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Catalytic Cooperativity, Nuclearity, and O_2/H_2O_2 Specificity of Multi-Copper(II) Complexes of Cyclen-Tethered Cyclotriphosphazene Ligands in Aqueous Media

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Abstract: Three ligands **L1**, **L2**, and **L3** with 2, 4, and 6, respectively, 1,4,7,10-tetraazacyclododecane (cyclen) moieties attached to a cyclotri-phosphazene core were synthesized, and oxidation activities of their Cu(II) complexes investigated. Aerobic oxidation of catechol by these complexes follows an *intramolecular dinuclear pathway* with significant cooperativity (i.e., $\theta \approx 1.5$ out of a maximum of 2 for two potential substrate binding sites) and kinetic constants (i.e., k_{cat} = 17.5 × 10^{-3} s^{-1}, K_m = 2.8 mM, and quite remarkable catalytic specificity k_{cat}/K_m 12.5 M^{-1} s^{-1} per di-Cu center), while that by untethered Cu(II)–cyclen follows a *bimolecular dinuclear pathway* without noticeable cooperativity (θ = 0.96) and 4-fold lower k_{cat}, despite their similar dinuclear mechanisms. The proximity of Cu(II) centers is suggested by EPR spectra and relaxations, showing a broad spectral component particularly in Cu_6**L3**. Thermodynamic parameters also indicate the significance of multi-Cu(II) sites in the oxidative catalysis. Air is a more specific oxidation agent for the representative complex Cu_2**L1**, showing 3.2-fold higher catalytic specificity k_{cat}/K_m than H_2O_2 toward a catechol substrate. The research provides further molecular basis for better understanding of O_2/H_2O_2-specific oxidation by multi-domain Cu complexes.

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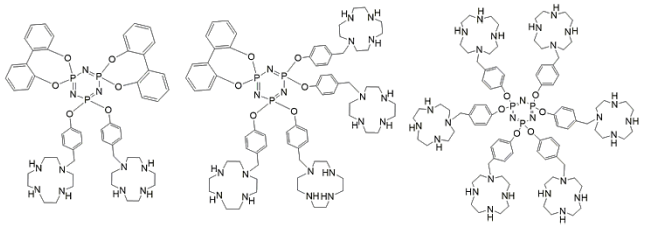
Introduction

Multi-domain and multi-subunit proteins are known to exhibit cooperativity, such as tetrameric hemoglobin but not monomeric myoglobin. Likewise, synthetic molecules with multi-domain structures might also exhibit such unique property as observed in dendrimers with identical metal-binding arms that exhibit significant metal-binding cooperativity, which is not seen in untethered ligands.[1] Cyclotriphosphazene (Cpz) is a versatile scaffold for incorporating up to six moieties to exhibit various chemical and biochemical properties,[2] which can serve as prototypical multi-domain molecules for investigation of metal-binding chemical cooperativity, protein-like ligand-binding cooperativity, and enzyme-like catalytic cooperativity. Metal complexes of various Cpz-containing ligands exhibit diverse structural features and physical and chemical properties.[3,4] The complexes of Cpz-containing ligands with tethered imidazole or other *N*-heterocyclic groups exhibit activities toward cleavage of plasmid DNA under physiological conditions.[5] Its Cu complexes exhibit significantly more effective and specific hydrolytic activity toward a phosphomonoester (K_d = 5 μM) than a phosphodiester, showing significant cooperativity with θ = 1.5 for a maximum of 2 for two potential binding sites and following a dinuclear mechanism that mimics alkaline phosphatase.[6]

In addition to hydrolytic reactions, dioxygen activation and oxidation processes mediated by transition metal centers play essential roles in biological systems (e.g., the respiratory chain, animal pigmentation, and browning of fruits and vegetables),[7] environmental chemistry (e.g., degradation of inert organic hazards),[8] medicinal chemistry,[9] and industrial processes.[10] Redox-active Type-3 dinuclear copper enzymes, such as catechol oxidase and tyrosinase, and their corresponding biomimetic complexes have been extensively studied.[7, 11, 12] Many di-Cu complexes can bind and activate O_2 and/or H_2O_2 to hydroxylate or oxidize catechol, phenol, and their derivatives, presumably by following the same dinuclear pathways as the type-3 enzymes.

A few approaches have been followed in mechanistic studies of the oxidation reactions by di-Cu–peroxo/oxo complexes, such as the assembly of mononuclear metal centers and the use of ligands with multi-metal binding capability with a hydroxo, alkoxo, phenoxo, oxo, or peroxo group as a bridging ligand.[12–14] Moreover, specific recognition[15] and a general acid/base[16] have been verified to be significant factors for effective catalysis by metal complexes. Macrocyclic polyamine ligands such as

1,4,7-triazacyclononane, 1,5,9-triazacyclododecane, and 1,4,7,10-tetraazacyclododecane (cyclen) and their derivatives can form multinuclear complexes[17] that exhibit various chemical activities,[18] and can be covalently linked to various scaffolds such as Cpz for building new multinuclear metal complexes. With the aim of further development of metal complexes possessing hydrolytic, oxidative, or other chemical activities under physiological conditions, three multi-domain ligands **L1**–**L3** with different numbers of cyclen linked to Cpz via a phenol-phosphoester bond were synthesized. The oxidation activities and the reaction mechanisms of catechol-type substrates by the Cu(II) complexes of these ligands were investigated and presented herein.



L1 **L2** **L3**

Results and Discussion

Cu(II) binding. Upon Cu(II) binding to the multi-dentate ligands **L1**, **L2**, and **L3**, a d-d transition at 600 nm is detected which is consistent with a type-2 Cu(II) center of a strong ligand field due to its all-N ligand environment. This transition is similar to those of Cu(II)-cyclen (599 nm)[19] and the Cu(II) complexes of several cyclen-containing ligands such as N,N'-*meta*-xylylenebis(cyclen) and its *para*-isomer (both at 594 nm),[20] which adopt a square pyramidal coordination geometry with the four N atoms at the basal positions [20, 21] and an axial group (even a very weak ligand such as NO_3^- and ClO_4^-) that may be involved in further coordination and/or H-bonding with a cyclen NH group based on crystallographic studies.[22] A molar absorptivity of ~200 M^{-1} cm^{-1} per Cu is consistent with a coordinated external ligand, such as OH^- or Cl^-.[19b] Analogous Cu-complexes with all-N square planar coordination geometry shows a transition at <600 nm, while all-N distorted octahedral geometry shows at >600 nm and a tetrahedral coordination is even lower in energy at >700 nm.[23] Despite our attempts, crystal structures of the Cu**L** complexes were not obtained which might be due to the significant flexibility of the cyclen-arms of the complexes. Nevertheless, a square planar/pyramidal coordination geometry can be expected for the metal centers in these Cu**L** complexes on the basis of the structures of the other cyclen-containing Cu-complexes. N-rich coordination sphere of different geometries in metalloproteins follows the same trend, such as the all-N (distorted tetrahedral 3 His') active sites of Cu(II)-substituted carbonic anhydrase at 750 nm[24] and Cu,Zn-superoxide dismutase at 680 nm (distorted square planar 4 His').[25]

Plotting the change in absorption as a function of [Cu(II)] affords metal-to-ligand stoichiometry of 2:1 (▼, Fig. 1), 4:1 (o), and 6:1 (●), respectively, for Cu(II) binding to **L1**, **L2**, and **L3**. The results can be well fitted to a simplified equilibrium M + cy' ⇌ M-cy', in which the cyclen arms cy' of the ligands **L's** are considered completely independent and act like a free cyclen ligand. The fitting affords apparent affinity constants of (3.0 ± 2.1) × 10^5, (4.0 ± 1.1) × 10^5, and (4.7 ± 1.1) × 10^5 M^{-1}. The similar magnitude of the affinity constants indicates Cu(II) binding to the multi-dentate ligands in a similar fashion. The lack of a sigmoidal shape before reaching the equivalents of 2, 4, and 6 indicates absence of detectable interactions between/among the cyclen arms that may potentially cause binding cooperativity. Such metal-binding cooperativity has previously observed for multi-domain ligands such as dendrimers,[1] which is more pronounced in proteins such as metal binding to dinuclear aminopeptidase.[26]

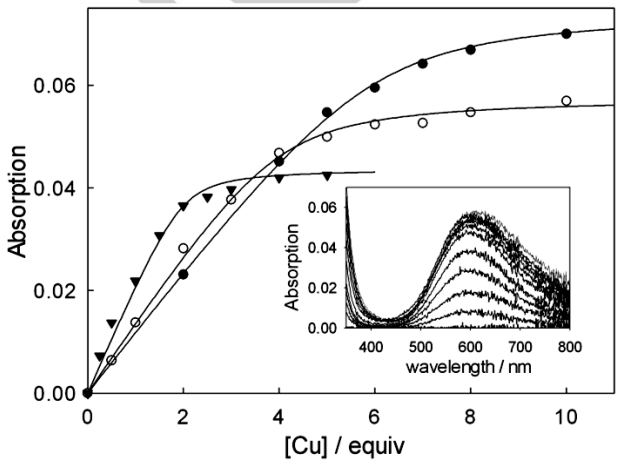


Figure 1. Optical titration of Cu(II) into **L1** (▼, 0.10 mM), **L2** (○, 0.05 mM), and **L3** (●, 0.05 mM) in 50% methanol/HEPES solution (100 mM at pH 7.0 and 25 °C), monitored at λ_{max} = 600 nm. The solid traces are the best fits to 2:1, 4:1, and 6:1 metal-to-ligand stoichiometry. The electronic spectra of the complexes have similar features with molar absorptivity of 203 ± 25 M^{-1} cm^{-1} per Cu, and follow similar spectral change upon Cu(II) binding to **L2** (inset).

Although the electronic spectra of these Cu(II) complexes are similar, the EPR spectra of the complexes are significantly different despite their identical metal-binding cyclen sites. The EPR spectrum of Cu_1**L1** at 5 K shows prototypical features of a type-2 Cu(II) center with a $d_{x^2-y^2}$ ground state in a distorted square pyramidal coordination environment,[27] which can be simulated with $g_{//}$ = 2.202, $A_{//}$ = 550 MHz, $g_{\perp}$ = 2.055, and $A_{\perp}$ = 55 MHz (Fig. 2B) similar to that of the Cu(II)-cyclen complex with $g_{//}$ = 2.202, $A_{//}$ = 554 MHz, $g_{\perp}$ = 2.055, and $A_{\perp}$ = 45 MHz (Fig. 2A). These parameters are consistent with those of cyclen-based Cu-complexes in a square planar coordination sphere.[23] The spectrum of Cu_2**L1** is quite different from that of Cu_1**L1** and is best simulated with three components: 48% of component **A** with $g_{//}$ = 2.202, $A_{//}$ = 554 MHz, $g_{\perp}$ = 2.056, and $A_{\perp}$ = 47 MHz similar to the spectrum of Cu_1**L1**, 12% of component **B** with $g_{//}$ = 2.416, $A_{//}$ = 389 MHz, $g_{\perp}$ = 2.0832, and $A_{\perp}$ = 14.5 MHz, and 40% of a broad component **C** with $g_{//}$ = 2.262, $A_{//}$ = 178 MHz, $g_{\perp}$ = 2.0480, and $A_{\perp}$ = 7.5 MHz and H-strain broadenings in the order of 400 MHz (Fig. 2C). The second component with relatively

large $g_{//}$ would indicate coordination to four oxygen ligands according to the Peisach-Blumberg rules[28] if it could be considered to have only four ligands but this is unlikely for Cu(II)–cyclen complexes as seen from recent structural data of similar complexes.[27, 29] It only accounts for 12% of the spectral intensity and was included in the simulation because it was quite pronounced in the echo-detected field sweep spectra of the same sample (cf. Fig. 3). In case of metalloproteins, the three-N(His) coordination sphere in Cu(II)-substituted carbonic anhydrase[24] shows $g_{//}$ = 2.31, and $g_{\perp}$ = 2.06 and the four-N(His) coordination sphere in Cu,Zn-superoxide dismutase exhibits $g_{//}$ = 2.268, and $g_{\perp}$ = 2.087.[25, 30]

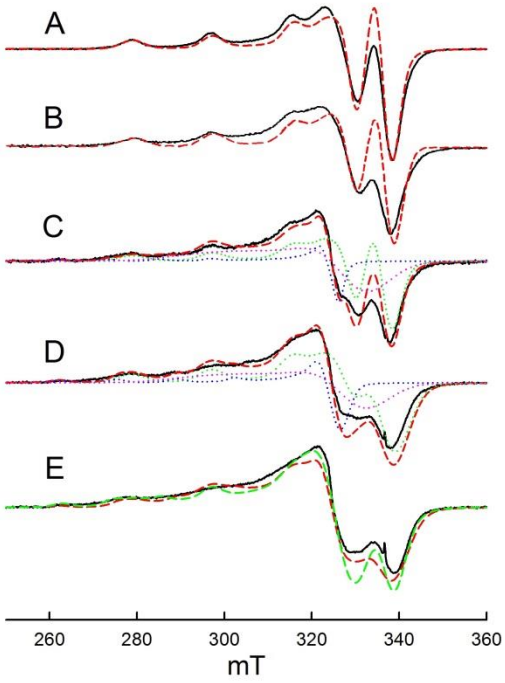


Figure 2. EPR spectra of Cu–cyclen (A), Cu_1**L1** (B), Cu_2**L1** (C), Cu_4**L2** (D), and Cu_6**L3** (E) in DMSO/100 mM HEPES buffer at pH 7.5 and 50 K, their simulated spectra (dashed traces), and the three components for the simulation in spectra C and D (dotted traces).

Notably, component **C** at 40% of the total intensity has to be introduced to the spectrum of Cu_2**L1** in order to generate a good fit. This component is practically absent in the echo-detected EPR spectrum indicating that its relaxation is too fast for echo detection. Together with the lack of clear features, component **C** reflects the signature of possible magnetic coupling due to proximity of the two Cu(II) centers in Cu_2**L1**. The spectra of Cu_4**L2** and Cu_6**L3** were simulated to some degree with the same three components in Cu_2**L1**, *i.e.*, 56% of **A**, 14% of **B**, and 30% of **C** for Cu_4**L1**, and 24% of **A**, 20% of **B**, and 56% of **C** for Cu_6**L3** (Figs. 2D,E). It should be noted that Cu_6**L3** presented the most difficulty for a reasonable fit with variations in the distribution of the components, possibly indicating changes in the broad complex spectrum that were not seen in the other cases. The spectral features suggest a common coordination environment of the metal centers (i.e., component **A**) in all the complexes with a tetragonally distorted octahedral geometry and a nitrogen-rich coordination environment.[28] The loss in resolution of hyperfine coupling with increasing amounts of Cu(II) may be attributed to magnetic interactions among the multiple Cu(II) centers within the complexes.

Spin-echo-detected EPR (se-EPR) spectra are sensitive to changes in electronic relaxation times and only relatively slow-relaxing signals can be better revealed. The se-EPR spectrum of Cu_1**L1** (Fig. 3B) is similar to that of Cu–cyclen (Fig. 3A) and can be fitted with similar spectral parameters of $g_{//}$ = 2.202 (vs. 2.002), $A_{//}$ = 554 MHz, $g_{\perp}$ = 2.055, and $A_{\perp}$ = 45 MHz, which concludes their similar geometric and magnetic properties. The se-EPR spectra of Cu_2**L1** (Fig. 3C) and Cu_4**L2** (Fig. 3D) are similar to each other, but significantly different from that of Cu_1**L1**, and can be reasonably simulated with the two components **A** and **B** already from their cw-EPR spectra (Fig. 3C), with component **A** resembling the spectrum of Cu_1**L1**. Although the EPR spectra of these complexes are different, the Cu(II) sites exhibit similar electronic spectra (Fig. 1) and kinetic parameters (see next section) with respect to each Cu(II) site which suggests that the different Cu(II) centers undergo fast exchange at room temperature to render one similar average site for all the complexes. The se-EPR spectra of Cu_2**L1** (Fig. 2C) and Cu_4**L2** (Fig. 2D) can be simulated with two components without the broad component, whereas that of Cu_6**L3** cannot. Further fitting of this complex was not attempted since anisotropy of the relaxation times can skew the shape of the underlying EPR spectra.

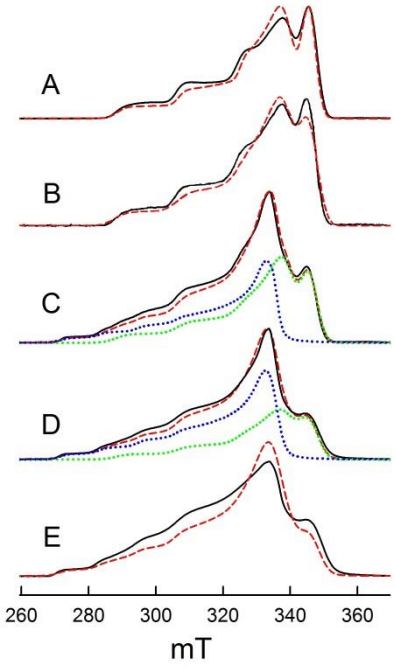


Figure 3. Spin-echo-detected EPR spectra of Cu–cyclen (A), Cu_1**L1** (B), Cu_2**L1** (C), Cu_4**L2** (D), and Cu_6**L3** (E) in DMSO/100 mM HEPES buffer at pH 7.5, simulated spectra (dashed traces), and the components (dotted traces).

Catalytic activities and cooperativity: The Cu(II) complexes of **L1**, **L2**, and **L3** exhibit significant activities toward catechol oxidation in 50% methanol aqueous solution of 100 mM HEPES at pH 7.0 and 25 °C, showing an enzyme-like saturation kinetic pattern which can be fitted to Eq. (2) to yield the kinetic constants k_{cat} and *K*' (Fig. 4A, Table 1) and significant first-order catalytic proficiencies (k_{cat}/k_o)[31] of (0.75–2.1) × 10^5 relative to catechol auto-oxidation (k_o). The k_{cat} values follows the order of Cu_2**L1** < Cu_4**L2** < Cu_6**L3**, but are close to each other after normalization with respect to Cu(II) concentrations, i.e., 0.0175 ± 0.0012 s^{-1} (Table 1); whereas the *K*' values remain close to each other (2.8 ± 0.3 mM). This observation indicates that the higher activities of Cu_4**L2** and Cu_6**L3** are attributed to their higher metal contents, and the similar k_{cat} and *K'* values per Cu center of these complexes are indicative of their similar catalytic mechanism.

The rate for the oxidation of 6 mM catechol by the complex Cu_1**L1** with one-half of the metal-binding sites occupied (2.2× 10^{-7} M/min) is about 7-fold slower than those of the other complexes with the metal-binding sites fully occupied, e.g., a rate of 1.5 × 10^{-6} M/min per Cu by Cu_2**L1** under the same conditions. The EPR spectrum of Cu_1**L1** is similar to that of the untethered Cu-cyclen complex (Figs. 2A,B & 3A,B), indicating their similar Cu environments in frozen state. These results suggest proximity of Cu sites in the Cu_{2n}**L1** complexes may be important for catalysis, possibly rendering dinuclear catalysis.

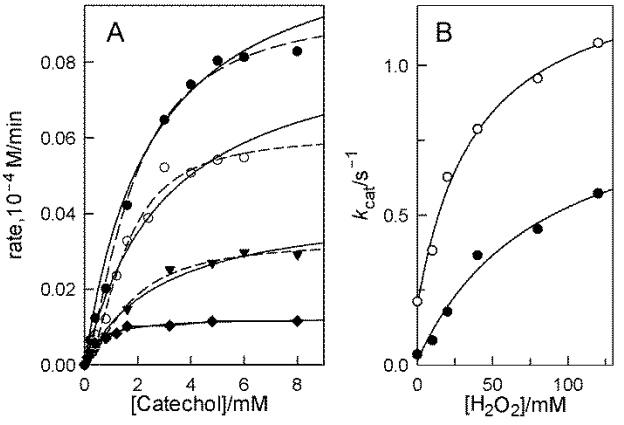


Figure 4. (A) Plots of the initial oxidation rates of catechol by Cu–cyclen (♦, 10 μM), Cu_2**L1** (▼, 5 μM), Cu_4**L2** (○, 5 μM), and Cu_6**L3** (●, 5 μM) in 100 mM HEPES (50% methanol) at pH 7.0 and 25 °C, and fitted to equation 2 (solid traces). The dashed traces are fittings to the Hill's equation to obtain the cooperativity coefficients. (B) Profiles of k_{cat} for oxidation of catechol by H_2O_2 (•) and DTBC (○) by Cu_2**L1** in 100.0 mM HEPES/menthol (1:1) at pH 7.0 and 25 °C, and fitted to pre-equilibrium kinetics Eqs. 1–2.

Table 1. Kinetic parameters for catechol oxidation by the Cu–**L** complexes[a]

Complex	k_{cat} (10^{-3} s^{-1})	*K*' (mM)	k_{cat}/K' (M^{-1} s^{-1})	CP[b] (k_{cat}/k_o)	θ
Cu_2**L1**	35.5 (17.8[c])	2.89	12.3	7.49 × 10^4	1.5
Cu_4**L2**	73.8 (18.5[c])	3.06	24.1	1.56 × 10^5	1.7
Cu_6**L3**	97.8 (16.3[c])	2.48	39.4	2.06 × 10^5	1.5
Cu(cyclen)	4.16	0.515	8.08	8.80 × 10^3	0.96

[a] In 50% methanol/100 mM HEPES buffer at pH 7.0 and 25 °C. [b] The background auto-oxidation rate constant k_o = 4.74 × 10^{-7} s^{-1}.[32] [c] Turn-over number per Cu(II) center.

The rates of catechol oxidation by the three Cu_{2n}**L** complexes are clearly sigmoidal and are better fitted to the Hill's equation with Hill coefficients θ of ~1.5 (dashed traces, Fig. 4A; Table 1) out of a maximum of 2 for two metal sites of potential dinuclear catalysis (discussed later). The close proximity of the Cu centers in the complexes with multiple domains may have rendered the significant cooperativity in the catalysis. Note that the Hill coefficient is around 2.5 in hemoglobin of four subunits, out of a maximum of 4 for the four possible binding sites. For comparison, the untethered Cu(II)-cyclen complex shows about 4-fold lower k_{cat} relative to the Cu_{2n}**L** complexes per Cu center without noticeable catalytic cooperativity (θ ≈ 0.96; ♦, Fig. 4A and Table 1). Similar to multi-domain/subunit proteins, synthetic molecules with multi-domain structures may also exhibit the unique property, such as the significant metal-binding cooperativity among the dendritic arms of dendrimers that is not seen in untethered arms.[1] The Cu**L** complexes herein do not exhibit metal-binding cooperativity (Fig. 1) but catalytic cooperativity (Fig. 4A), suggesting that metal binding to the cyclen sites in these complexes is independent and random whereas the catalysis follows cooperative dinuclear reaction mechanism which is further discussed later.

Substrate specificity: The specificity of the prototypical complex Cu_2**L1** was further investigated with various catechol-containing substrates, 3,5-di-*t*-butylcatechol (DTBC) and the catecholamine neurotransmitters dopamine, epinephrine, and norepinephrine. These substrates are effectively oxidized by this complex with high catalytic proficiencies of 155 to 75,000-fold higher than uncatalyzed reactions (Table 2), which however do not seem to show significant cooperativity (cf. Fig. 5A). The 2-order smaller catalytic proficiencies for the three catecholamines relative to catechol and DTBC seem to correlate with their biological roles as neurotransmitters to be stable enough to resist non-regulated oxidative transformation. Nevertheless, their metal-mediated oxidation reflects that they may be victimized by oxidative stress

caused by misregulated redox-active metal centers, such as the oxidation chemistry of the Alzheimer's disease-related Cu-β-amyloid.[32] In the proposed mechanism for di-Cu catechol oxidase,[33] the catechol substrate is oxidized by the met-di-Cu(II) form of the enzyme via two-electron transfer to afford the quinone product and reduced di-Cu(I)-enzyme, followed by binding of O_2 to form an active (di-Cu)-O_2 center which binds and oxidizes the second substrate. The aerobic oxidation of catechol and its derivatives by the Cu(II) complexes may follow the same dinuclear pathway as catechol oxidase, detailed in a later section.

Table 2. Oxidation of catechol-containing substrates by Cu_2**L1**[a], unless as noted.

Substrate	k_{cat} (10^{-3} s^{-1})	K' (mM)	k_{cat}/K' (M^{-1} s^{-1})	CP[b] (k_{cat}/k_o)	k_o (s^{-1})
Catechol	35.5	2.89	12.3	7.49×10^4	4.74×10^{-7}[c]
Catechol[b]	944	84.2	11.2	2.79×10^5	3.38×10^{-6}[d]
Catechol[e]	8.32	0.515	16.2	1.76×10^4	4.74×10^{-7}[c]
DTBC	212	2.24	94.6	1.41×10^4	1.50×10^{-5}[c]
DTBC[b]	1160	39.4	29.4	1.16×10^4	9.95×10^{-5}[d]
Dopamine	1.72	0.628	2.74	195	8.8×10^{-6}[c]
Epinephrine	0.266	0.403	0.660	205	1.3×10^{-6}[c]
Norepinephrine	0.372	0.586	0.635	155	2.4×10^{-6}[c]

[a] In 100 mm HEPES buffer/methanol (1:1) solution at pH 7.0 and 25 C. [b] In the presence of saturating amounts of H_2O_2 extrapolated from the fittings in Fig. 4B. [c] The background rates constants for the oxidation of the substrates, Reference [32]. [d] The auto-oxidation rate of the substrates in the presence of 100 mM H_2O_2. [e] Kinetic parameters for catechol oxidation by Cu(cyclen) normalized to 2[Cu(cyclen)] under the same conditions as in [a].

Catalytic specificity and mechanism: Hydrogen peroxide is an environmentally clean oxidation agent,[34] which however frequently requires further activation in order to perform effective oxidation reactions by various chemical and biochemical systems, such as peroxidases, the met-forms (di-Cu^{II}) of catechol oxidase and tyrosinase,[33, 35] and di-Cu(II) complexes.[36] The k_{cat} values for H_2O_2-mediated oxidation of catechol and DTBC by Cu_2**L2** were determined at certain fixed H_2O_2 concentrations. A plot of k_{cat} as a function of [H_2O_2] follows a saturation kinetic pattern (Fig. 4B), reflecting direct H_2O_2 binding to the metal center in the presence of the substrate *S* to afford a ternary peroxo–([di-Cu^{II}]–*S*) intermediate. Fitting of the k_{cat} values as a function of [H_2O_2] to Eq (2) yields the overall k_{cat} = 0.944 and 1.16 s^{-1}, K' = 84.2 and 39.4 mM, and catalytic proficiencies (k_{cat}/k_o) of 2.79×10^5 and 1.16×10^4, respectively, for oxidation of catechol and DTBC by H_2O_2 (Fig. 4B; Table 2), which are the rate constants with saturating amounts of catechol/DTBC and H_2O_2.

This kind of H_2O_2-saturation kinetic pattern was previously suggested to fallow an alternative pathway with a possible change in the rate determining step in the presence of H_2O_2 from first-order to zero-order kinetics with respect to H_2O_2 in which the oxidation of the substrate by the di-Cu(II) center is considered the rate-limiting step.[36] Regardless, the proposed pathway has the same kinetic pattern as saturation kinetics with a reversible H_2O_2 binding (instead of H_2O_2 dissociation at *path a* in reference [36]) followed by DTBC oxidation by the bound H_2O_2 (*path c* in reference [36]). If the change in rate would be attributed to the previously described mechanism, the mechanism would not necessarily follow a bi-substrate pathway with both the catechol substrate *S* and H_2O_2 bound to the metal center to form the ternary complex peroxo–[Cu_2**L1**]–*S*, but regenerate the di-Cu(II) center via oxidation of the di-Cu(II) center by H_2O_2 after the oxidation of the substrate[36] which however is inconsistent with the systems herein as discussed below. Both k_{cat} and K' in catechol oxidation increase significantly with [H_2O_2] (Table 2, Fig. 4B), resulting in no net gain in catalytic specificity k_{cat}/K'. Conversely, the catalytic specificity of DTBC oxidation is much smaller in the presence of saturating amounts of H_2O_2, indicating Cu_2**L1** is more specific toward DTBC oxidation by air than that by H_2O_2.

To determine the patterns and affinities for the binding of the catechol substrate and H_2O_2 to the Cu(II) center, the rate constants of DTBC under different concentrations of H_2O_2 were determined (Fig. 5A) and analyzed with the Hanes plots (Eq. 3, Figs. 5B,C).[9, 37] The secondary plots of the slopes and y-intercepts as functions of 1/[*B*] (Fig. 5D) from the Hanes plots (Fig. 6B) yield K_{DTBC} = 5.15 mM, $K_{H_2O_2}$ = 28.5 mM, and K_{iDTBC} = 2.47 mM with *A* = DTBC and *B* = H_2O_2 in Eq. (3). The intrinsic dissociation constant K_{iDTBC} = 2.47 mM obtained from the Hanes analysis is close to K' = 2.24 mM for DTBC oxidation without H_2O_2 (Table 2), corroborating the bi-substrate mechanism. The ratio K_{DTBC}/K_{iDTBC} = 2.08 (>1.0) indicates the binding of H_2O_2 to the metal center slightly decreases DTBC binding affinity to exhibit some exclusive nature between the two substrates H_2O_2 and DTBC.

A similar Hanes analysis of the rate-vs.-[H_2O_2] plots at various [DTBC]s (Fig. 5C; with A and B switched in Eq. 3) and its secondary plots yields K_{DTBC} = 6.10 mM, $K_{H_2O_2}$ = 37.5 mM, and $K_iH_2O_2$ = 16.4 mM. The ratio $K_{H_2O_2}/K_iH_2O_2$ = 2.29 indicates the binding of DTBC also decreases H_2O_2 affinity to the metal center corroborating the slightly exclusive nature of their binding to the metal. The results are consistent with a random bi-substrate mechanism, wherein H_2O_2 and catechol bind to the metal centers in the Cu_n**L** complexes independently and slightly exclusively to form the active ternary complexes DTBC–(Cu_n**L**)–H_2O_2.

Likewise, Hanes analysis of H_2O_2-mediated oxidation of catechol by Cu_2**L1** yields K_{CA} = 4.17 (and 3.79) mM, $K_{H_2O_2}$ = 200 (and

158) mM, K_{iCA} = 1.69 mM (relative to *K'* = 2.89 mM in the absence of H_2O_2, Table 2), and $K_iH_2O_2$ = 42.7 mM (Table 3). The ratios K_{CA}/K_{iCA} = 2.46 and $K_{H_2O_2}/K_iH_2O_2$ = 3.70 indicate the binding of catechol to the metal center slightly decreases H_2O_2 binding and vice versa, as observed in DTBC oxidation discussed above.

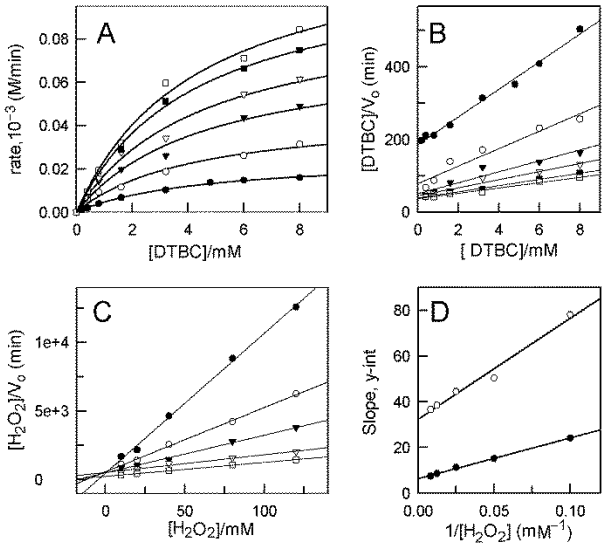


Figure 5. Oxidation of DTBC in the presence of 0, 10, 20, 40, 80, and 120 mM H_2O_2 (from top) at 25 °C and pH 7 by 2 μM Cu_2**L1** (A) and the Hanes plot against [DTBC] (B); (C) Hanes plot of (A) against [H_2O_2] with 0.2, 0.4, 0.8, 2.0, and 4.0 mM DTBC (from top); and (D) the slope (○) and y-intercept (●) from (B) as a function of 1/[H_2O_2].

Table 3. Hanes plot constants for DTBC, catechol (CA), and H_2O_2 from Eq. 3

	A = DTBC *B* = H_2O_2	*A* = H_2O_2 B = DTBC	*A* = *CA* *B* = H_2O_2	*A* = H_2O_2 B = CA
K_B (mM)	28.5	6.10	200	3.79
K_A (mM)	5.15	37.5	4.17	158
K_{iA} (mM)	2.47	16.4	1.69	42.7
K_A/K_{iA}	2.08	2.29	2.46	3.70

Comparison with other systems: The oxidative activities of the Cu-**L** complexes toward catechol and DTBC with k_{cat} = 0.036–1.16 s^{-1} and *K'* = 0.52–39 mM are comparable to or much better than many Cu(II) complexes of macrocyclic or multidentate ligands with k_{cat} in the range of (0.33–2.5) ×10^{-4} to 1.5 s^{-1} and *K'* = 0.071–2.0 mM.[36, 38] Several Cu(II)-containing multinuclear complexes catalyze DTBC oxidation with k_{cat} = 0.038–9.0 s^{-1} and *K'* = 0.35–5.0 mM.[11a, 14a, 18a, 39] Detailed comparison with various model complexes is not quite feasible as the reactions were conducted under different conditions, e.g., different solvents, pHs, and/or temperatures. Some best performing catechol oxidase models showed turnover numbers of 2.75–9.83 s^{-1} toward catechol oxidation,[40] while the catechol oxidase from *Ipomoea batatas*[41] exhibits k_{cat} = 2,293 s^{-1} and K_m = 2.5 mM that is expectedly not closely matched by any of the model complexes. Notably, the activities of the Cu_n**L** complexes herein are comparable to the catechol oxidase activity of hemocyanin (which has a similar di-Cu coordination site as catechol oxidase) from various sources,[42] i.e., k_{cat} = 0.0035–0.183 s^{-1}, K_m = 2.6–250 mM, and k_{cat}/K_m = 0.2–9.0 M^{-1} s^{-1}.

The catalytic activity of Cu_2**L1** toward dopamine oxidation (k_{cat} = 1.72 × 10^{-3} s^{-1} and *K'* = 0.628 mM) is close to those of the Cu(II) complexes of β-amyloid peptides (k_{cat} = (0.748–28.0) × 10^{-3} s^{-1} and *K'* = 0.27–0.90 mM at pH 7.0 and 25 °C)[32b] and the Cu(II) complex of an octapeptide fragment of prion[43] (k_{cat} = 1.1 × 10^{-3} s^{-1} and *K'* = 0.25 mM in the presence of 73 mM H_2O_2 in 80% methanol aqueous solution at pH 7.5 and 30 °C), but expectedly much less than that of tyrosinase from the cephalopod *Illex argentinus* (k_{cat} = 120 and 393 s^{-1} and K_m = 1.3 and 0.39 mM).[44] Although the di-Cu(II) met-form of tyrosinase exhibits H_2O_2-mediated oxidation catalysis, it does not catalyze oxidation/oxygenation by the use of air as the oxidation agent; whereas the complexes herein perform effective aerobic oxidation of catechol and derivatives.

Cu_2**L1** shows a significantly higher catalytic specificity for aerobic oxidation than oxidation by H_2O_2 toward DTBC with a ratio R_{cs} = $CS_{O_2}/CS_{H_2O_2}$ = 322% (CS_{O_2} and $CS_{H_2O_2}$ are the catalytic specificities of aerobic oxidation and H_2O_2-mediated oxidation), while the R_{cs} ratio of 110% for catechol is only slightly in favor of oxidation by air than by H_2O_2. Conversely, the Cu(II) complex of a 4-vinylpyridine-*co*-acrylamide copolymer (Cu**P1**) shows R_{cs} = 64% toward DTBC oxidation by air/H_2O_2 (1:1 methanol/25 mM MES buffer at pH 6.0 and 25 °C) and R_{cs} = 48% toward 1,2,3- trihydroxybezene;[45] and Cu-β-amyloid(1–20) exhibits R_{cs} = 440/1510 = 29% toward DTBC oxidation by air/H_2O_2 (25 mM H_2O_2 in 100 mM HEPES buffer at pH 7.0).[32a]

The axial ligand in some Cu-cyclen complexes forms a H-bond with an amine-NH group of cyclen.[22] The higher O_2-specificity of the Cu**L** complexes might be contributable to H-bonding between a bound O_2 and the NH group. Such stabilization of a bound O_2 via H-bonding is best known in hemoglobin and myoglobin, in which the bound O_2 forms a H-bond with the NH group of the distal His side chain. A bound H_2O_2 in the form of OOH^- may also form H-bonding with an acceptor such as an carbonyl group found in Cu**P1** (i.e., the amide group) and Cu-β-amyloid (i.e., various amino acid residues and the peptide bond) which can enhance oxidation activity by H_2O_2, whereas a bound peroxide O_2^{2-} can form H-bonds with an NH group. The Cu**L** complexes herein show higher R_{cs} toward O_2 than H_2O_2, suggesting that a bound O_2 may form H-bonding with the cyclen ligand better than H_2O_2. Owing to their high specificity toward oxidation by air than by H_2O_2 of catechol substrates, these Cu_n**L** complexes may thus serve as prototypes for further design of catalysts using air as an oxidation agent.

Catalytic mechanism and nuclearity: If all the Cu(II) centers in these complexes are equivalent and follow a similar pathway toward catechol oxidation, Cu_1**L1** would show 50% activity of Cu_2**L1**. However, the rate of oxidation of 6-mM catechol by Cu_1**L1** is 6.8-fold lower than that by Cu_2**L1**, suggesting that the mononuclear Cu_1**L1** complex does not contain the same structural motif as Cu_{2n}**L** complexes for an effective catalysis that presumably follows a dinuclear pathway. Since the substrate-bound intermediate [Cu**L**]–*S* in Eq. (1) is the catalytically active form, the mechanistically important stoichiometry of the intermediate can be determined by means of the "mechanistic Job plot"[6, 46b, 47] to reveal the nuclearity during the catalysis. The oxidation of catechol by Cu_2**L1**, Cu_4**L2**, and Cu_6**L3** exhibit a maximum at X_{CuL} ~ 0.5 (■, Fig. 6), 0.33 (○), and 0.25 (●) in their mechanistic Job plots, affording the Cu**L**:catechol ratios of 0.5:0.5, 0.33:0.67, and 0.25:0.75 (i.e., 1:1, 1:2, and 1:3), respectively. Since the complexes Cu_2**L1**, Cu_4**L2**, and Cu_6**L3** contain 2, 4, and 6 metal centers, the ratios of 1:1, 1:2, and 1:3 reflect 2-to-1 metal-to-substrate stoichiometry in the intermediates [Cu**L**]–*S* for the oxidation of catechol by these three complexes, indicating a dinuclear catalytic pathway.

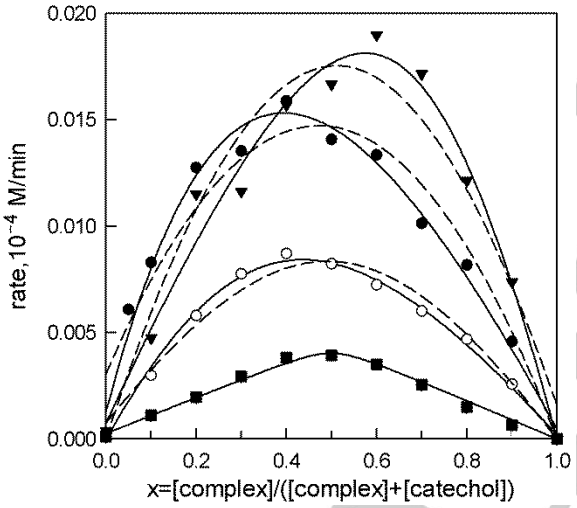


Figure 6. "Mechanistic Job plots" for the oxidation of catechol by Cu_2**L1** (■), Cu_4**L2** (○), and Cu**L3** (●) at a total concentration ([complex] + [catechol]) of 100 μM and by Cu–cyclen (▼) at a total concentration of 500 μM, and fitted to complex-to-catechol ratios of 1:1, 1:2, 1:3, and 2:1, respectively (solid traces). The dashed traces are fittings to a complex-to-catechol ratio of 1:1.

For comparison, the activity of Cu(II)-cyclen, which resembles a metal-binding arm in the ligands **L1**–**L3**, was determined under the same conditions. Cu(II)–cyclen catalyzes aerobic catechol oxidation with $k_{cat} = 4.16 \times 10^{-3}$ s^{-1} and $k_{cat}/K_m = 8.08$ s^{-1} M^{-1} per Cu center under the same conditions which are significantly smaller than those of Cu_2**L1** (Fig. 4A; Table 1). Moreover, Cu(II)–cyclen does not seem to show catalytic cooperativity toward catechol oxidation as the three Cu_2**L** complexes (Fig. 4A). The mechanistic Job plot of catechol oxidation by Cu–cyclen shows a maximum at $X_{[Cu–L]}$ ~ 0.67, opposite to those of the Cu**L** complexes, which affords the stoichiometry of [Cu–cyclen]:catechol = 2:1 (▼, Fig. 6), reflecting a dinuclear catalytic pathway by assembling two Cu centers to form the intermediate $[Cu–cyclen]_2$–catechol.

Thermodynamic Study: The k_{cat} values for the oxidation of catechol by the cyclen-containing Cu**L** complexes were acquired at different temperatures and the activation energy E_a determined according to the Arrhenius equation $k_{cat} = A\ exp(-E_a/RT)$ which yields the enthalpy of activation $\Delta H^{\ddagger}$ (= $E_a - RT$) with the trend Cu_6**L3** < Cu_4**L2** < Cu_2**L1** < Cu(cyclen). The Gibbs free energy of activation $\Delta G^{\ddagger}$ was obtained from k_{cat} according to the Eyring–Polanyi equation $k_{cat} = (k_B T/h)\ exp(-\Delta G^{\ddagger}/RT)$ with k_B being the Boltzmann constant and *h* the Planck constant, from which the entropy of activation $\Delta S^{\ddagger}$ was calculated, following the trend Cu_6**L3** > Cu_4**L2** > Cu_2**L1** > Cu(cyclen) for the $-\Delta S^{\ddagger}$ values (Table 4). The "normalized k_{cat} values" (thus the "normalized $\Delta G^{\ddagger}$ values") with respect to a single di-Cu center in the three Cu_{2n}**L** complexes are similar, resulting in more pronounced $-\Delta S^{\ddagger}$ values for Cu_4**L2** and Cu_6**L3** (Table 4). The observation of pre-equilibrium kinetics for catechol oxidation by these complexes indicates formation of the enzyme-like Michaelis complex [*S*–Cu**L**] prior to product formation. The negative $\Delta S^{\ddagger}$ values and their trend indicate that the transition state $TS^{\ddagger}$ is better organized than the prior step, probably due to the involvement of more metal sites in Cu_4**L2** and Cu_6**L3** to stabilize and organize the substrate than in Cu_2**L1** and Cu(cyclen).

Table 4. Activation parameters for catechol oxidation by Cu**L** complexes at 298 K[a]

	E_a (kJ/mol)[b]	$\Delta H^{\neq}$ (kJ/mol)	$\Delta G^{\neq}$ (kJ/mol)	$\Delta S^{\neq}$ (J/mol K)
Cu_2**L1**	29.2	26.7	81.2	−183
Cu_4**L2**	21.7	19.2	79.5 (81.2[c])	−202 (−208[c])
Cu_6**L3**	17.6	15.1	78.7 (81.5[c])	−214 (−223[c])
Cu(cyclen)	43.4	40.5	(86.6[c])	(−155[c])

[a] In 100 mM pH 7.0 HEPES buffer/methanol (1:1) solution. [b] obtained from temperature range 25–55 °C. [c] Normalized based on a di-Cu center relative to Cu_2**L1**.

Concluding Remarks

We describe herein the activities, mechanism, and catalytic specificities of the copper(II) complexes of the multidentate ligands **L1**, **L2**, and **L3** toward oxidation of catechol and derivatives. The studies reveal the involvement of intramolecular dinuclear Cu(II) centers during catalysis in the Cu_{2n}**L** complexes

with much higher activities than the mono-Cu(II) complexes Cu$_1$**L1** and Cu(cyclen). Although many dinuclear Cu(II) complexes show oxidation activity by dioxygen and/or H_2O_2, it is still challenging to obtain complexes that incorporate catalytic cooperativity, specific substrate recognition, and O_2/H_2O_2 specificity. The complexes herein possess all these properties. Moreover, the Cpz core is quite versatile for (a) systematic studies of the nature of the ligands (such as imidazole[6] and cyclen-containing arms herein), (b) assembling various di-Cu centers (such as Cu$_2$**L1**, Cu$_4$**L2**, and Cu$_6$**L3**), (c) building substrate specificity (described herein and the specific phosphomonoester recognition and hydrolysis[6]), (d) rendering catalytic cooperativity in oxidation (Fig. 5A) and hydrolysis,[6] (e) exhibiting more specific oxidation reactions by O_2 than by H_2O_2 (Table 2), (f) possibly building a potential substrate recognition site in the proximity of the metal center, and (g) exploration of other activities by various metal complexes of Cpz-containing ligands in the future.

Experimental Section

Materials and Ligand Syntheses are described in the Supporting Information. The Cu(II) complexes were prepared by mixing stoichiometric amounts of Cu(II) (99.999%) and the ligands **L**1–3 in 1:1 menthol/100.0 mM HEPES buffer at pH 7.0 for optical and kinetic studies, and in DMSO/100 mM HEPES buffer at pH 7.5 for EPR experiments. The choice of the solvents was partially empirical for better detection and observation, and for better formation of glass-state at low temperatures for EPR. All the samples were prepared right prior to measurements.

EPR Measurements. EPR spectra were obtained with a Bruker Elexsys E580 X-band spectrometer at ~20 K using an Oxford ESR900 cryostat and the Bruker standard rectangular TE_{102} resonator, typically with a microwave frequency at ~9.4 GHz, field modulation around 2 G, and time constant/conversion time of 40/80 ms. The g and A values were obtained from spectral simulations performed with the "EasySpin" toolbox for Matlab.[48] Since a few Cu(II)-substituted metalloproteins[49] and Cu(II) complexes[50] were determined to have pK_a values of around 6–7 for the coordinated water, it is thus appropriate to choose a pH value away from the pK_a values to avoid possible coexistence of both protonated and deprotonated forms of the coordinated water.[51] The spectra of Cu(II)-cyclen and Cu$_1$**L1** with a single component demonstrate this viewpoint. Pulsed EPR spectra were obtained with the same spectrometer and a Bruker Flexline cryostat at a temperature of 5 to 6 K. Spin-echo-detected field-swept spectra were obtained using the 2-pulse Hahn echo sequence 90°–τ–180°–τ–AQ where 'AQ' stands for the observed echo. Spin lattice relaxation times were obtained with the inversion-recovery sequence 180°–T–90°–τ–180°–τ–AQ with echo-detection. The traces were fitted to a first-order relaxation or a combined two-step relaxation to yield the relaxation times of the Cu(II) centers in the complexes.

Kinetic Measurements. The initial oxidation rates of the substrates by 5 μM Cu(II) complexes of **L1**, **L2**, and **L3** in buffered aqueous-methanol solution (50% 100 mM HEPES at pH 7.0) were determined by monitoring the change in absorption at 500 nm due to the formation of the product adduct *o*-quinone-MBTH as previously described[9, 32] (ε = 32,500 M^{-1} cm^{-1} for catechol,[52] 27,200 M^{-1} cm^{-1} for dopamine, and 27,500 M^{-1} cm^{-1} for epinephrine and norepinephrine).[53] The oxidation of 3,5-di-t-butylcatechol (DTBC) is directly monitored at 420 nm (ε = 1,910 M^{-1} cm^{-1}).[54] The *para*-quinone isomer does not generate a significant 500-nm absorption in the experimental time frame to interfere the detection of the *ortho* isomer under the same conditions.

In enzyme-like pre-equilibrium kinetics, the substrate *S* binds to the catalytic Cu center of the Cu**L** complexes to form the intermediate *S*–Cu**L** complex (analogous to the enzyme-substrate ES complex) which is followed by conversion to products as described in Equation (1). The rate law for this reaction mechanism is expressed as Equation (2), with $K' = (k_{-1} + k_{cat})/k_1$ being the apparent dissociation constant of the *S*–Cu**L** complex analogous to the Michaelis constant K_m in enzyme catalysis, assuming that the concentration of this complex is much lower than that of the unbound substrate. The initial rates for the oxidation of the substrate *S* at different concentrations are fitted to Equation (2) with non-linear regression to find the rate constants k_{cat} and *K*'.

$$\mathrm{CuL} + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S\text{–CuL} \xrightarrow{k_{cat}} \mathrm{CuL} + \mathrm{P} \quad (1)$$

$$\text{rate} = \frac{k_{cat}\,[\mathrm{CuL}]\,[S]}{K' + [S]} \quad (2)$$

The stoichiometry of a metal complex M–L$_n$ in the equilibrium M + nL $\rightleftharpoons$ M–L$_n$ can be determined by means of the Job plot, wherein the total concentration [M] + [L] is kept constant while the metal-to-ligand ratio is varied and the optical density of the complex is monitored.[46] Likewise, the presence of the pre-equilibrium Cu**L** + *S* $\rightleftharpoons$ *S*–Cu**L** in the catalysis of catechol oxidation allows the use of the "mechanistic Job plot[6, 47]" to determine the stoichiometry of the substrate-bound complex *S*–Cu**L** in the reaction mechanism by monitoring the reaction rate that directly reflects the concentration of the intermediate (i.e., rate = k_{cat}[*S*–Cu**L**]), analogous to the optical density of the complex in the conventional Job plot. In this case, the total concentration ([Cu**L**] + [*S*]) is kept constant while the ratio [Cu**L**]/[*S*] varies and the oxidation rate of the substrate *S* is monitored. The maximum reaction rate in the Job plot reflects the stoichiometry of the ternary complex, e.g, a maximum at mole fraction $X_{Cu–L}$ = 0.66 reflects that the stoichiometry of the ternary complex *S*–Cu**L** is $X_{Cu–L}$:X_S = 0.66:0.33 = 2:1 which indicates a dinuclear catalysis.

For a random bi-substrate catalysis, such as H_2O_2-mediated oxidation of catechol by the Cu(II) complexes herein with both H_2O_2 and catechol as the substrates,[] the dissociation constants for each substrate can be determined from the Hanes plots (Eq. 3)[9, 37] by measuring the reaction rate of one substrate *S1* with systematic variation in concentration of the other substrate *S2*. The rate law for this catalysis is shown as Eq (3),

$$\frac{[A]}{V_o} = \frac{(1 + K_B/[B])}{V_{max}}[A] + \frac{K_A}{V_{max}}\left[1 + \frac{K_{iA}K_B}{[B]K_A}\right] \quad (3)$$

in which *A* can be fixed to either *S1* or *S2* and *B* is the other substrate once *A* is fixed, K_A and K_B are apparent dissociation constants for the ternary complex with both *A* and *B* bound to the catalytic center, i.e., *A*–(Cu**L**)–*B* $\rightleftharpoons$ (Cu**L**)–*B* + *A* and *A*–(Cu**L**) + *B*, respectively, and K_{iA} is the intrinsic dissociation constant in *A*–(Cu**L**) $\rightleftharpoons$ Cu**L** + *A*. A secondary plot of the slope $(1 + K_B/[B])/V_{max}$ or the *y*-intercept $(K_A/V_{max})(1 + K_{iA}K_B/K_A[B])$ as function of [*B*] yields the dissociation constants. If the binding of one substrate influences the binding of the other, a significant difference between $K_{A(B)}$ and $K_{iA(B)}$ would be detected.

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Keywords: catechol oxidase • copper • cyclen • multinuclear • oxidation

References

[1] a) J. D. Epperson, L.-J. Ming, B. D. Woosley, G. R. Baker, G. R. Newkome, *Inorg. Chem.* **1999**, *38*, 4498–4502; b) J. D. Epperson, L.-J. Ming, G. R. Baker, G. R. Newkome, *J. Am. Chem. Soc.* **2001**, *123*, 8583–8592.

[2] H. R. Allcock, *Chemistry and Applications of Polyphosphazenes.* Wiley: Hoboken, NJ, **2003**.

[3] a) R. Kagit, M. Yildirim, O. Ozay, S. Yesilot, H. Ozay, *Inorg. Chem.* **2014**, *53*, 2144–2151; b) V. Chandrasekhar, T. Senapati, A. Dey, S. Das, M. Kalisz, R. Clerac, *Inorg. Chem.* **2012**, *51*, 2031–2038; c) E. W. Ainscough, A. M. Brodie, P. J. B. Edwards, G. B. Jameson, C. A. Otter, S. Kirk, *Inorg. Chem.* **2012**, *51*, 10884–10892; d) E. W. Ainscough, H. R. Allcock, A. M. Brodie, K. C. Gordon, M. D. Hindenlang, R. Horvath, C. A. Otter, *Eur. J. Inorg. Chem.* **2011**, 3691–3704; e) M. R. Rao, A. Ghosh, M. Ravikanth, *Inorg. Chim. Acta* **2011**, *372*, 436–441; f) R. Horvath, C. A. Otter, K. C. Gordon, A. M. Brodie, E. W. Ainscough, *Inorg. Chem.* **2010**, *49*, 4073–4083; g) M. R. Rao, G. Gayatri, A. Kumar, G. N. Sastry, M. Ravikanth, *Chem. Eur. J.* **2009**, *15*, 3488–3496; h) V. Chandrasekhar, G. T. S. Andavan, R. Azhakar, B. M. Pandian, *Inorg. Chem.* **2008**, *47*, 1922–1924; i) E. W. Ainscough, A. M. Brodie, A. Derwahl, S. Kirk, C. A. Otter, *Inorg. Chem.* **2007**, *46*, 9841–9852; j) E. W. Ainscough, A. M. Brodie, R. J. Davidson, C. A. Otter, *Inorg. Chem. Commun.* **2007**, *10*, 1005–1008; k) A. Ouali, R. Laurent, A.-M. Caminade, J.-P. Majoral, M. Taillefer, *J. Am. Chem. Soc.* **2006**, *128*, 15990–15991.

[4] a) V. Chandrasekhar, S. Nagendran, *Chem. Soc. Rev.* **2001**, *30*, 193–203; b) V. Chandrasekhar, P. Thilagar, B. M. Pandian, *Coord. Chem. Rev.* **2007**, *251*, 1045–1074.

[5] L. Wang, Y. Ye, S. Zhong, Y. Zhao, *Chin. Chem. Lett.* **2009**, *20*, 58–61.

[6] L. Wang, Y. Ye, V. Lykourinou, A. Angerhofer, L.-J. Ming, Y. Zhao, *Eur. J. Inorg. Chem.* **2011**, 674–682.

[7] a) E. I. Solomon, U. M. Sundaram, T. E. Machonkin, *Chem. Rev.* **1996**, *96*, 2563–2606; b) E. A. Lewis, W. B. Tolman, *Chem. Rev.* **2004**, *104*, 1047–1076; c) S. Hikichi, M. Akita, Y. Moro-oka, *Coord. Chem. Rev.* **2000**, *198*, 61–87.

[8] N. Duran, E. Esposito, *Appl Catal B*: **2000**, *28*, 83–99.

[9] G. F. Z. da Silva, W. M. Tay, L.-J. Ming, *J. Biol. Chem.* **2005**, *280*, 16601–16609.

[10] a) C. Limberg, *Angew. Chem. Int. Ed.* **2003**, *42*, 6112–6136; b) R. Hage, A. Lienke, *Angew. Chem. Int. Ed.* **2006**, *45*, 206–222.

[11] a) K. Born, P. Comba, A. Daubinet, A. Fuchs, H. Wadepohl, *J. Biol. Inorg. Chem.* **2007**, *12*, 36–48; b) C. Gerdemann, C. Eicken, B. Krebs, *Acc. Chem. Res.* **2002**, *35*, 183–191.

[12] L. M. Mirica, X. Ottenwaelder, T. D. P. Stack, *Chem. Rev.*, **2004**, *104*, 1013–1045.

[13] a) N. Kitajima, *Adv. Inorg. Chem.* **1992**, *39*, 1-77; b) N. Kitajima, Y. Morooka, *Chem. Rev.* **1994**, *94*, 737–757; c) N. Kitajima, W. B. Tolman, *Prog. Inorg. Chem.* **1995**, *43*, 419–531; d) Y. Morooka, K. Fujisawa, N. Kitajima, *Pure Appl. Chem.* **1995**, *67*, 241–248; e) W. B. Tolman, *Acc. Chem. Res.* **1997**, *30*, 227–237; f) P. L. Holland, W. B. Tolman, *Coord. Chem. Rev.* **1999**, *192*, 855–869; g) H. C. Liang, M. Dahan, K. D. Karlin, *Curr. Opin. Chem. Biol*. **1999**, *3*, 168–175; h) V. Mahadevan, R. J. M. K. Gebbink, T. D. P. Stack, *Curr. Opin. Chem. Biol.* **2000**, *4*, 228–234; i) B. A. Jazdzewski, W. B. Tolman, *Coord. Chem. Rev.* **2000**, *200*, 633–685; j) A. G. Blackman, W. B. Tolman, *Struc. Bond.* **2000**, *97*, 179–211; k) W. B. Tolman, D. J. E. Spencer, *Curr. Opin. Chem. Biol.* **2001**, *5*, 188–195; l) P. Gamez, P. G. Aubel, W. L. Driessen, J. Reedijk, *Chem. Soc. Rev.* **2001**, *30*, 376–385; m) E. Kim, E. E. Chufan, K. Kamaraj, K. D. Karlin, *Chem. Rev.* **2004**, *104*, 1077–1133; n) I. A. Koval, P. Gamez, C. Belle, K. Selmeczi, J. Reedijk, *Chem. Soc. Rev.* **2006**, *35*, 814–840.

[14] a) K. Selmeczi, M. Réglier, M. Giorgi, G. Speier, *Coord. Chem. Rev.* **2003**, *245*, 191–201; b) L. Que, Jr., W. B. Tolman, *Nature*, **2008**, *455*, 333–340.

[15] a) L. Kovbasyuk, R. Kramer, *Chem. Rev.* **2004**, *104*, 3161–3187; b) A. M. O'Donoghue, S. Y. Pyun, M.-Y. Yang, J. R.Morrow, J. P. Richard,*J. Am. Chem. Soc.* **2006**, *128*, 1615–1621.

[16] E. Kovari, R. Kramer, *J. Am. Chem. Soc.* **1996**, *118*, 12704–12709.

[17] R. M. Izatt, K. Pawlak, J. S. Bradshaw, R. L. Bruening, *Chem. Rev.* **1995**, *95*, 2529–2586.

[18] a) N. Sengottuvelan, D. Saravanakumar, V. Narayanan, M. Kandaswamy, K. Chinnakali, G. Senthilkumar, *Bull. Chem. Soc. Jpn.* **2004**, *77*, 1153–1159; b) S. Aoki, M. Shiro, T. Koike, E. Kimura, *J. Am. Chem. Soc.* **2000**, *122*, 576–584; c) P. V. Bernhardt, *Inorg. Chem.* **1999** *38*, 3481–3483; d) T. H. Bennur, D. Srinivas, S. Sivasanker, *J. Mol. Cat A Chem.* **2004**, *207*, 163–171; e) M. Woods, A. D. Sherry, *Inorg. Chem.* **2003**, *42*, 4401–4408.

[19] a) K. Myoshi, H. Tanaka, E. Kimura, S. Tsuboyama, S. Murata, H. Shimizu and K. Ishizu, *Inorg. Chim. Acta*, **1983**, *78*, 23–30. b) S. Lacerda, M. P. Campello, I. C. Santos, I. Santos, R. Delgado, *Polyhedron* **2007**, *26*, 3763–3773.

[20] a) S. El Ghachtouli, C. Cadiou, I. Dechamps-Olivier, F. Chuburu, M. Aplincourt, W. Turcry, M. Le Baccon, H. Handel, *Eur. J. Inorg. Chem.* **2005**, *2005*, 2658-2668; b) M. Soibinet, I. Dechamps-Olivier, E. Guillon, J. P. Barbier, M. Aplincourt, F. Chuburu, M. Le Baccon, H. Handel, *Eur. J. Inorg. Chem.* **2003**, *2003*, 1984-1994.

[21] a) J.-F. Guo, W.-F. Yeung, S. Gao, G.-H. Lee, S.-M. Peng, M. H.-W. Lam, T.-C. Lau, *Eur. J. Inorg. Chem.* **2008**, 158-163; b) J. Hormann, C. Perera, N. Deibel, D. Lentz, B. Sarkar, N. Kulak, *Dalton Trans.* **2013**, *42* 4357-4360; c) W.-F. Yeung, W.-T. Wong, J.-L. Zuo, T.-C. Lau, *J. Chem. Soc., Dalton Trans*. **2000**, 629-631; d) R. Clay, P. Murrayrust, J. Murrayrust, *Acta Crystallogr. SecB-Struc. Sci.* **1979**, *35*, 1894-1895; e) T.-H. Lu, J.-L. Lin, W.-J. Lan, C.-S. Chung, *Acta Crystallogr. Sec. C-Cryst. Struc. Commun.* **1979**, *53*, 1598-1600; f) D. Rohde, K. Merzweiler, *Acta Crystallogr. Sec. E-Stru. Rep. Online* **2010**, *66*, M894-U351; g) S. El Ghachtouli, C. Cadiou, I. Dechamps-Olivier, F. Chuburu, M. Aplincourt, T. Roisnel, *Eur. J. Inorg. Chem.* **2006**, 3472-3481.

[22] a) L. M. P. Lima, D. Esteban-Gómez, R. Delgado, C. Platas-Iglesias, R. Tripier, *Inorg. Chem.*, **2012**, *51*, 6916–6927; b) G. Gasser, M. J. Belousoff, A. M. Bond , L. Spiccia, *Inorg. Chem.*, **2007**, *46*, 3876–3888; c) L. Gray, D. L. Gerlach, E. T. Papish, *Acta Crystallogr. Sec. E-Crystallogra. Commun.* **2017**, 73, 31–34.

[23] T. Sawada, K. Fukumaru, H. Sakurai, *Chem. Pharmaceu. Bull.* **1996**, *44*, 1009-1016.

[23] I. Bertini, E. Borghi, C. Luchinat, *J. Am. Chem. Soc.* **1979**, *101*, 7069-7071.

[24] a) R. J., Carrico, H. F. Deutsch, *J. Biol. Chem.* **1969**, *244*, 6087-6093; b) G. Rotilio, A. F. Agro, L. Calabrese, F. Bossa, P. Guerrieri, B. Mondovi, *Biochemistry* **1971**, ***10***, 616–621.

[25] a) R. J., Carrico, H. F. Deutsch, *J. Biol. Chem.* **1969**, *244*, 6087-6093. b) G. Rotilio, A. F. Agro, L. Calabrese, F. Bossa, P. Guerrieri, B. Mondovi, *Biochemistry* **1971**, *10*, 616 – 621.

[26] a) C. Hasselgren, H. I. Park, L.-J. Ming, *J. Biol. Inorg. Chem.* **2001**, *6*, 120–127; b) L.-Y. Lin, H. I. Park, L.-J. Ming, *J. Biol. Inorg. Chem.* **1997**, *2*, 744–749.

[27] a) A. Bencini, D. Gatteschi, D. in: *Inorganic Electronic Structure and Spectroscopy, Vol. I, Methodology* (Eds.: E. I. Solomon, A. B. P. Lever), Wiley, **2006**, pp. 93–159; b) C. V. Esteves, L. M. P. Lima, P. Mateus, R. Delgado, P. Brandao, V. Felix, *Dalton Trans.* **2013**, *42*, 6149–6160.

[28] J. Peisach, W. E. Blumberg, *Arch. Biochem. Biophys.* **1974**, *165*, 691–708.

[29] C. V. Esteves, P. Lamosa, R. Delgado, J. Costa, P. Desogere, Y. Rousselin, C. Goze, F. Denat, *Inorg. Chem.* **2013**, *52*, 5138–5153.

[30] a) R. A. Lieberma, J. A. Fee, *J. Biol. Chem.* **1973**, *248*, 7617–7619; b) G. Rotilio, L. Calabrese, B. Mondovi, W. E. Blumberg, *J. Biol. Chem.* **1974**, *249*, 3157–3160.

[31] a) A. Radzicka, R. Wolfenden, *Adv. Enzymol. Relat. Areas Mol. Biol.* **1999**, *73*, 25–55.

[32] a) G. F. Z. da Silva, L.-J. Ming, *Angew. Chem. Int. Ed.* **2005**, *44*, 5501–5504; b) G. F. Z. da Silva, L.-J. Ming, *Angew. Chem. Int. Ed.* **2007**, *46*, 3337–3341.

[33] a) C. Gerdemann, C. Eicken, B. Krebs, *Acc. Chem. Res*. **2002**, *35*, 183–191; b) M. Güell, P. E. M. Siegbahn, *J. Biol. Inorg. Chem.* **2007**, *12*, 1251–1264.

[34] a) J. Piera, J. E. Backvall, *Angew. Chem. Int. Ed.* **2008**, *47*, 3506–3523; b) R. Noyori, M. Aoki, K. Sato, *Chem. Commun.* **2003**, 1977–1986.

[35] A. Sánchez-Ferrer, J. N. Rodríguez-López, F. García-Cánovas, F. García-Carmona, *Biochim. Biophys. Acta* **1995**, *1247*, 1–11.

[36] E. Monzani, L. Quinti, A. Perotti, L. Casella, M. Gullotti, L. Randaccio, S. Geremia, G. Nardin, P. Faleschini, G. Tabbi, *Inorg. Chem.* **1998**, *37*, 553–562.

[37] V. Leskovac, *Comprehensive Enzyme Kinetics*, Kluwer, Boston, **2002**.

[38] a) A. Granata, E. Monzani, L. Casella, *J. Biol. Inorg. Chem*., **2004**, *9*, 903–913; b) S. Sreedaran, K. S. Bharathi, A. K. Rahiman, K. Rajesh, G. Hirmala V. Narayanan, *J. Coord. Chem.*, **2008**, *61*, 3594–3609; c) K. Born, P. Comba, A. Daubinet, A. Fuchs, H. Wedepohl, *J. Biol. Inorg. Chem.*, **2007**, *12*, 36–48; d) I. A. Koval, K. Selmeczi, C. Belle, C. Philouze, E. Saint-Aman, I. Gautier-Luneau, A. Schuitema, M. Van Vliet, P. Gomez, O. Roubeau, M. Luken, B. Krebs, M. Lutz, A. Spek, J. Pierre, J. Reedjik, *Chem. Eur. J.* **2006**, *12*, 6138–6150; e) V. J. Bhardwaj, N. Aliaga-Alcalde, M. Corbella, G. Hundal, *Inorg. Chim. Acta*, **2010**, *363*, 97–106.

[39] a) J. H. Qiu, Z. R. Liao, X. G. Meng, L. Zhu, Z. M. Wang, K. B. Yu, *Polyhedron* **2005**, 24, 1617–1623; b) V. K. Bhardwaj, N. Aliaga-Alcalde, M. Corbella,G. Hundal, *Inorg. Chim. Acta*. **2010**, *363*, 97–106; c) L. Gasque, V. M. Ugalde-Saldivar, I. Membrillo, J. Olguıin, E. Mijangos, S. Bernes, I. Gonzalez, *J. Inorg. Biochem.* **2008**, *102*, 1227–1235; d) M. Merkel, N. Moller, M. Piacenza, S. A. Rompel, B. Krebs, *Chem. Eur. J.* **2005**, *11*, 1201–1209; e) C. Jocher, T. Pape, W. W. Seidel, P. Gamez, J. Reedijk, F. E. Hahn, *Eur. J. Inorg. Chem.* **2005**, *24*, 4914–4923; f) A. Granata, E. Monzani, L. Casella, *J. Biol. Inorg. Chem*. **2004**, *9*, 903–913.

[40] a) K. Banu, T. Chattopadhy, A. Benarjee, S. Bhattacharya, E. Suresh, M. Nethajl, E. Zangrando, D Das, *Inorg. Chem.*, **2008**, *47*, 7083–7093; b) P. Nanda, V. Bertolasi, G. Aromi, D. Ray, *Polyhedron* **2009**, *28*, 987–993.

[41] C. Eicken, F. Zippel, B. Krebs, K. Buldt-Karentzopoulos, *FEBS Let.* **1998**, *436*, 293–299.

[42] a) A. Dolashki, W. Voelter, P. Dolashka, *Comp. Biochem. Physiol. B* **2011**, *160*, 1–7; b) R. Hristova, A. Dolashki, W. Voelter, S. Stevanovic, P. Dolashka-Angelova, *Comp. Biochem. Physiol. B* **2008**, *149*, 439–446; c) N. I. Siddiqui, R. F. Akosung, C. Gielens, *Biochem. Biophys. Res. Commun.* **2006**, *348*, 1138–1144.

[43] C. Madhavaiah, S. Verma, *Bioorg. Med. Chem.* **2005**, *13*, 3241–3248.

[44] T. Naraoka, H. Uchisawa, H. Mori, H. Matsue, S. Chiba, A. Kimura, *Eur. J. Biochem.* **2003**, *270*, 4026–4038.

[45] V. Lykourinou, A. I. Hanafy, G. F. Z. da Silva, K. S. Bisht, R. W. Larsen, B.T. Livingston, A. Angerhofer, L.-J. Ming,*Eur. J. Inorg. Chem.* **2008**, 2584–2592.

[46] a) C. Y. Huang, *Meth. Enzymol.* **1982**, *87*, 509–525; b) J. S. Renny, L. L. Tomasevich, E. H. Tallmadge, D. B. Collum, *Angew. Chem. Int. Ed.* **2013**, *52*, 11998–12013.

[47] W. M. Tay, J. D. Epperson, G. F. Z. da Silva, L.-J. Ming, *J. Am. Chem. Soc.* **2010**, *132*, 5652–5661.

[48] S. Stoll, A. Schweiger, *J. Magn. Res.* **2006**, *178*, 42–55.

[49] a) H. I. Park, L.-J. Ming, *J. Biol. Inorg. Chem.* **2002**, *7*, 600–610. b) G. F. Z. da Silva, R. L. Reuille, L.-J. Ming, B. T. Livingston, *J. Biol. Chem.* **2006**. *281*, 10737–10744.

[50] a) A. I. Hanafy, V. Lykourinou-Tibbs, K. S. Bisht, L.-J. Ming, *Inorg. Chim. Acta* **2005**, *358*, 1247–1252. (b) L. Wang, Y. Ye, V. Lykourinou, A. Angerhofer, L.-J. Ming, Y. Zhao, *Eur. J. Inorg. Chem.* **2011**, 674–682.

[51] F. Jian, J. Peisach, L.-J. Ming, L. Que, L., Jr., V. J. Chen, *Biochemistry* **1991**, *30*, 11437–11445.

[52] S. G. Srivatsan, P. Nigam, M. S. Rao, S. Verma, *Appl. Catal. A* **2001**, *209*, 327–334.

[53] J. N. Rodriguez-Lopez, J. Escribano, F. Garcia-Canovas, *Anal. Biochem.* **1994**, *216*, 205–212.

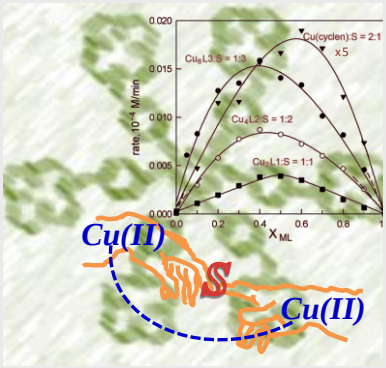
[54] S. Grabiec, W. Tomassi, T. Podbielski, *Bull. Acad. Pol. Sci. Biol.* **1974**, *22*, 187–191.

Entry for the Table of Contents (Please choose one layout)

Layout 1:

FULL PAPER

Catechol oxidation by three Cu(II) complexes of 1,4,7,10-tetraaza-cyclododecane (cyclen) attached to a cyclotriphosphazene core follows enzyme-like saturation kinetics and an *intramolecular dinuclear pathway* with significant cooperativity ($\theta \approx 1.5$ with a maximum of 2 for two binding sites), but not so for the untethered Cu(II)–cyclen with a lower k_{cat}/K_m. The studies also show that O_2 is a more specific oxidation agent than H_2O_2 for catechol oxidation.



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Catalytic Cooperativity, Nuclearity, and O_2/H_2O_2 Specificity of Multi-Copper(II) Complexes of Cyclen-Tethered Cyclotriphosphazene Ligands in Aqueous Media