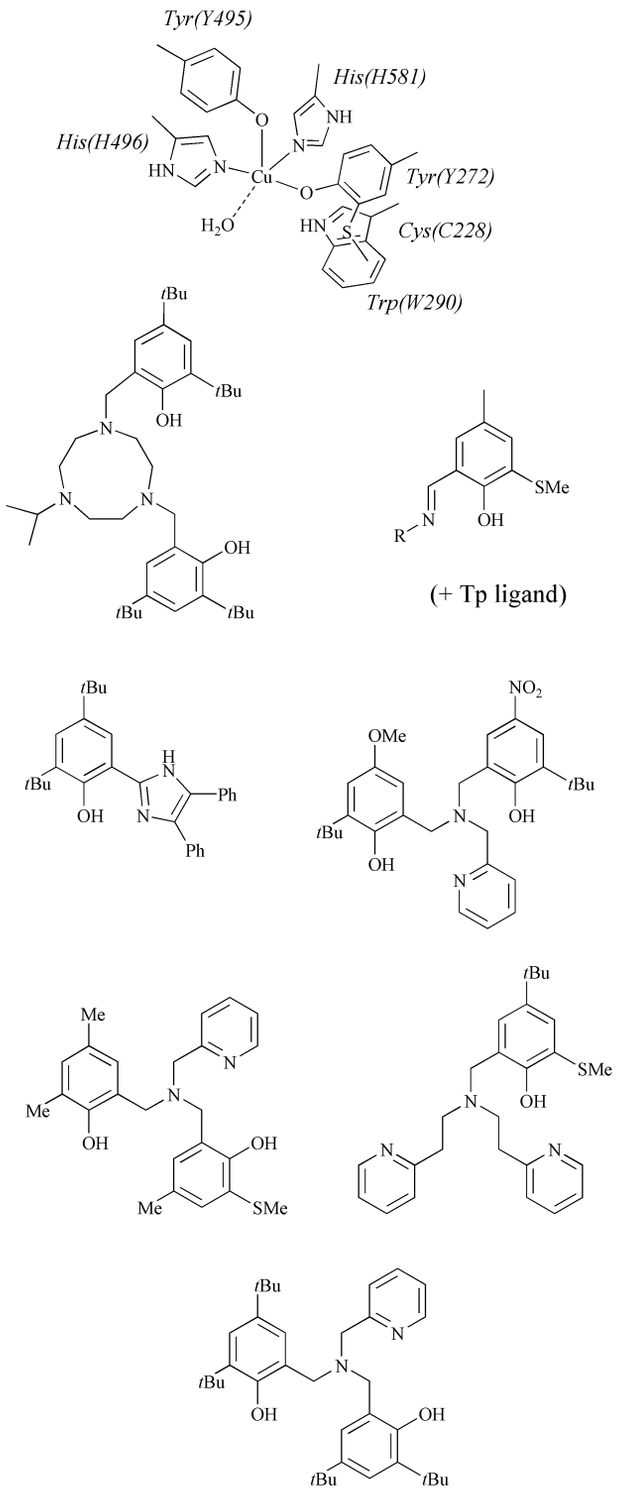
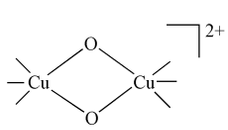



**Fig. 2** Active site of of galactose oxidase and representative ligands (phenol forms) of model complexes.[36]



**Scheme 6**

$Cu^{II}(\mu\text{-}\eta^2\text{:}\eta^2\text{-}O_2)Cu^{II}$ as reactive intermediate in tyrosinase enzyme activity continues to be investigated experimentally and theoretically.[34,35]

The presumed mechanism of galactose oxidase reactivity includes alcohol substrate binding, deprotonation and oxidation by the $Cu^{II}$–$Y^{\bullet}$ form, followed by its regeneration through binding of $O_2$ to $Cu^{I}$–$Y^{-}$ and formation of $H_2O_2$.[3,31]

Several model studies regarding the galactose oxidase structure and function, especially metal–phenoxyl interaction, have been put forward and reviewed over the last few years (Fig. 2).[36]

Stabilisation through chelate coordination, sulfur involvement and steric shielding have been employed to mimic the unusual persistence of the copper(II)–phenoxyl combination.[32] The question of spin–spin coupling and its conformational dependence was discussed especially by Wieghardt's group,[37a] considering the antiferromagnetic coupling and EPR silence of the oxidised state of the native enzyme.[31,32] Additional identification of coordinated phenoxyl and related species can come from DFT-supported structural and vibrational studies[37b,c] which reveal a typical alternance of C–O and intra-ring bond lengths in agreement with a *p*-quinonoid resonance structure and a singly occupied molecular orbital (SOMO) of the $\pi^*_{sym}$ type (Fig. 3).

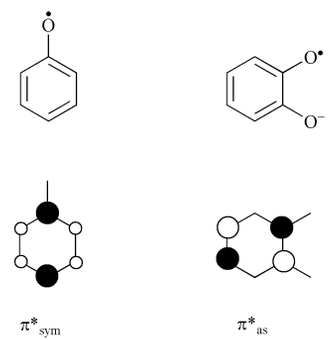



**Fig. 3** Resonance formulations of phenoxyl and *o*-semiquinone radicals and types of benzene π* SOMOs.

Bulky substituents and an additional donor atom for chelate coordination in iminosemiquinone radical ligands[37d] have led to copper complex systems which catalyse reaction (14).[38] The introduction of a donor function in the *ortho* position to the phenolate oxygen, either synthetically or enzymatically through copper-dependent tyrosinase enzymes[30] and subsequent Cu-supported oxidation (Fig. 4), leads to the *o*-quinone two-step redox system (Fig. 5)[39] for which copper–radical interactions have also been established.

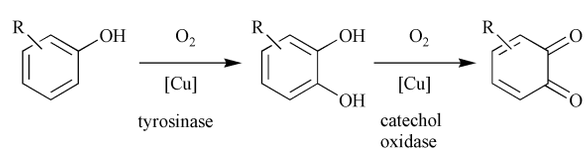



**Fig. 4** Enzymatic pathway of *o*-quinone formation from phenol precursors.[2b,3,30]

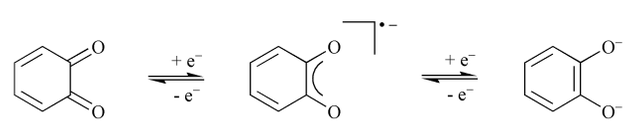
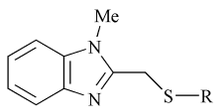



**Fig. 5** The *o*-quinone/catecholate (1,2-dioxolene) redox system.[39]

## 5. Amine oxidases and the *o*-quinone/*o*-semiquinone/catecholate ($Q/Q^{\bullet -}/Q^{2-}$) redox system

There has been a long known dichotomy in the $(Q^{n-})Cu^{n+}L$ complex series, with strong π acceptors, L = CO, CNR, $ER_3$ (E = P, As), favouring the copper(I)-semiquinone form ($n = 1$) and conventional (non-π-acceptor) ligands such as amines stabilising the copper(II)-catechol state ($n = 2$).[40] The observation that thioethers as rather weak π acceptors still favour the copper(I)-radical state albeit with EPR spectroscopically detectable increased metal contributions[40e] has led to the design of

mixed-N,S-donor ligands such as 1-methyl-2-(methylthioalkyl)-1*H*-benzimidazoles (Scheme 7) which were found, under the right conditions,[41] to yield the valence tautomeric (redox isomeric) complex forms (19) coexisting in a temperature-dependent equilibrium.




mmb: 1-methyl-2-(methylthiomethyl)-1*H*-benzimidazole
mtb: 1-methyl-2-(*tert*-butylthiomethyl)-1*H*-benzimidazole

**Scheme 7**

$$(L)Cu^{I}(Q^{\bullet -}) \rightleftharpoons (L)Cu^{II}(Q^{2-}) \qquad (19)$$

The capability of 1-methyl-2-(alkylthiomethyl)-1*H*-benzimidazoles to tolerate both the structurally and electronically quite diverse oxidation states (+I and +II) of copper (Fig. 6) has also been demonstrated through DFT-reproduced structural similarities (nearly linear N–Cu–N angle) and fully reversible electrochemical conversion, indicating an unusually small reorganisation energy.[42]

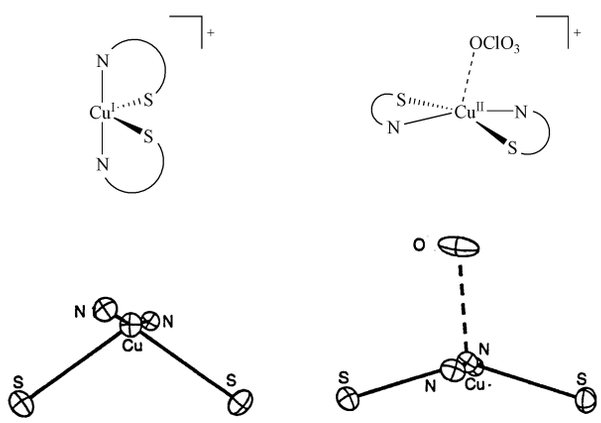
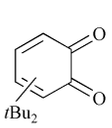
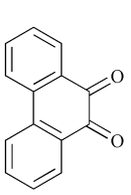



**Fig. 6** Metal coordination in $[Cu^{I}(mmb)_2]^+$ (left) and $[Cu^{II}(mmb)_2(ClO_4)]^+$ (right).[42]

Due to the very different EPR characteristics (*g* factors, $^{63,65}$Cu hyperfine splitting) between $Cu^{II}$ with its $3d^9$ configuration (large *g*, $a_{Cu}$) and $Cu^{I}$-containing organic π radicals (small *g*, $a_{Cu}$) the equilibrium (19) could be well analysed using high-resolution EPR at X-band frequency (Fig. 7).[41] *o*-Semiquinones show structural and EPR features which confirm a SOMO of the $\pi_{as}$* type (Fig. 3).[37d,40,41] Variation of components Q and L (Scheme 8) has indicated a remarkable sensitivity of this equilibrium towards perturbation: electron-rich quinones favour the

$(L)Cu^{n}Q^{n-}$; n = 1,2

| L | Q |
|---|---|
| mmb, mtb |  |
| $(py)_2$ |  |

**Scheme 8**

$Cu^{I}$-radical form and electron deficient quinones stabilise the $Cu^{II}$-catecholate state, with only a few combinations exhibiting a detectable equilibrium situation (Fig. 7).[41]

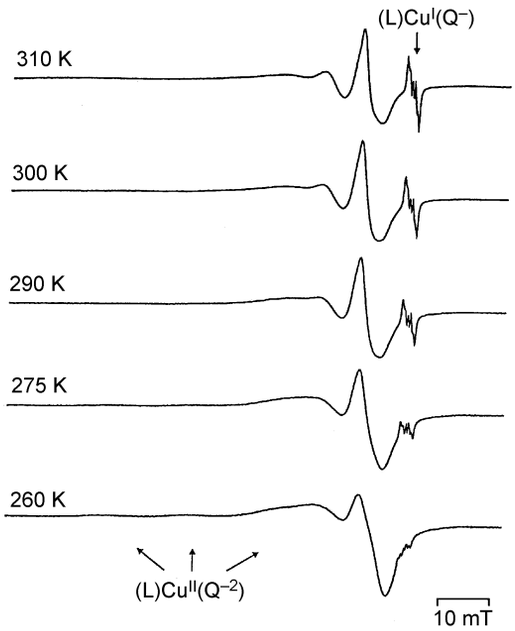



**Fig. 7** EPR spectra illustrating the temperature dependent redox isomerism (19), with L = mtb and Q = 3,5-di-*tert*-butyl-*o*-quinone, in THF solution.[41]

Such intramolecular electron transfer (redox isomer)[43a] equilibria involving *ortho*-quinonoid (dioxolene) chelate ligands and transition metals (Fig. 8) have been discovered and studied mainly for manganese and cobalt complexes.[43a] Only recently have some corresponding copper systems (19) been reported.[41,43]

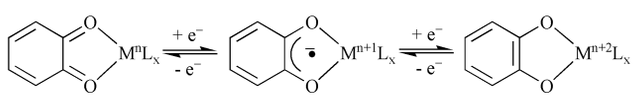



**Fig. 8** General redox isomerism in complexes of 1,2-dioxolenes.[43a]

A dynamic equilibrium (19) had been observed before, however, in a study of copper-dependent amine oxidases which, in the substrate-reduced form, display temperature-dependent EPR signals of a copper(II) species (at low *T*) and of a radical form (at high *T*).[44]

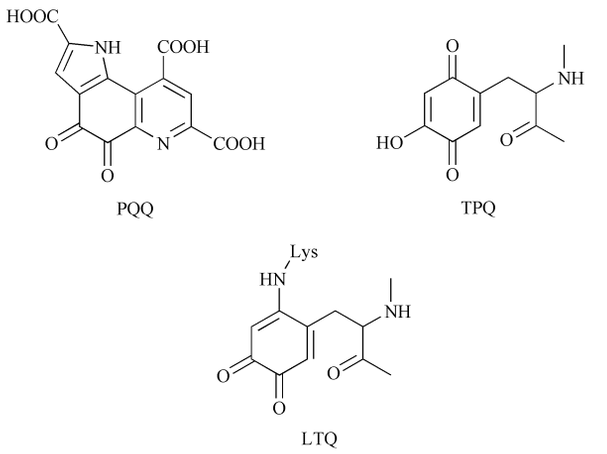
Copper-dependent amine oxidases are ubiquitous enzymes which catalyse the oxidation of amines to aldehydes (20).[45,46]

$$RCH_2NH_3^+ + O_2 + H_2O \longrightarrow RCHO + H_2O_2 + NH_4^+ \qquad (20)$$

Amine oxidases are typically homodimeric enzymes ($M_r$ = 70–90 kDa) with one Cu centre per subunit. Their biological roles within the general amine metabolism include developmental functions such as growth regulation and connective tissue maturation; stress response and defense functions *via* $H_2O_2$ production are also being discussed. Among the biogenic amine substrates for the amine oxidases are neurotransmitters, hormones and allergens. The two-electron oxidation of primary amines to aldehydes is set off by the two-electron reduction of $O_2$ to $H_2O_2$ (20).

The catalysis of a two-electron process such as (20) requires a corresponding catalytic site. Since mononuclear biological copper only adopts the oxidation states I and II the single type 2 copper centre[46] in each subunit requires coupling with a redox-active cofactor. For some time that quinonoid cofactor had been assumed to be pyrroloquinoline quinone (PQQ, methoxatin),[47] however, subsequent work has led to a reformulation, revealing the 2,5-quinone form (topaquinone, TPQ) of

2,4,5-trihydroxyphenylalanine as covalently linked, post-translationally modified cofactor.[45] A related enzyme, lysyl oxidase, uses the similar cofactor lysine tyrosylquinone (LTQ, Scheme 9).[45b,c,48]




**Scheme 9**

The electronic coupling and mechanistic cooperation between the single copper centre and the quinonoid cofactor is facilitated by the close proximity of the metal and TPQ as evident from structural analysis.[46] Protein crystal structures were reported of copper-dependent amine oxidases from prokaryotic (*E. coli*) and eukaryotic sources (pea seedling). They agree in placing the topaquinone and the metal in close proximity in the active site, however, the actual arrangements differ in details. In the structure of the enzyme from *E. coli*[46a] an "active" crystal form shows triply histidine-coordinated copper $Cu^{II}(His)_3$ with two additional water ligands very close to TPQ while an "inactive" crystal form contains TPQ directly coordinated to the $Cu^{II}(His)_3$ group *via* the oxygen atom in the 4-position. In the structure of an enzyme from a pea seedling[46b] there is also a loose connection between the metal and the quinonoid ring, suggesting some, probably even essential, flexibility in the metal–cofactor interaction. Whereas the triple histidine coordination of the copper centre remains a constant feature, the obvious flexibility of TPQ with respect to metal bonding is probably essential for enzymatic catalysis.[46b] EXAFS results of $Cu^{I}$ and $Cu^{II}$ forms confirmed the metal–cofactor interaction.[49]

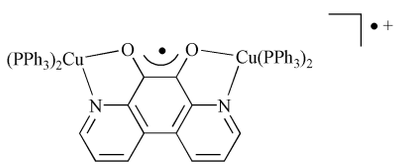
The requirement for a metal such as copper (or a flavin cofactor in metal-free amine oxidases) may come from the necessity to activate the dioxygen co-substrate in its triplet ground state, $^3O_2$. The need to generate $Cu^{I}$ starting from the enzyme resting state which involves $Cu^{II}$ and the aromatic 5-aminated form of the quinone requires an enzymatically (conformationally?) controlled intramolecular electron transfer (19) from the $Cu^{II}$-catecholate form to the $Cu^{I}$-semiquinone state. Such an equilibrium had indeed been deduced from EPR spectroscopic studies of substrate-reduced forms of amine oxidases from various sources which revealed a low-temperature $Cu^{II}$ EPR signal and a narrow EPR line at higher temperatures; the latter was attributed to a $Cu^{I}$-semiquinone, *i.e.* an organic radical species.[44] Added cyanide was shown to stabilize the copper(I)-semiquinone form. Detailed studies of enzyme kinetics confirmed that the copper(I)-semiquinone state is a viable intermediate,[50] however, the essentiality of this option for enzyme action has been challenged following investigations on metal-substituted *i.e.* copper-free analogues.[51] Kinetic studies by Kamau and Jordan have shown that simple aqueous copper(II) can oxidise catechols although reduced forms (for $O_2$ activation) accelerate the reaction.[52] Thus, the primary role of the metal appears to be the electrostatic activation of the TPQ cofactor with the intramolecular electron transfer alternative providing an additional reaction pathway.

In addition to the perhaps crucial intramolecular electron transfer step, the overall enzymatic mechanism[3,45] involves $O_2$ addition and its reduction by $Cu^{I}$, the oxidation of the aromatic 5-amino derivative of TPQ to the quinonoid species with formation of $H_2O_2$ and ammonium ion (deamination step), the reaction of an activated carbonyl group at the generated quinone with the primary amine substrate, and the conversion of the quinoneimine intermediate to the aldehyde and the aromatic form.

Introducing more basic NR functions (Scheme 10) to replace one or both *o*-quinone oxygen atoms [*cf.* the analogy in eqns. (5) and (6)] leads to *o*-semiquinoneimines (iminosemiquinones) and *o*-semiquinonediimines.


**Scheme 10**

Iminosemiquinone complexes of copper(I) (Scheme 11) have been studied by EPR and, in one case, also structurally characterised (Fig. 9);[53] iminosemiquinone complexes of copper(II) were investigated with respect to spin–spin interaction.[37b] *o*-Quinonediimines such as tautomerised azophenine are good π-acceptor ligands for electron-rich copper(I), however, the bis(triphenylphosphine)copper(I) complex (Fig. 10) of the azophenine tautomer could not be converted reversibly to a corresponding *o*-semiquinonediimine.[54] Stabilisation of *o*-semiquinone complexes is also achieved through coordination with ancillary donors.[55] A dinuclear copper(I) complex has thus been obtained.[56]




**Scheme 11**

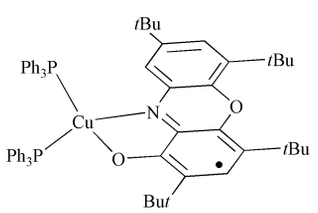
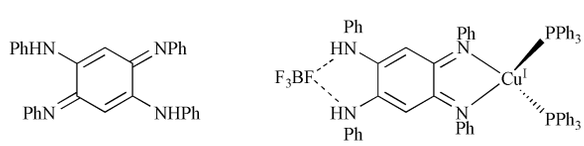



**Fig. 9** Structurally characterised copper(I) complex of an iminosemiquinone ligand.[53]




azophenine (*p*-quinonoid tautomer) — azophenine complex (*o*-quinonoid tautomer)

**Fig. 10** Azophenine (*p*-quinonediimine ground state) and a copper(I) complex of the tautomerised *o*-quinonediimine form.[54]

On the other hand, *o*-semiquinone complexes of copper(II) such as $Cu^{II}(Q^{\bullet -})_2$ are stable and have found interest because of the magnetic interaction between the three spins.[57]

These examples demonstrate that *o*-semiquinone ligands are truly ambivalent intermediates, being able to undergo electron transfer in both directions (21, 22).

**Table 1** Radicals and their reactivity towards copper compounds

| Radical | Character of singly occupied orbital [a] | General electron transfer reactivity | Typical reaction with $Cu^{I}$ | Typical reaction with $Cu^{II}$ |
|---|---|---|---|---|
| $O_2^{\bullet -}$ | $(\pi^*)^3$ (13 v.e.) | Mainly oxidising Metastable | e.t. ($\longrightarrow Cu^{II} + O_2^{2-}$) [12] | Complexes possible [14,15] and e.t. ($\longrightarrow Cu^{I} + O_2$) [12] |
| $(RN)_2^{\bullet -}$ | $(\pi^*_{azo})^1$ | Mainly reducing | Complexes possible [18,19] | e.t. ($\longrightarrow Cu^{I} + RNNR$) [18,20a] |
| $NO^{\bullet}$ | $\pi^1$ (11 v.e.) | More reducing | Complexes possible [24] | e.t. ($\longrightarrow Cu^{I} + NO^{+}$) [25] |
| $Y^{\bullet}$ [b] | $(\pi^*_{sym})^1$ | Oxidising | e.t. ($\longrightarrow Cu^{II} + Y^{-}$) [31] | Complexes possible [31,36] |
| $Q^{\bullet -}$ [c] | $(\pi^*_{as})^1$ | Mainly reducing | Complexes possible *and* e.t. ($\longrightarrow Cu^{II} + Q^{2-}$) [41,43] | Complexes possible *and* e.t. ($\longrightarrow Cu^{I} + Q_N{}^{o}$) [54,55] |

[a] v.e. = valence electrons; π* orbitals of the benzene molecule (Fig. 3). [b] Phenoxyl. [c] *o*-Semiquinone.

$$Cu^{o}\text{-}Q^{o} \longleftrightarrow Cu^{I}\text{-}Q^{\bullet -} \longleftrightarrow Cu^{II}\text{-}Q^{2-} \quad (21)$$

$$+e^{-} \quad -e^{-}$$

$$Cu^{I}\text{-}Q^{o} \longleftrightarrow Cu^{II}\text{-}Q^{\bullet -} \longleftrightarrow Cu^{III}\text{-}Q^{2-} \quad (22)$$

## 6. Conclusion

Table 1 summarises the results obtained when combining copper redox pairs with redox systems involving organic or inorganic radical intermediates.

The list in Table 1 demonstrates that the interaction between O or N centred radicals and copper in its oxidation states I or II is quite variable. The radical component can include diatomic open-shell species as well as larger organic radicals with significantly different electronic structures. Chemically, $Cu^{I}$ and $Cu^{II}$ show a remarkable tolerance towards both reducing and oxidising radical intermediates without undergoing degradation to unreactive products. This quality is probably a consequence of the "semi-noble" character of copper; neither reduction to the metal nor the formation of oxides is an energetically very favourable option. The structural peculiarities of copper(I) ($\longrightarrow$ linear, trigonal or tetrahedral configuration) and copper(II) ($\longrightarrow$ square planar or pyramidal configuration) and the problems of the transition between them do not appear to constitute a principal obstacle to the electron transfer involving radical species, once the right ligand environment is provided. Given the enormous progress within biocopper chemistry during the last decade the potential for natural or synthetic copper–radical interactions may not yet be exhausted. For example, a currently debated question is the bis[oxocopper(III)] configuration (Scheme 6) and its implication in ligand activation *via* electron transfer according to the formulation $O^{-II}$–$Cu^{III} \leftrightarrow O^{-I}$–$Cu^{II}$.[34,35,58]

In contrast to carbon-based radicals *e.g.* in the function of coenzyme $B_{12}$ or to the oxygen- or sulfur-based radicals in ribonucleotide reductases[11] the oxygen-containing radicals associated with copper proteins do not exhibit a (controlled) intrinsic radical reactivity such as hydrogen abstraction but serve as parts of redox series at relatively high potentials. In fact, for all cases discussed the protein environment has been shown to be particularly suited to tolerate these radicals chemically but allowing their electron transfer interaction with the copper centre(s).

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