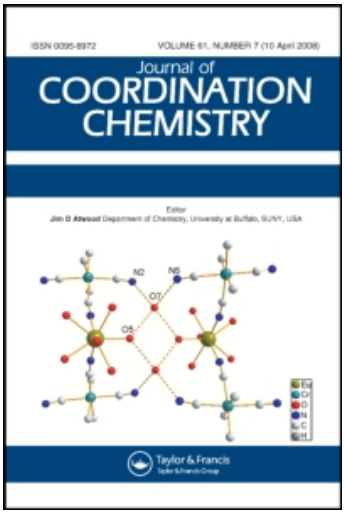

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Monooxygenation of external phenolic substrates in small-molecule dicopper complexes: implications on the reaction mechanism of tyrosinase

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Monooxygenation of external phenolic substrates in small-molecule dicopper complexes: implications on the reaction mechanism of tyrosinase†

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This review describes the reactions of synthetic dicopper complexes with external monophenolic substrates in the presence of O_2, generating *o*-diphenols (catechols) or *o*-quinones. Such systems are of interest as structural and functional models of the type 3 copper enzyme tyrosinase, which mediates the *o*-hydroxylation of tyrosine to DOPA and the subsequent two-electron oxidation to dopaquinone. The known dicopper systems mediating the aromatic hydroxylation of monophenolic substrates are described. Systems based on μ-$\eta^2:\eta^2$ peroxo, bis-μ-oxo, and *trans*-μ-1,2-peroxo dicopper cores are considered separately. After reviewing the stoichiometric conversion of phenolic substrates, the available catalytic systems are described. The implications for the reaction pathway of tyrosinase are discussed.

Keywords: Tyrosinase; Copper enzymes; Bioinorganic chemistry

1. Introduction

Tyrosinases are ubiquitous copper enzymes mediating the *o*-hydroxylation of monophenols to *o*-diphenols and the subsequent two-electron oxidation to *o*-quinones by O_2 (scheme 1) [1]. The physiological role of tyrosinases is the conversion of tyrosine to dopaquinone, which constitutes the first step of melanine synthesis [2, 3]. As evident from the spectroscopic data and a recent X-ray crystal structure determination [4], the active site of tyrosinase contains a binuclear copper center coordinated by six histidines which are provided by four α-helices (figure 1a). In the *oxy* form of the active site, dioxygen is bound as peroxide (O_2^{2-}) in a characteristic side-on bridging (μ-$\eta^2:\eta^2$) fashion, whereby the Cu(I) centers of the *deoxy* states are converted to Cu(II). This type of binuclear (type 3) copper center is analogous to the active sites of catechol oxidase and hemocyanin [3].

The enzymatic activity of tyrosinase is conventionally described by two interpenetrating reactive cycles (scheme 2). Starting from the *oxy* form of tyrosinase, monophenols are converted to *o*-diphenols (monophenolase cycle) [5]; subsequently,

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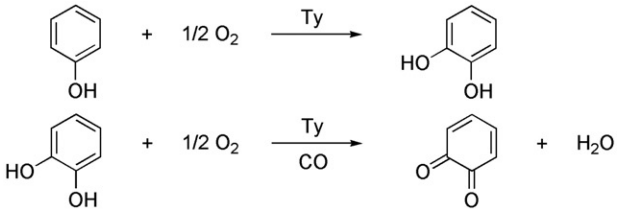


Scheme 1. The two reaction steps mediated by tyrosinase (Ty). The second reaction is also mediated by catecholoxidase (CO).

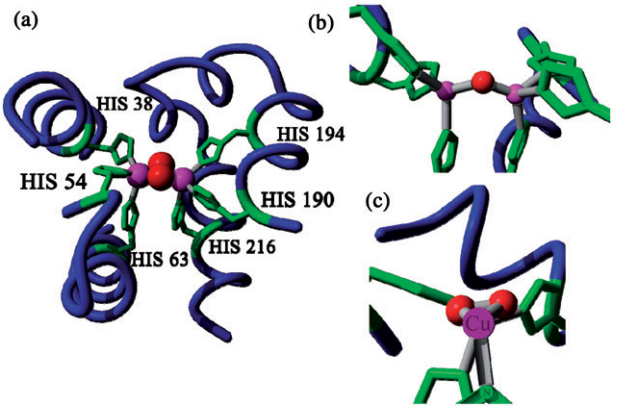
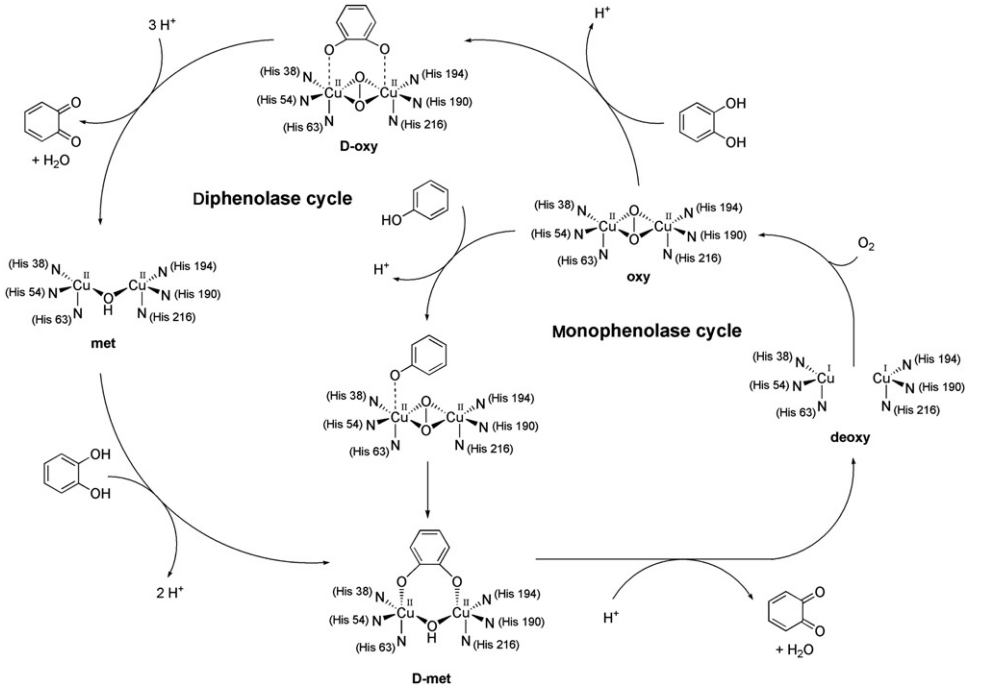


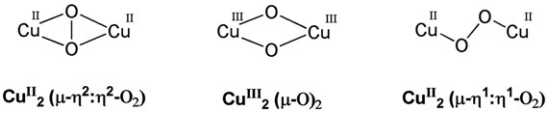
Figure 1. Active site of *Streptomyces castaneoglobisporus* tyrosinase: (a) complete picture; (b) view along the O–O bond; and (c) view along the Cu–Cu bond (equatorial histidines of the copper in the foreground omitted for clarity). Magenta, copper; red, oxygen; green, histidine residue; blue, α helix.

the *o*-diphenol (catechol) intermediates are converted to *o*-quinones, reforming the *deoxy* site which is able to bind O_2. A second reactivity of the *oxy* site is oxidation of external catechols to *o*-quinones (diphenolase activity), generating the *met* derivative of tyrosinase. This intermediate is in turn capable of two-electron oxidizing catechols such that in the course of the diphenolase cycle, two molecules of diphenol (catechol) are converted to *o*-quinone. Whereas tyrosinase mediates both mono- and diphenolase reactions, the enzymatic activity of catechol oxidase is restricted to the latter conversion [6].

While the described mechanistic scheme accounts for the kinetics and the inhibition patterns of tyrosinase [2], open questions with respect to the detailed mechanism of tyrosinase exist. These, in particular, relate to the most interesting step of tyrosinase activity, the *o*-hydroxylation of phenolic substrates. The copper-mediated oxygenation of phenols has mostly been interpreted in terms of an electrophilic substitution mechanism [6, 7], explaining general trends in reactivity observed for the enzyme as well as for low-molecular weight model systems in a satisfactory way [8, 9]. This view has recently been challenged, proposing (on the basis of DFT calculations) a radical mechanism for tyrosinase [10, 11]. Although radical pathways have been observed in binuclear copper dioxygen chemistry under certain conditions [9], the notion of a radical mechanism applying to tyrosinase would certainly represent a total paradigm



Scheme 2. Monophenolase and diphenolase cycles of tyrosinase.

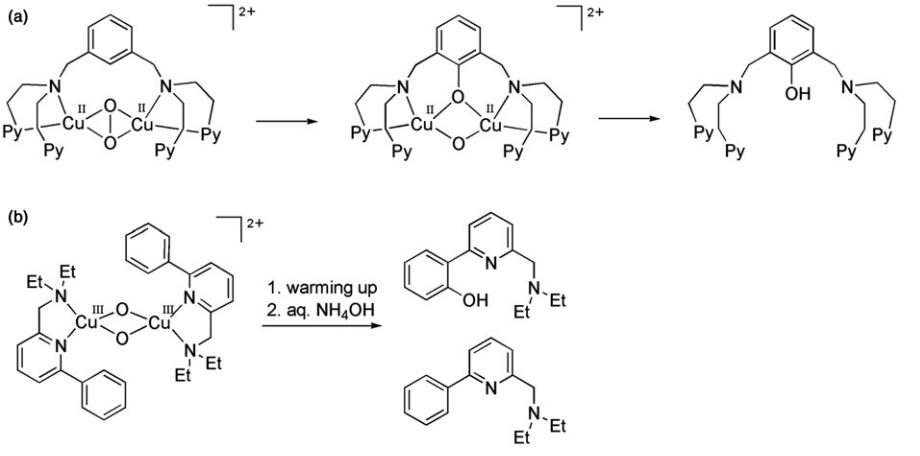


Scheme 3. Different types of reactive copper–O_2 intermediates.

change for this key enzymatic reaction. The question of the molecular mechanism of tyrosinase, thus, still needs clarification.

Apart from enzymatic and quantum-chemical studies, important insights into the reactivity of tyrosinase has been obtained from the synthesis and mechanistic investigation of binuclear copper model systems [7, 12–16]. In this context, it has to be mentioned that besides the (tyrosinase-like) μ-$\eta^2:\eta^2$ peroxo geometry, two alternative, bridging coordination modes of dioxygen have been evidenced in such systems: the bis-μ-oxo and the *trans*-μ-1,2-peroxo geometry (scheme 3). Moreover, apart from oxygenation reactions of phenolic or aromatic substrates, evidence has also been found for monooxygenations of *aliphatic* residues in copper–dioxygen systems. Although these reactions are mechanistically related to the (physiological) aromatic hydroxylation reactions, they are not of direct relevance to tyrosinase; thus, we exclude them from this review.

The earliest examples of biomimetic oxygenation reactions mediated by dicopper peroxo cores were provided by systems undergoing hydroxylations of the ligand



Scheme 4. (a) Cu_2(XYL) system of Karlin and coworkers [17] and (b) dicopper(III)-bis-μ-oxo complex supported by the ligand 2-(diethylaminomethyl)-6-phenylpyridine by Tolman and coworkers [18].

frame [17–21]. The first evidence of this type of reactivity was found by Karlin *et al*. [17] for the Cu_2(XYL) system. Starting from the side-on peroxide-bridged structure, the aromatic spacer of the binucleating XYL ligand was oxygenated, leading to a μ-phenoxo μ-hydroxo structure (scheme 4a). A related reactivity based on a dicopper(III) bis-μ-oxo core was later evidenced by Tolman and coworkers [18] (scheme 4b). The systems laid the foundations for a mechanistic understanding of the tyrosinase reaction and are described in several excellent reviews [13, 14, 19, 20].

The scope of this review is the oxygenation of *external* monophenolic substrates by dicopper–O_2 complexes, which has more and more shifted into the focus of interest [8, 21–26]. In contrast to the large number of copper model systems exhibiting catechol oxidase reactivity [27], the number of systems being able to convert externally added monophenols to catechols or *o*-quinones in a tyrosinase-like fashion has remained small. This might be due to the fact that the active site of tyrosinase, although always being pictured in a fairly symmetric C_{2h} or C_{2v} geometry with four equatorial histidine ligands and two axial ligands arranged in a *trans*- or *cis*-configuration, in fact is quite asymmetric. The ''real'' configuration applying to tyrosinase can rather be approximated as deriving from the symmetric geometry by rotating the two copper-histidine units against each other around the Cu–Cu axis (figure 1b and c), leading to a ''twisted'' binuclear copper configuration. This also gives rise to a *nonplanar* $(His)_2Cu–O_2–Cu(His)_2$ unit which should be less stable and thus chemically more reactive than its planar counterpart, in particular, toward external substrates. Synthetic modeling of this asymmetry is not trivial, but has been attempted in a few cases [13, 14, 28–30]. Crystal structure determinations of various forms of *S. castaneoglobisporus* tyrosinase have further shown that the type 3 copper active site of this enzyme is structurally very flexible and undergoes major structural changes during the catalytic cycle [5]. While this might also be crucial to the catalytic activity, it is again difficult to reproduce synthetically.

Nevertheless, a couple of systems with the capability to convert monophenolic substrates to *o*-diphenols or *o*-quinones have been synthesized and investigated.

In section 2.1, the existing systems exhibiting a μ-$\eta^2:\eta^2$ peroxo intermediate are discussed. In addition, binuclear copper complexes exhibiting the bis-μ-oxo structure have been discovered, which are able to mediate aromatic hydroxylation reactions in analogy to tyrosinase. Although a bis-μ-oxo intermediate has not been observed in the enzyme as a static intermediate, it may be relevant to the reactivity of tyrosinase (cf. section 2.2). Very recently, evidence has also been found for aliphatic as well as aromatic hydroxylation reactions mediated by *trans*-μ-1,2-peroxo dicopper cores [29, 31–33]. The case of an aromatic hydroxylation mediated by a *trans*-μ-1,2-peroxo intermediate is considered in section 2.3.

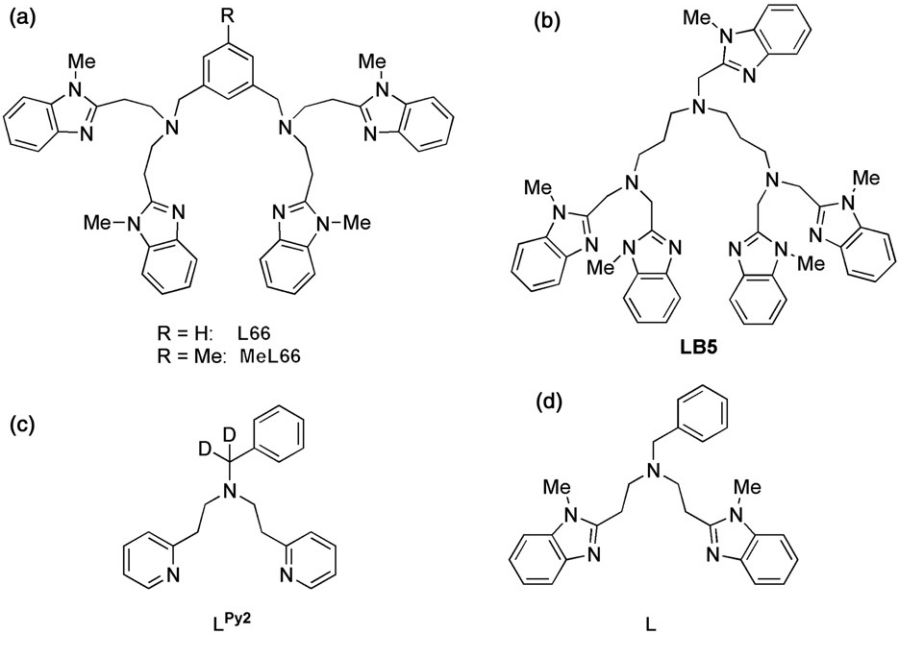
An additional challenge in copper–dioxygen chemistry has been the synthesis of *catalytic* model systems of tyrosinase. Apart from a patent [34], however, only two reports exist in the chemical literature referring to this point, one by Réglier and the other by Casella. In section 3, we will describe these systems and compare them to their stoichiometric counterparts in order to define the mechanistic features that might be essential for catalytic action of the investigated system. It is anticipated that this comparison will also provide fundamental insights into the reactive cycle of the biological system and spur further development of catalytic (and, perhaps, more effective) model systems of tyrosinase. This aspect is further considered in section 4, which also summarizes the results of this review.

2. Stoichiometric model systems of tyrosinase

2.1. *Hydroxylation via Cu(II)$_2$-μ-$\eta^2:\eta^2$-peroxo intermediates*

The interaction of organic substrates with Cu–O_2 systems has been studied for a long time. Brackman and Havinga [35] paved the way for binuclear copper systems with monophenolase activity by their observation that different copper salts mediate the hydroxylation of external phenolic substrates. In a similar fashion, Maumy and Capdevielle [36] established that Cu_2Cl_2 in the presence of copper(0) and sodium hydride could stoichiometrically oxygenate phenols in acetonitrile to *o*-quinones. Afterwards, several attempts were made to improve the biomimetic features of copper-based systems capable of hydroxylating external substrates by introducing *N*-donor ligands with, e.g., pyridine and/or phenanthroline functions [37–39]. These systems, however, always gave mixtures of different products, complicating understanding of the central hydroxylation step. It should be mentioned that attempts to hydroxylate external substrates in a biomimetic fashion were also performed by Kitajima *et al*. [40], who shortly after the historic discovery of the side-on peroxo-bridged dicopper complex $[Cu[HB(3,5\text{-}Me_2pz)_3]]_2(O_2)$ investigated the reactivity of this new dioxygen adduct toward added phenols. These reactions, however, invariably produced C–C coupling instead of physiological hydroxylation products, suggesting the presence of a radical pathway [41].

One of the first studies reporting copper-mediated, *selective* formation of *o*-catechol after addition of an external monophenolic substrate was published by Casella and Monzani [42]. In this study, copper(I) complexes of different benzimidazole-based ligands were shown to mediate the conversion of the tetra-*n*-butylammonium salt of methyl 4-hydroxybenzoate to the corresponding *o*-diphenol upon oxygenation at

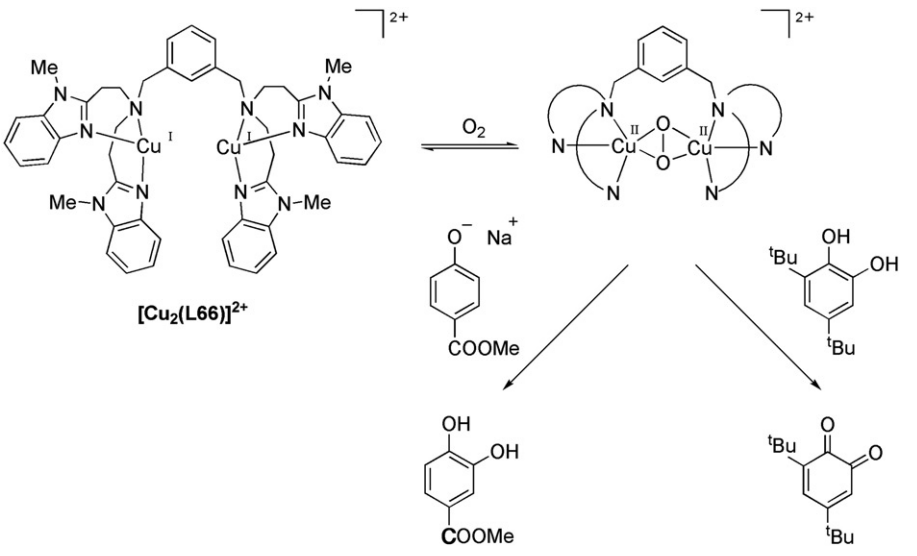


Scheme 5. (a) Ligands L66 and MeL66 by Casella and coworkers [42, 50]; (b) ligand LB5 by Casella and coworkers [43]; (c) ligand L^{Py2} by Itoh and coworkers [8]; and (d) ligand L by Casella and coworkers [47].

low temperature. The systematic variation of the length of the ligand side chains was shown to influence the amount of the hydroxylation product. The highest yield (~37%) was observed for the binuclear copper(I) complex of the most flexible *m*-xylene-bridged ligand L66 (scheme 5a), which previously had been reported to mediate the catalytic conversion of sodium 2,4-di-*tert*-butyl phenolate to 3,5-di-*tert*-butyl quinone by reaction with dioxygen (*vide* section 3). This finding provided an early hint for the notion (later supported by the crystal structure of tyrosinase; see above) that the active site of this enzyme mediating the aromatic hydroxylation reaction in unrivaled efficiency is highly flexible [4, 5]. All systems reported in the mentioned study, however, also gave by-products like Michael adducts if the oxygenation was performed at a higher (e.g., room) temperature.

In a later study, Casella and coworkers [43] investigated the monophenol oxygenase activity of the $Cu(I)_2$LB5 system (scheme 5b). Interestingly, this system was found to mediate the selective *o*-hydroxylation of tetra-*n*-butylammonium 4-(carbomethoxy)phenolate at ambient temperature *without* the occurrence of side reactions observed for $Cu(I)_2$L66. Moreover, monooxygenase activity was also observed if phenols are reacted with $Cu(I)_2$LB5 instead of phenolates. The L66 system, in contrast, lacks this ability. This difference in reactivity was attributed to the fact that $Cu(I)_2$LB5 bears additional *N*-donor functions which do not coordinate to the Cu(I) centers and thus can deprotonate the phenol before coordination to the copper center. Such an internal base was also postulated for tyrosinase, but cannot be identified in the enzyme (see below) [4, 5].

Although the described systems revealed monooxygenase activity in a tyrosinase-like fashion, no corresponding copper–O_2 intermediate was detected. This gap was closed



Scheme 6. Reactivity of $Cu(I)_2L66$ as established by Casella and coworkers [44].

by Casella and coworkers [44] in 2000 by re-examining the O_2 reactivity of $Cu(I)_2L66$. In this study, oxygenation at $-80^{\circ}C$ of $[Cu_2(L66)]^{2+}$ in dry acetone led to reversible formation of the corresponding $Cu(II)_2$-μ-$\eta^2:\eta^2$-peroxo intermediate (scheme 6). This species was further shown to both convert electron-poor phenolates to catechols (monophenolase activity) and electron-rich catechols to quinones (diphenolase activity). Consequently, this system was the first small-molecule model complex that both forms a stable dioxygen adduct and performs a subsequent hydroxylation of external phenolates. Unfortunately, the yield of catechol was rather low at 20% or 40%, respectively, if the amount of isolated organic phase was standardized to 100% (the total recovery of catechol and phenol was ~50%). Furthermore, by-products were observed in the NMR spectra showing that the hydroxylation was not selective.

Shortly after Casella, Itoh *et al.* [8] investigated the O_2 reactivity of $Cu(I)L^{Py2}$ (scheme 5c). $Cu(I)L^{Py2}$ is the benzyl-di-deuterium analog of the $[Cu(RPY2)]^+$ complex of Karlin and coworkers [45] that showed formation of a $Cu(II)_2$-μ-$\eta^2:\eta^2$-peroxo intermediate in dry dichloromethane upon oxygenation at low temperature. Furthermore, after stirring a mixture of Cu(I)RPY2 in dichloromethane in the presence of dioxygen for 4d at low temperature and subsequent warming to room temperature, aliphatic hydroxylation in benzyl position with subsequent *N*-dealkylation had been observed in 20% yield. To slow this reaction – which is undesired if reaction of the peroxo intermediate with *external* substrates is intended – Itoh *et al.* [8] substituted deuterium for the benzylic hydrogen in the RPY2 ligand. The correspondingly modified system $Cu(I)L^{Py2}$ also formed a $Cu(II)_2$-μ-$\eta^2:\eta^2$-peroxo intermediate after reaction with dioxygen at $-94^{\circ}C$ in dry acetone. Moreover, subsequent reaction with lithium salts of different *p*-substituted phenols gave the corresponding catechols in high yields. The catechol derivative of lithium *p*-chlorophenolate, e.g., was isolated in 90% yield per dicopper unit. This hydroxylation was shown to be selective because neither quinone nor C–C or C–O coupling products were found in the reaction mixture. The kinetics of

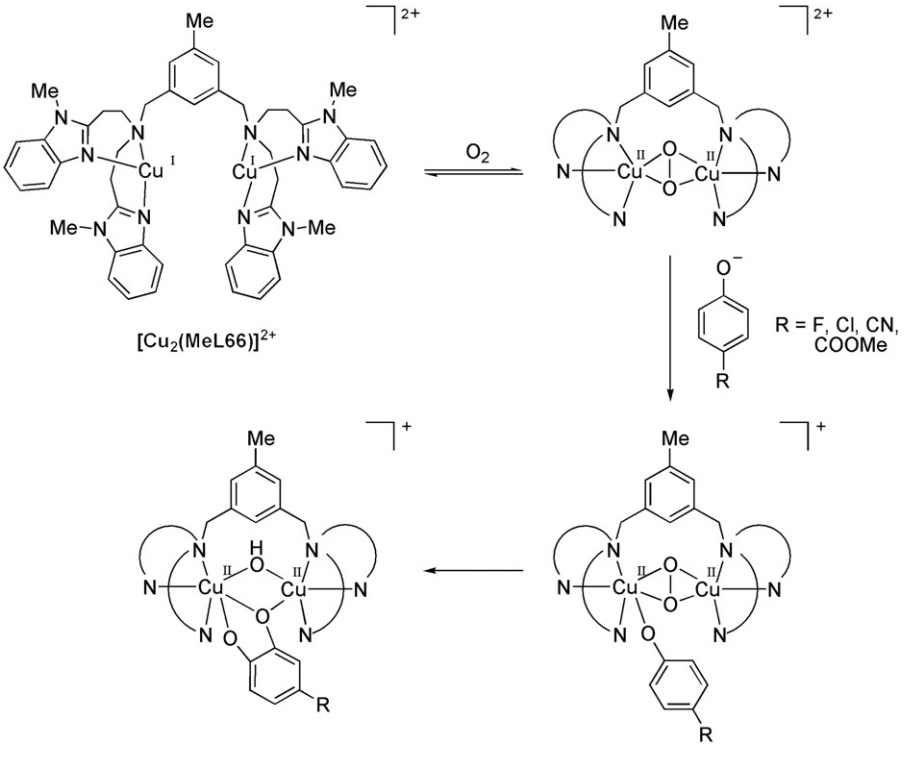
the hydroxylation step was consistent with a rate-determining electrophilic attack of the μ-$\eta^2:\eta^2$-peroxo dicopper(II) intermediate on the aryl ring of the substrate, being coordinated to one copper center in a ternary complex. This result is in agreement with one of the possible mechanisms that were discussed for the monophenolase activity of tyrosinase [1, 5, 46].

Two years later, the reactivity of the same system toward external phenols was studied by Itoh and coworkers [9]. In contrast to the reaction with phenolates, the $Cu(II)_2$-μ-$\eta^2:\eta^2$-peroxo intermediate of $Cu(I)L^{Py2}$, upon reaction with neutral phenols, neither gives catechol nor quinone, but the C–C coupling product in nearly 50% yield based on the dicopper unit. The authors concluded that in this case the peroxo intermediate formally acts as a one-electron oxidant, generating phenoxyl radicals that immediately dimerize. This finding once more raised the question of how the undesired radical-based side reactions are avoided in the enzyme; i.e., whether (and, if so, how) a deprotonation of the substrate occurs before the aromatic hydroxylation step takes place.

Shortly after this study, Casella and coworkers [47] investigated the O_2 reactivity of another mononuclear Cu(I) complex $[Cu(I)L]^+$ (scheme 5d) at low temperature. Sayre and coworkers [48] had shown in 2000 that upon oxygenation at room temperature, this system mediates the conversion of ethyl 4-hydroxybenzoate to the corresponding *o*-catechol, followed by a C–O coupling reaction with the starting phenol. Casella and coworkers now treated $[Cu(I)L]^+$ with an excess of dioxygen at −85°C in anhydrous acetone, resulting in partial formation of a $Cu(II)_2$-μ-$\eta^2:\eta^2$-peroxo complex. Using an ε-value of 20,000 $(mol\,L^{-1})^{-1}\,cm^{-1}$ for the 356 nm absorption band of the peroxo complex, the yield of this intermediate was estimated as 30%. Subsequent addition of sodium 4-carbomethoxyphenolate to the peroxo intermediate under a constant O_2 pressure of 1 atm for 1 h at low temperature, followed by an acid quench and HPLC analysis of the resulting products gave 88% of catechol (based on the amount of the reactive dicopper unit) with no quinone, C–C or C–O coupling byproducts. This yield of catechol almost exactly corresponds to the amount of catechol formed by the Itoh system $Cu(I)L^{Py2}$.

In the mentioned study, kinetic experiments were also performed. Formation of the peroxo complex was shown to follow a first order (in copper) kinetics, followed by a fast reaction of the peroxo intermediate with the added phenolate, and a subsequent slower formation of the catecholato species $[Cu_2(L)_2(Cat)(OH)]^+$. In agreement with Itoh *et al.* [8] (*vide supra*), these results were interpreted as evidence for an electrophilic attack of the peroxo intermediate on the arene ring of the phenolate. In contrast to the Itoh system, however, the rate-limiting step was found to be the continuous formation of the peroxo complex and not the electrophilic attack itself. If the reaction was carried out at room temperature, the C–O coupling product was formed in analogy to the L66 system.

In 2005, Casella and coworkers [26] studied the hydroxylation of various *p*-substituted phenolates mediated by the slightly modified $Cu(I)_2$L66 analog, $Cu(I)_2$MeL66 (schemes 5a and 7). Performance of this reaction in acetone at −55°C again indicated an electrophilic aromatic substitution pathway, as evident from a plot of the reaction rate *versus* the corresponding Hammett substituent constants, giving a Hammett constant $\rho = -1.84$. The MeL66 system is better soluble in acetone at low temperature than its L66 analog, allowing study of O_2 uptake and phenol hydroxylation at different temperatures. Importantly, for both the oxygenation and the hydroxylation



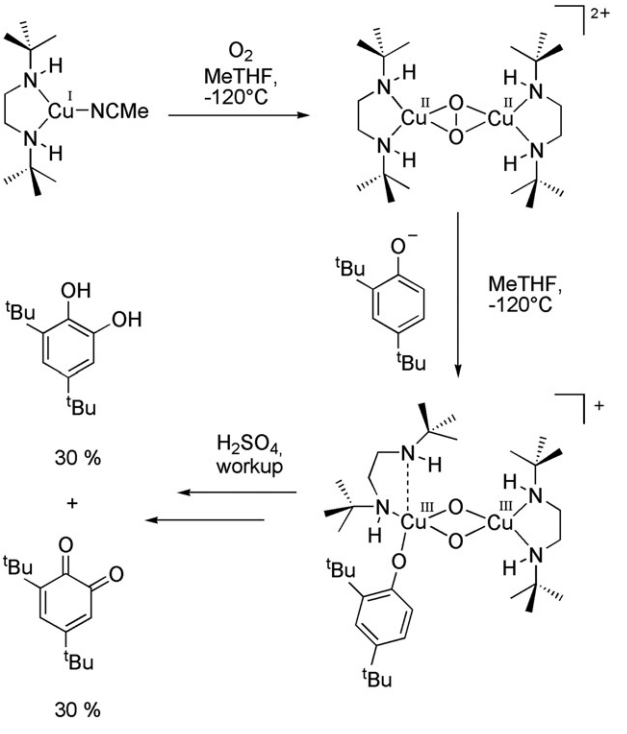
Scheme 7. Reactivity of $Cu(I)_2$MeL66 as established by Casella and coworkers [26].

steps, i.e., the complete monophenolase reaction of this system, activation and binding parameters could be determined, showing a large activation entropy and a weak phenolate binding constant, in contrast to tyrosinase.

2.2. *Hydroxylation via Cu(III)$_2$-bis-μ-oxo cores*

In the model complexes presented in the previous section, the aromatic hydroxylation of phenolic substrates was found to be mediated by $Cu(II)_2$-μ-$\eta^2:\eta^2$-peroxo intermediates. As demonstrated by Tolman and coworkers [49], this species under certain conditions is in equilibrium with its bis-μ-oxo-dicopper(III) isomer (cf. section 1). Since the discovery of the bis-μ-oxo core, various attempts were made to perform the *o*-hydroxylation of external phenolic substrates by such intermediates. These experiments largely lead to unphysiological, radical-based C–C and C–O coupling products which was explained by the tendency of the bis-μ-oxo-dicopper(III) unit for H-atom abstraction [9, 13, 49].

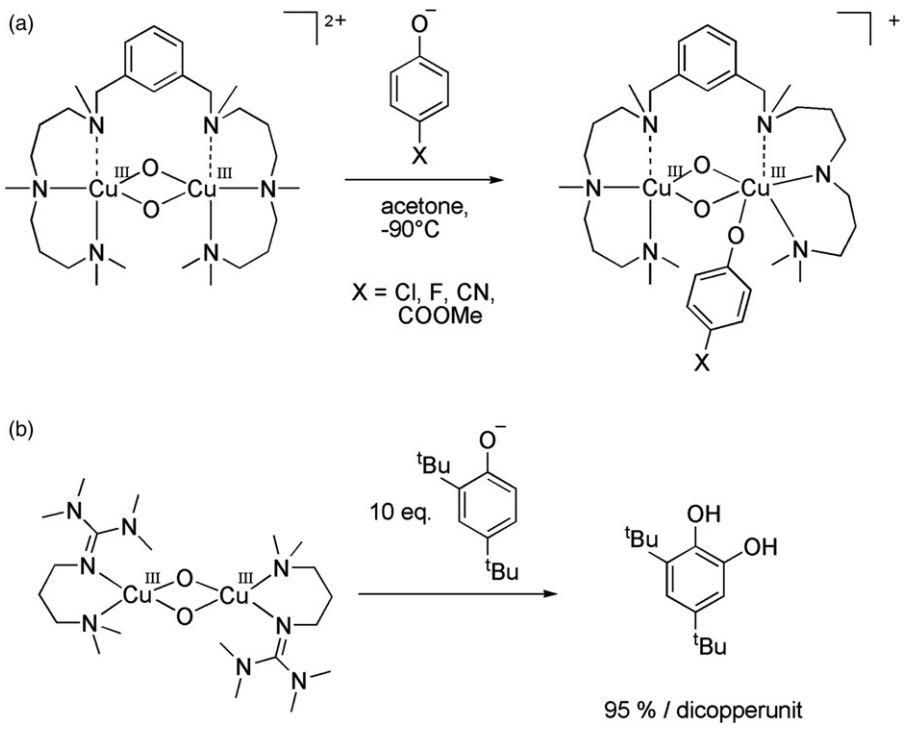
The first true monooxygenase activity of a bis-μ-oxo intermediate toward an external phenolate was reported by Stack and coworkers [22]. Upon oxygenation at −120°C in 2-methyl-tetrahydrofuran, the Cu(I)BDED system (scheme 8) forms a $Cu(II)_2$-μ-$\eta^2:\eta^2$-peroxo complex. After addition of sodium 2,4-di-*tert*-butylphenolate at −120°C, O–O bond cleavage occurs, concomitant with coordination of the phenolate to the



Scheme 8. Reactivity of the Cu(I)BDED system as established by Stack and coworkers [22].

bis-μ-oxo-dicopper(III) core. This highly interesting ternary intermediate was fully characterized by EXAFS, UV-Vis and resonance Raman spectroscopy [22]. Upon warming and subsequent acid quench, the corresponding catechol and *o*-quinone were isolated in 30% yield each. The 1 : 1 ratio of catechol and quinone suggested that the substrate might in fact be released as semiquinone upon protonation, followed by disproportionation to catechol and quinone [50]. This hypothesis was supported by spectroscopic studies and DFT calculations of Solomon and coworkers [24] in 2009. In this study, two intermediates were postulated to form after the hydroxylation reaction mediated by the ternary intermediate. According to this scenario, the phenolato-bis-μ-oxo-dicopper(III) complex after 3 h of reaction converts to an asymmetric μ-hydroxo-μ-$\kappa^1:\kappa^2$-catecholato-dicopper(II) intermediate which upon addition of one equivalent of protons dissociates into a mononuclear Cu(I)–aquo complex and a mononuclear copper(II)-semiquinone species. Upon mineral acid quench, the coordinated semiquinone presumably disproportionates to the corresponding quinone and catechol. Detailed kinetic studies of the DBED system further suggested that the hydroxylation step is accomplished by an electrophilic aromatic substitution, in analogy to the dicopper(II) μ-$\eta^2:\eta^2$-peroxo intermediates discussed earlier.

The Stack system described above is a μ-$\eta^2:\eta^2$ complex which undergoes conversion to the bis(μ-oxo) structure only in the presence of a bound substrate. This provides a new scenario for the hydroxylation reaction in the enzyme as it indicates that the O–O



Scheme 9. (a) Reactivity of the bis-μ-oxo-dicopper(III) core supported by the ligand (*m*-XYL^{MeAN}) as established by Company *et al.* [51]; and (b) reactivity of the bis-μ-oxo-dicopper(III) core supported by the ligand ^{2}L as established by Herres-Pawlis and coworkers [52].

bond of side-on bound peroxide in *oxy*-tyrosinase may be cleaved *prior* to hydroxylation [46]. Model chemistry shows, however, that genuine Cu(III)$_2$-bis-μ-oxo complexes (i.e., having this constitution also in the absence of coordinated substrate) do have monooxygenase activity. The first example of such a system was presented by Company *et al.* [51] in 2008. The Cu(I)$_2$(*m*-XYL^{MeAN}) complex (scheme 9a) forms a bis-μ-oxo-dicopper(III) intermediate upon oxygenation in THF, diethyl ether, dichloromethane, and acetone at low temperature. Addition of an excess of sodium *p*-chlorophenolate followed by acid quench, HPLC workup, and NMR spectroscopy leads to the formation of *o*-catechol in 67% yield without occurrence of quinone products or C–C/C–O coupling dimers. Thus, this system is not only a rare example for an efficient hydroxylation of a phenolate *via* a bis-μ-oxo-dicopper(III) core, but also the first bis-μ-oxo system that shows *selective* formation of catechol. Raman and UV-Vis monitoring of the addition of the substrate to the bis-μ-oxo complex at −90°C reveals a metastable intermediate with a phenolate bound to the bis-μ-oxo species. Formation of this intermediate was too fast to be monitored, even by stopped-flow experiments, but the subsequent decay of this species was shown to follow first-order behavior. The Hammett plot for the thermal decay ($\rho = -1.9$) again indicates an electrophilic substitution pathway.

An even more efficient hydroxylation of phenolates *via* a bis-μ-oxo-dicopper(III) core was observed by Herres-Pawlis and coworkers [52] in 2009. The Cu(I) complex

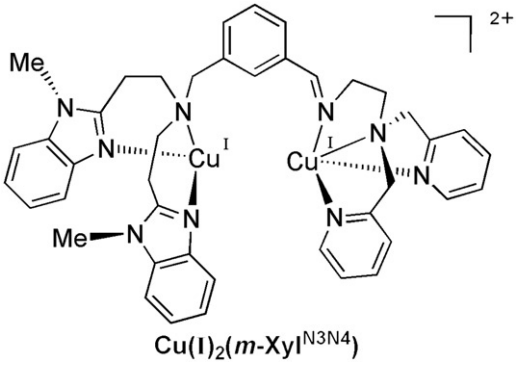
supported by the hybrid permethylated-amine-guanidine ligand ^{2}L was shown to form a bis-μ-oxo intermediate in polar aprotic solvents at low temperature (scheme 9b). This species reacts with an excess of sodium 2,4-di-*tert*-butyl phenolate under formation of the corresponding catechol in >95% yield based on the amount of the dicopper complex.

The three model systems described in this section indicate that the hydroxylation of an external substrate in the enzymatic system can also be mediated by a bis-μ-oxo-dicopper(III) core. Importantly, O–O bond cleavage of the μ-$\eta^2:\eta^2$ intermediate in this case would occur *before* electrophilic attack on the arene ring, representing an alternative scenario to the electrophilic attack by the side-on peroxo-coordinated dicopper(II) unit described earlier.

2.3. *Hydroxylation via Cu(II)$_2$-μ-$\eta^1:\eta^1$-peroxo complexes*

Very recently, Garcia-Bosch *et al.* [29] demonstrated that end-on coordinated μ-$\eta^1:\eta^1$-peroxo intermediates exhibit monooxygenase reactivity toward external phenolates as well. Upon oxygenation at −90°C in acetone, the asymmetric Cu(I)$_2$(*m*-Xyl^{N3N4}) complex (scheme 10) forms a Cu–O_2 adduct which was characterized by UV-Vis and resonance Raman spectroscopy. Based on spectroscopic features, the authors favored formulation of this intermediate as an asymmetric μ-$\eta^1:\eta^1$-peroxo species. Addition of three equivalents of sodium *p*-chlorophenolate to this intermediate leads to the formation of a short-lived species, presumably a ternary complex. The authors concluded from the UV-Vis features that the Cu(II)$_2$-μ-$\eta^1:\eta^1$-peroxo character is retained in the new intermediate. Decomposition of this species with subsequent acid quenching and HPLC–MS analysis confirmed the formation of catechol in 39% yield. Kinetic experiments showed a decay of the ternary intermediate following first-order behavior, and the Hammett plot ($\rho = -0.6$) suggested an electrophilic attack on the aromatic ring.

Although the presence of a Cu(II)$_2$-μ-$\eta^1:\eta^1$-peroxo intermediate in the enzymatic system would be rather surprising due to restrictions on the geometry of the active site (the Cu–Cu distance in the μ-$\eta^1:\eta^1$-peroxo species of the (*m*-Xyl^{N3N4}) system was calculated as 4.31 Å), this intriguing result represents a new mechanistic scenario for the

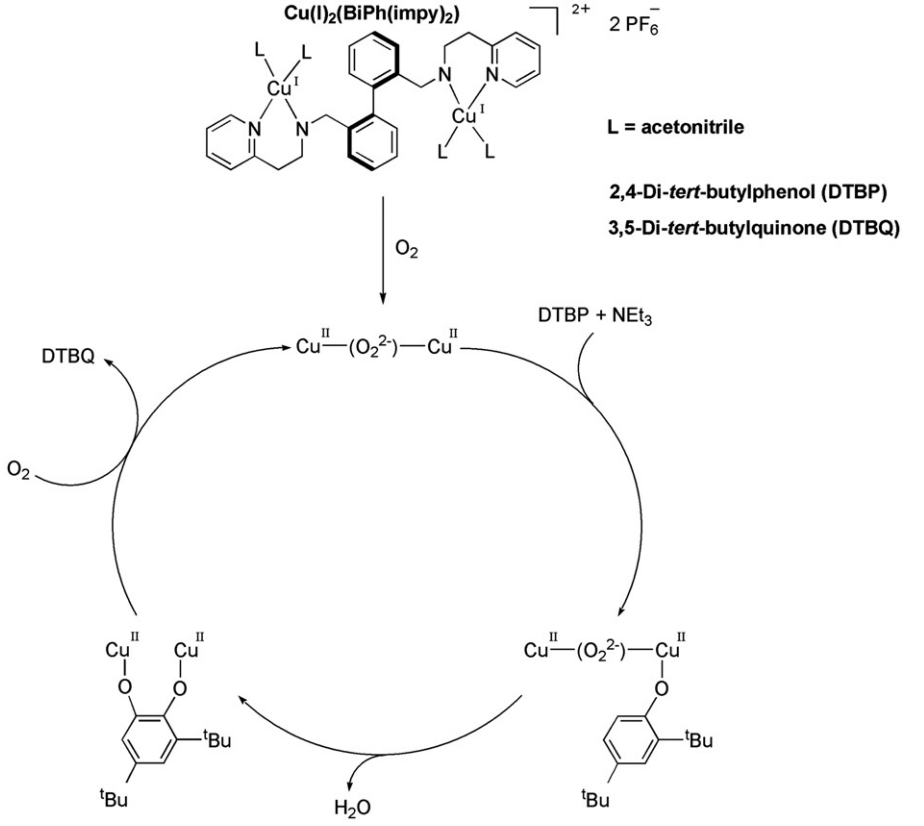


Scheme 10. Binuclear copper(I) complex of the ligand (*m*-Xyl^{N3N4}) by Garcia-Bosch *et al.* [29].

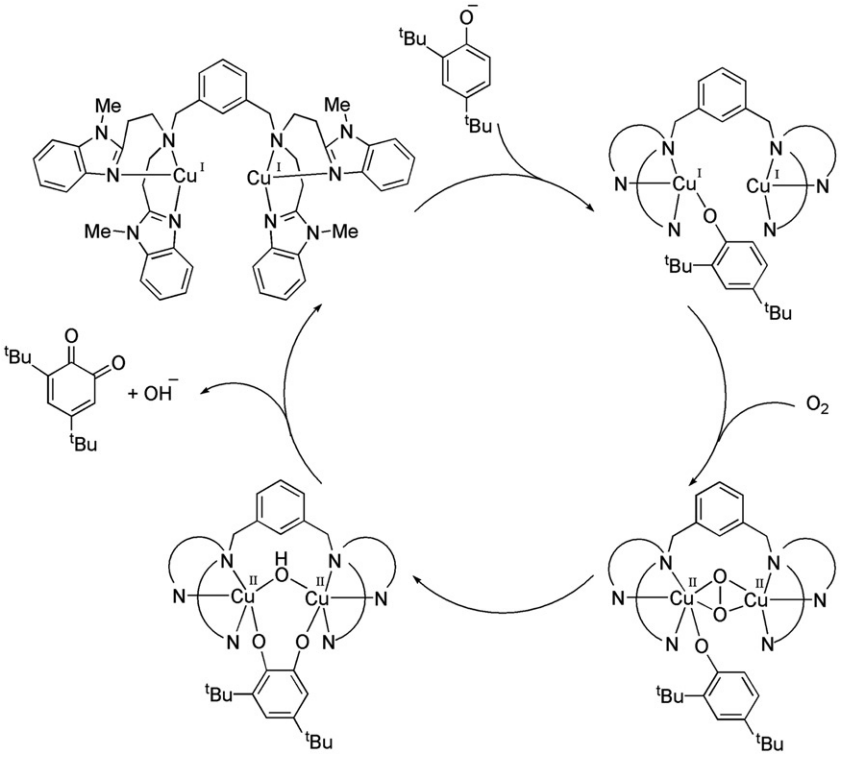
hydroxylation of external phenolic substrates. It should be mentioned that recently also evidence for the hydroxylation of *aliphatic* substrates by μ-$\eta^1:\eta^1$-peroxo cores has been found [31–33].

3. Catalytic model systems of tyrosinase

Model systems performing both the *o*-hydroxylation *and* subsequent two-electron oxidation to *o*-quinone of external phenolic substrates in a *catalytic* fashion are scarce [23, 25, 36]. The first corresponding model system was presented by Réglier *et al*. in 1990 [23]. The $Cu(I)_2(BiPh(impy)_2)$ complex (scheme 11) consists of a binucleating four-dentate *N*-donor ligand ($BiPh(impy)_2$), which contains two pyridylethylimine sidearms bridged by a biphenyl spacer. It was demonstrated that a mixture of ($BiPh(impy)_2$) with two equivalents of Cu(I) salt, 100 equivalents of 2,4-di-*tert*-butylphenol (DTBP), and 200 equivalents of triethylamine (NEt_3) leads to catalytic generation of quinone with a turnover number (TON) of 16, monitored *via* the optical absorption band of 3,5-di-*tert*-butyl-*o*-quinone (DTBQ) at ~400 nm. After 1 h, the reaction stopped, presumably by



Scheme 11. Catalytic monophenolase cycle proposed for the reaction of $Cu(I)_2(BiPh(impy)_2$ with DTBP proposed by Réglier and coworkers [23].

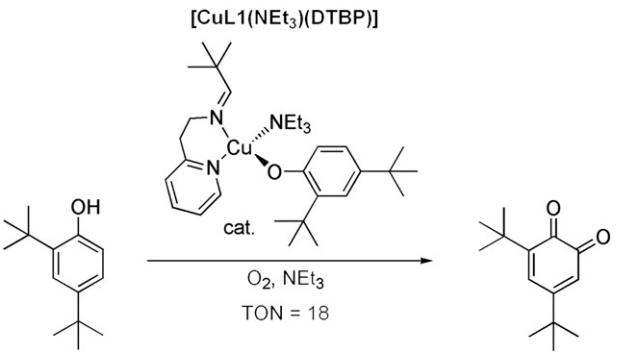


Scheme 12. Catalytic cycle proposed for the reaction of $Cu(I)_2L66$ by Casella and coworkers (peroxo adduct modified to μ-$\eta^2:\eta^2$) [21].

formation of an oxo-bridged complex. A mechanism was proposed for this reaction, involving the formation of a binuclear peroxo complex and a catechol-bridged intermediate (scheme 11).

Shortly after Réglier, Casella *et al.* [21] described another catalytic model system of tyrosinase based on the ligand L66 (scheme 5a). As already described in section 2.1, this system was found to mediate the hydroxylation of carbomethoxyphenol to carbomethoxycatechol in a stoichiometric mode (TON = 1). In addition, the binuclear copper(I) complex of L66 was demonstrated to catalytically oxygenate sodium 2,4-di-*tert*-butyl phenolate containing two-electron donating *tert*-butyl groups. A mechanistic cycle was proposed for this reaction (scheme 12). The complex, however, apparently also catalyzes polymerization–condensation reactions of the formed quinone with the starting phenol, leading to by-products. A good yield of quinone was obtained by reacting sodium 2,4-di-*tert*-butyl phenolate and $Cu(I)_2L66$ in a molar ratio of 1.5 : 1.0 with dioxygen in acetonitrile for 3 min. After subsequent mineral acid quench and chromatography, the quinone was isolated as the major product with a TON > 1.

Both catalytic systems described are based on *binuclear* copper(I) complexes bridged by a *m*-xylyl or a biphenyl spacer. Just recently, a catalytic tyrosinase model system supported by the ligand L1 (scheme 13) was found [53]. Although this system is the first catalytic tyrosinase model complex based on a *mononuclear* Cu(I) complex, it was



Scheme 13. Reactivity of the Cu(I)L1 system by Rolff *et al.* [53].

shown to follow a *binuclear* pathway in a tyrosinase-like fashion. By reacting a 20-μmol L^{-1} solution of [Cu(I)L1(NEt_3)(DTBP)] with molecular oxygen in the presence of 50 equivalents of DTBP and 100 equivalents of NEt_3, DTBQ was catalytically generated with a TON of 18. Importantly, the reactive cycle could also be performed in a stoichiometric fashion, allowing isolation and characterization of a binuclear catecholato species which upon acid treatment forms quinone. Alternatively, quinone is released upon addition of an excess of monophenol, reproducing the reaction conditions that are also present in tyrosinase.

4. Summary and conclusions

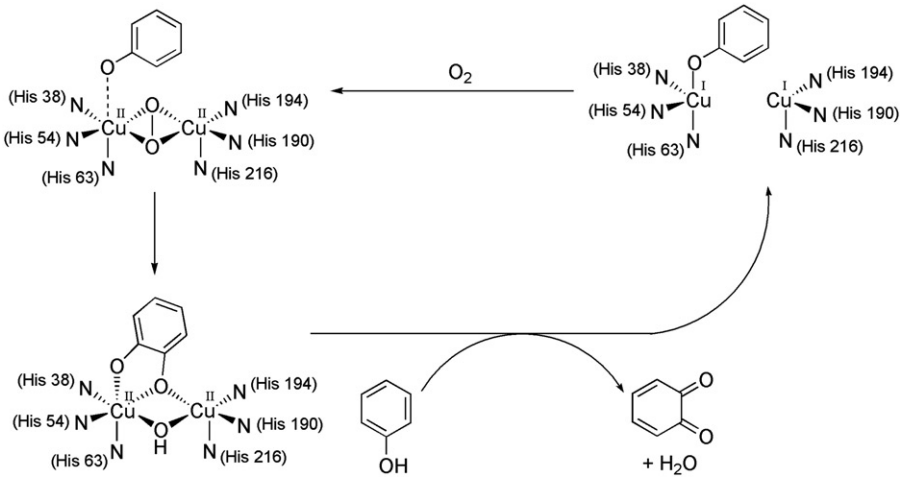
In the preceding sections, a number of small-molecule dicopper systems that are able to stoichiometrically or catalytically mediate the monooxygenation of external phenols with O_2 have been reviewed. From the observation that such reactions occur both in systems forming side-on peroxo intermediates and in systems exhibiting bis-μ-oxo species, it appears that the actual presence of one isomer or the other may be less critical to the ensuing chemistry and both scenarios are associated with elementary reactions that are relevant to tyrosinase. Importantly, the combined experimental evidence indicates that hydroxylation in both cases is mediated by an electrophilic substitution mechanism. A new variant of these reactions is provided by the discovery that *trans*-μ-1,2-peroxo intermediates may also be capable of hydroxylating aromatic substrates. It remains to be shown, however, that this corresponds to a viable mechanistic scenario for tyrosinase.

The question of whether phenols or phenolates are employed as substrates in copper-mediated oxidation/oxygenation reactions is critical with respect to the type of products found in such conversions. As already shown by Kitajima, the addition of phenols to $Cu–O_2$ systems in the absence of base invariably leads to C–C coupling products. This can be explained by H-atom abstraction reactions mediated by dicopper μ-peroxo or bis-μ-oxo cores, leading to phenoxy radicals, which give rise to C–C and C–O coupling products. This reaction course has also been realized in a catalytic fashion, generating, e.g., 4,4′,6,6′-tetra-*tert*-butyl-2,2′-bisphenol from DTBP in the presence of anhydrous

$CuCl_2$ and tetramethylethylene diamine and O_2 [54]. As described in the previous sections, radical reactions can more or less be avoided if, instead of free phenols, phenolate salts are employed in oxygenation reactions. In these cases, the detection of C–C coupling products can be attributed to workup procedures employing acids. The problem associated with the use of phenolate salts in oxygenation reactions is, on the other hand, that in this case only stoichiometric but not catalytic conversions can be achieved, as the protons required for the formation of water are lacking (cf. scheme 2).

Another important, but as yet unanswered question relates to the point which structural or electronic factors are required to turn a stoichiometric system into a catalytic one. Clearly, the reaction mechanism of tyrosinase (scheme 2) dictates that only those model systems can be catalytic that quantitatively lead to the generation of quinone. Only in this case, the Cu(I) $\cdots$ Cu(I) (deoxy) intermediate is regenerated which can bind O_2, forming the central peroxo (or bis-μ-oxo) intermediate. Whereas (as described in this review) a number of systems has been found in which the mechanistically demanding hydroxylation of monophenols to *o*-diphenols can be achieved, the formation of *quinone* in these systems has either not been observed at all or to $\leq$50%. In the catalytic Cu(I)L1 system described in section 3, on the other hand, it was found that the bound catechol is quantitatively released as quinone in the presence of an excess of substrate (phenol). This could have an interesting consequence with respect to the enzymatic reaction.

In general, it is assumed that phenols bind to the *oxy* form of tyrosinase (cf. scheme 2 and section 1) [2]. It has been difficult to understand, however, why addition of (free) phenols to synthetic binuclear peroxo complexes always leads to unphysiological radical-coupling products, whereas in tyrosinase, the same reaction leads to hydroxylation and two-electron oxidation [9]. In synthetic systems, the radical reactions can be avoided if phenolates are used instead of phenols (*vide supra*). The question, therefore, arises as to whether phenols also become deprotonated in the enzyme prior to coordination to the oxy site. It has repeatedly been proposed [55–57], but never been proven that the coordinating histidines of tyrosinase can be protonated. On the



Scheme 14. Alternative pathway for the monophenolase cycle of tyrosinase.

contrary, it has been shown that the histidines of *met*-tyrosinase are not protonated at low pH [58, 59]. Alternatively, the incoming phenol could be deprotonated by a nearby basic residue. Scanning the immediate vicinity of the active site in tyrosinase gives, however, no hint as to the presence of such a residue, with the exception of a conserved non-coordinating His residue that is sequentially positioned directly adjacent to a His reside coordinating to CuB (in *S. castaneoglobisporus* tyrosinase His215 and His216, respectively) [4]. The way His215 could be involved in a proton shuttle with the active site is, however, unclear. If, on the other hand, phenol binding was coupled with release of the bound catechol as quinone, the incoming phenol could be deprotonated by the bound hydroxide of the catecholate intermediate, forming water. A corresponding mechanism in the enzyme (scheme 14) would (1) render a basic amino acid residue close to the binuclear copper center under turnover conditions unnecessary and (2) imply that the phenol *is already bound in the deoxy state of tyrosinase, prior to binding of* O_2. Experiments are underway to check this assumption [60].

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