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Catalytic Phenol Hydroxylation with Dioxygen: Extension of the Tyrosinase Mechanism Beyond the Protein Matrix

Alexander Hoffmann[1], **Cooper Citek**[2], **Stephan Binder**[3], **Arne Goos**[3], **Michael Rübhausen**[3], **Oliver Troeppner**[4], **Ivana Ivanović-Burmazović**[4], **Erik C. Wasinger**[5], **T. Daniel P. Stack**[*,2], and **Sonja Herres-Pawlis**[*,1]

[1]Department of Chemistry, Ludwig-Maximilians-Universität München, 81377 Munich, Germany, and Fakultät Chemie, Technische Universität Dortmund, 44227 Dortmund, Germany

[2]Department of Chemistry, Stanford University, Stanford, California 94305, USA

[3]Institute for Applied Physics and Center for Free Electron Laser, University of Hamburg, 22607 Hamburg, Germany

[4]Department of Chemistry and Pharmacy, University of Erlangen-Nuremberg, 91058 Erlangen, Germany

[5]Department of Chemistry and Biochemistry, California State University, Chico, California 95929, USA

A pinnacle of bio-inorganic chemistry is the ability to leverage insights gleaned from metalloenzymes toward the design of small analogs capable of effecting catalytic reactivity outside the context of the natural system.[1,2] Structural mimicry of active sites is an attempt to insert a synthetic catalyst into an enzymatic mechanism. Such a mechanism evolves by selection pressures for efficiency and traverses an energetic path with barriers and wells neither too high nor too deep in energy – a critical factor of catalytic turnover.[3] An advantage of metalloenzymes over small metal complexes is the site-isolation of the metal center in the protein matrix with its attendant ability to attenuate destructive decay processes – reaction sinks. This protection provides access to thermal regimes that allows barriers and wells to be traversed. Synthetic complexes too must avoid any deleterious reactions, often necessitating deliberate incorporation of protective superstructures.[4,5] Such limitations make reproducing enzymatic catalytic reactivity in a synthetic complex with native substrates a significant challenge, as evidenced by the dearth of good examples, despite decades of effort.

The intellectual investment of translating a catalytic mechanism from an active site to a synthetic system is justified by a comparison of Nature's dexterity with dioxygen to perform catalytic monooxygenase-type chemistry[6–9] and the dependence of synthetic chemists on

Corresponding authors: Professor S. Herres-Pawlis, Department of Chemistry, Ludwig-Maximilians-Universität München, 81377 Munich, Germany, sonja.herres-pawlis@cup.uni-muenchen.de. Professor T. Daniel P. Stack, Department of Chemistry, Stanford University, Stanford, CA 94305, stack@stanford.edu.

Supplementary Information:
Materials and Methods, synthetic procedures, Raman measurements, XAS spectroscopy details, TD-DFT details, optimized coordinates of **1**.

Author contribution statement
A.H. and S.H.-P. were the major contributors of this investigation (UV-vis, reactivity, kinetic analysis, DFT), while C.C. and T.D.P.S. contributed in the design and implementation of several key experiments and drafting of the manuscript. S.B., A.G. and M.R. collected and analyzed the Raman data. O.T. and I.I.-B. collected and analyzed the cryo-ESI mass spec data. C.C. and E.C.W. collected and analyzed the Cu K-edge X-ray absorption data.

exotic reagents for oxygen-atom insertion reactions. Tyrosinase is a ubiquitous dinuclear copper enzyme that catalyzes the hydroxylation of phenols to catechols and the subsequent oxidation of catechols to quinones by activating dioxygen in the form of a side-on bonded peroxide dicopper(II) species, crystallographically[10] and spectroscopically identified.[11,12] The seemingly simple, regiospecific transformation mediated by tyrosinase is not reproduced easily by synthetic methods, though its importance has been acknowledged by recent efforts to move beyond stoichiometric oxidants,[13] as these sometimes multistep syntheses are often low to moderate yielding and frequently unselective.[14–17] Limited examples of *stoichiometric* phenolate hydroxylation exist with synthetic, tyrosinase-like side-on peroxide complexes[18–22] formed by oxygenation of copper(I) complexes. Only two complexes achieve *significant* catalytic phenol hydroxylation using dioxygen: a dinucleating, polydentate imine complex reported by Réglier,[23] and a mononucleating analogue reported by Tuczek[24] both capable of *ca.* 16 turnovers in the presence of triethylamine. Neither study could identify the operative oxygenated copper species nor illuminated key mechanistic hallmarks that support a tyrosinase-like mechanism. The possibility of a synthetic side-on peroxide as a catalytic oxidant is suggested by the work of Casella with a dinucleating copper-benzimidazole complex, achieving 1.2 turnovers with readily oxidized phenols.[21,25]

Here we report a synthetic catalyst, capable of hydroxylating a wide variety of phenols using dioxygen, that proceeds through a room temperature (RT) stable analog of oxygenated tyrosinase – a side-on peroxide complex, possessing ligation and spectroscopic attributes similar to those of the enzymatic active site. Efficient stoichiometric oxidation of phenolates to catecholates at −78°C is shown, as well as catalytic oxidation of phenols to quinones at RT with triethylamine, through a reaction pathway consistent with the generally accepted enzymatic mechanism.[11] Conditions are shown in which catalytic turnover is halted and restored at catecholate-product adduct. This investigation demonstrates that simple structural mimicry suffices to not only move the inherent enzymatic catalytic reactivity into a synthetic complex, but that bioinspiration is a viable stategy of pursuing selective transformations of substrates beyond scope of the enzyme.[26]

Oxygenation of [Cu(I)bis(3-*tert*-butyl-pyrazolyl)pyridylmethane]SbF_6 in dichloromethane (CH_2Cl_2) at −78°C results in near quantitative formation (*vide infra*) of $[Cu_2O_2(HC(3\text{-}tBuPz)_2(Py))_2](SbF_6)_2$ (**1**), a side-on peroxide dicopper(II) complex (Figure 1A), as evidenced by the characteristic O-O stretch at 750 cm^{-1} in its resonance Raman spectrum, which shifts by 39 cm^{-1} upon $^{18}O_2$ substitution (Figure 1C).[27,28] The expected mass signature and isotope pattern observed by cryo-ESI-TOF also shifts appropriately upon oxygenation with $^{18}O_2$ (Figure S3). The ligand-to-metal-charge-transfer (LMCT) features at 350 nm (20 mM^{-1} cm^{-1}) and 550 nm (1 mM^{-1} cm^{-1}) have a 20:1 intensity ratio,[22,27] similar to those of oxy-tyrosinase and oxy-hemocyanin,[29] and the feature near 412 nm (0.9 mM^{-1} cm^{-1}) is tentatively assigned to a pyrazole/pyridine $\pi^* \rightarrow d_{xy}$ charge transfer transition, based on a natural transition orbital analysis of a TD-DFT calculated spectrum (Figure 1B).[22,30] The DFT optimized structure of **1** predicts a planar Cu_2O_2 core with a Cu-Cu separation of 3.57 Å, in line with the 3.51 Å distance determined by Cu K-edge extend x-ray absorption fine structure models (Table S2 and Figure S5).[27,28] Taken together, these data fully support the structural homology between tyrosinase and **1**.

The formation of **1** is effectively quantitative (> 95%) in a variety of solvents at −78°C, as assessed by iodometric titrations of the released peroxide after treatment with trifluoroacetic acid.[22,31] The complex is stable for weeks in CH_2Cl_2 at −78°C, yet decays within 1 day in tetrahydrofuran or acetone. Compound **1** reacts within 60 min with a wide variety of sodium phenolate salts (5 equiv), both electron-rich and deficient, efficiently consuming the side-on peroxide oxidant and forming catechol products after an acidic workup (Table 1A). Using a

1:1 oxidant:phenolate stoichiometry, impressive catecholate yields (> 90%) are possible at −78°C, albeit reaction times of nearly 1 week are required. Expectedly, the mass of the *p*-methoxy-1,2-catechol product is shifted by 2 a.u. if **1** is formed with $^{18}O_2$, indicating dioxygen as the oxygen atom source.[32]

From kinetic data, the hydroxylation of phenolate to catecholate by **1** is best understood as a second order process: first order in [**1**] and first-order in [phenolate], with a pre-equilibrium binding event and a rate limiting oxidation step, presumably C-O bond formation. An intramolecular competitive kinetic isotope effect of 1.2(2) measured at −78°C with 2-D-4-*tert*-butylphenolate excludes rate limiting C-H bond cleavage. The observed rate constants k_{obs} saturate with respect to added phenolate (Figure 2), consistent with an initial phenolate-binding equilibrium K_{eq}, followed by an intramolecular rate-determining oxidation step k_{ox} from a substrate-complex adduct (Table S1),[18,32–33] as electron-deficient phenolates clearly react more slowly. A plot of ln(k_{ox}) versus σ_p^+ for a variety of phenolates gives a Hammett $\rho = -0.99$ (Figure 2), consistent with the trend reported for tyrosinase ($\rho = -1.8$ to -2.2) [34–35] and in line with an electrophilic aromatic substitution mechanism.

At RT in CH_2Cl_2, **1** is quantitatively formed but irreversibly decays with a half-life of 30 min. However, this rare stability allows for the catalytic hydroxylation of phenols to quinone products at RT. Only two other synthetic side-on peroxide species are more thermally stable, but no exogenous substrate reactivity has been reported.[36,37] With 25 equiv of *p*-methoxyphenol and 50 equiv of triethylamine under 1 atm of O_2,[23,24,38] 10 equiv of quinone are formed in 1 hr or 15 equiv in 24 hr (Table 1B), as assessed by the characteristic 400 nm quinone optical feature (Figure S1). Slower catalytic reaction rates are observed with more electron-deficient phenols. At these higher temperatures, the oxidation of the catechol to quinone and its subsequent release is proposed to drive the reduction of the Cu(II) centers back to a Cu(I) state, permitting re-oxygenation to **1** (Figure 3).

Three conditions appear to be necessary for catalytic turnover and support operation through a tyrosinase mechanism: ambient reaction temperatures (25°C), proton management, and excess dioxygen. Under catalytic reaction conditions at −78°C, rapid formation of a green species is observed, tentatively assigned as a Cu(II)-catecholate complex (Figure 3), as one equiv of *p*-methoxy-1,2-catechol is formed as the sole product upon an acidic quench at this temperature. If warmed to 25°C, this green species yields quinone rather than a catecholate product, suggesting catecholate-binding inhibition at low temperatures (Figure 3). Phenol deprotonation and binding to a copper center is essential in the catalytic oxidation process, as exclusion of triethylamine returns unreacted phenol at −78°C over hours. After phenol deprotonation, the resultant triethylammonium cation presumably provides the necessary proton for hydroxide protonation to water as catecholate reduces Cu(II) to Cu(I).

Beyond the ability to catalytically oxidize a tyrosinase substrate, N-acetyl-tyrosine methyl ester,[39] to its dopaquinone form, the oxidative reactivity of **1** can be extended to more complicated phenols, outside the typical substrate scope of the enzyme (Table 1B). Estrone, an estrogenic hormone, is regioselectively *ortho*-hydroxylated to 3,4-estrone-*o*-catechol in >90% yield at −78°C in less than 5 min with 5 equiv of its sodium salt relative to **1** (Table 1A). The 3,4-estrone-*o*-quinone is formed under the catalytic conditions at 25°C with 4 turnovers.[32] While mushroom tyrosinase itself can oxidize estrone,[40] 8-hydroxyquinoline is not a viable substrate,[32] possibly due to the extreme steric demands of the fused ring structure positioned *ortho* to the phenol oxygen (Table 1B).[26] Compound **1** oxidizes 8-hydroxyquinoline both stoichiometrically to the catechol (7,8-dihydroxyquinoline) at −78°C, and catalytically to its quinone at 25°C (quinoline-7,8-dione; 8 turnovers; Table 1B), extending the substrate scope beyond that possible in the enzymatic system.[2] The substrate flexibility of **1** may thus make it a potentially useful reagent for efficient stoichiometric

conversion of a wide variety of phenolates to catechol or a catalyst for the multi-turnover oxidation of phenols by dioxygen to quinones, which are readily reduced back to catechols.[17]

Oxygen-insertion reactions that use dioxygen directly are extremely limited, despite the indisputable advantages to using Earth's ready supply of dioxygen. The difficulty lies in directing the oxidative power of dioxygen and in assuring that reactions profiles do not have insurmountable barriers or thermodynamically over-stabilized intermediates. Here, the essence of the tyrosinase enzyme mechanism, founded on the oxidizing power of dioxygen, is translated from its protein environment into a small complex through only approximate structural mimicry of the oxygenated active site, yet selective and efficient catalytic ortho-hydroxylation reactivity results. This strategy of copying pre-existing reactivity from Nature opens the door not only to challenging organic transformations, but for developing useful synthetic tools with substrate scopes beyond those of biological systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Que L Jr, Tolman WB. Nature. 2008; 455:333–340. [PubMed: 18800132]
2. Mahadevan V, Gebbink RJM, Stack TDP. Curr Op Chem Biol. 2000; 4:228–234.
3. Kozuch S, Shaik S. Acc Chem Res. 2010; 44:101–110. [PubMed: 21067215]
4. Collmann JP. Acc Chem Res. 1977; 10:265–272.
5. Momentau M, Reed CA. Chem Rev. 1994; 94:659–698.
6. Itoh S, Fukuzumi S. Acc Chem Res. 2007; 40:592–600. [PubMed: 17461541]
7. Karlin, KD.; Itoh, S.; Rokita, S. Coppper-Oxygen Chemistry. John Wiley & Sons, Inc; Hoboken, New Jersey: 2011.
8. Meunier, B., editor. Metal-Oxo and Metal-Peroxo Species in Catalytic Oxidations (Structure and Bonding). Springer; Berlin and Heidelberg: 2000.
9. Hatcher LQ, Karlin KD. Adv in Inorg Chem. 2006; 58:131–184.
10. Matoba Y, Kumagai T, Yamamoto A, Yoshitsu H, Sugiyama M. J Biol Chem. 2006; 281:8981–8990. [PubMed: 16436386]
11. Solomon EI, Sundaram UM, Machonkin TE. Chem Rev. 1996; 96:2563–2605. [PubMed: 11848837]
12. Rolff M, Schottenheim J, Decker H, Tuczek F. Chem Soc Rev. 2011; 40:4077–4098. [PubMed: 21416076]
13. Huang C, Ghavtadze N, Chattopadhyay B, Gevorgyan V. J Am Chem Soc. 2011; 133:17630–17633. [PubMed: 21999512]
14. Hansen TV, Skattebøl L. Tetrahedron Lett. 2005; 46:3357–3358.

15. Gigante B, Santos C, Silva AM, Curto MJM, Nascimento MSJ, Pinto E, Pedro M, Cerqueira F, Pinto MM, Duarte MP, Laires A, Rueff J, Goncalves J, Pegadod MI, Valdeirad ML. Bioorg Med Chem. 2003; 11:1631–1638. [PubMed: 12659748]
16. Itoh S, Ogino M, Haranou S, Terasaka T, Ando T, Komatsu M, Ohshiro Y, Fukuzumi S, Kano K, Takagi K, Ikeda T. J Am Chem Soc. 1995; 117:1485–1493.
17. Pezzella A, Lista L, Napolitano A, d'Ischia M. Tetrahedron Lett. 2005; 46:3541–3544.
18. Itoh S, Kumei H, Taki M, Nagatomo S, Kitagawa T, Fukuzumi S. J Am Chem Soc. 2001; 123:6708–6709. [PubMed: 11439064]
19. Santagostini L, Gullotti M, Monzani E, Casella L, Dillinger R, Tuczek F. Chem Eur J. 2000; 6:519–522. [PubMed: 10747419]
20. Mirica LM, Vance M, Jackson Rudd D, Hedman B, Hodgson KO, Solomon EI, Stack TDP. J Am Chem Soc. 2002; 124:9332–9333. [PubMed: 12167002]
21. Palavicini S, Granata A, Monzani E, Casella L. J Am Chem Soc. 2005; 127:18031–18036. [PubMed: 16366554]
22. Citek C, Lyons CT, Wasinger EC, Stack TDP. Nat Chem. 2012; 4:317–322. [PubMed: 22437718]
23. Réglier M, Jorand C, Waegell B. J Chem Soc, Chem Commun. 1990:1752–1755.
24. Rolff M, Schottenheim J, Peters G, Tuczek F. Angew Chem. 2010; 122:6583–6587.Angew Chem, Int Ed. 2010; 49:6438–6442.
25. Casella L, Gullotti M, Radaelli R, Di Gennaro P. J Chem Soc, Chem Commun. 1991:1611–1612.
26. del Mar Garcia-Molina M, Muñoz-Muñoz F, Garcia-Molina F, García-Ruiz PA, Garcia-Canovas F. J Agric Food Chem. 2012; 60:6447–6453. [PubMed: 22670832]
27. Mirica LM, Ottenwaelder X, Stack TDP. Chem Rev. 2004; 104:1013–1045. [PubMed: 14871148]
28. Lewis EA, Tolman WB. Chem Rev. 2004; 104:1047–1076. [PubMed: 14871149]
29. Himmelwright RS, Eickman NC, LuBien CD, Solomon EI. J Am Chem Soc. 1980; 102:5378–5388.
30. Martin RL. J Chem Phys. 2003; 118:4775–4777.
31. Sanya I, Karlin KD, Strange RW, Blackburn NJ. J Am Chem Soc. 1993; 115:11259–11270.
32. Materials and methods are available as supporting material.
33. Herres-Pawlis S, Verma P, Haase R, Kang P, Lyons CT, Wasinger EC, Flörke U, Henkel G, Stack TDP. J Am Chem Soc. 2009; 131:1154–1169. [PubMed: 19119846]
34. Yamazaki SI, Itoh S. J Am Chem Soc. 2003; 125:13034–13035. [PubMed: 14570470]
35. Muñoz-Muñoz JL, Berna J, del Mar Garcia-Molina M, Garcia-Molina F, García-Ruiz P Antonio, Varon R, Rodriguez-Lopez JN, Garcia-Canovas F. Biochem Biophys Res Commun. 2012; 424:228–233. [PubMed: 22732412]
36. Hu Z, Williams RD, Tran D, Spiro TG, Gorun SM. J Am Chem Soc. 2000; 122:3556–3557.
37. Kodera M, Katayama K, Tachi Y, Kano K, Hirota S, Fujinami S, Suzuki M. J Am Chem Soc. 1999; 121:11006–11007.
38. Bulkowski, JE. US patent. 4545937. 1984.
39. Kahn V, Ben-Shalom N. Pigment Cell Res. 1998; 11:24–33. [PubMed: 9523332]
40. Westerfeld WW. Biochem J. 1940; 34:51–58. [PubMed: 16747136]

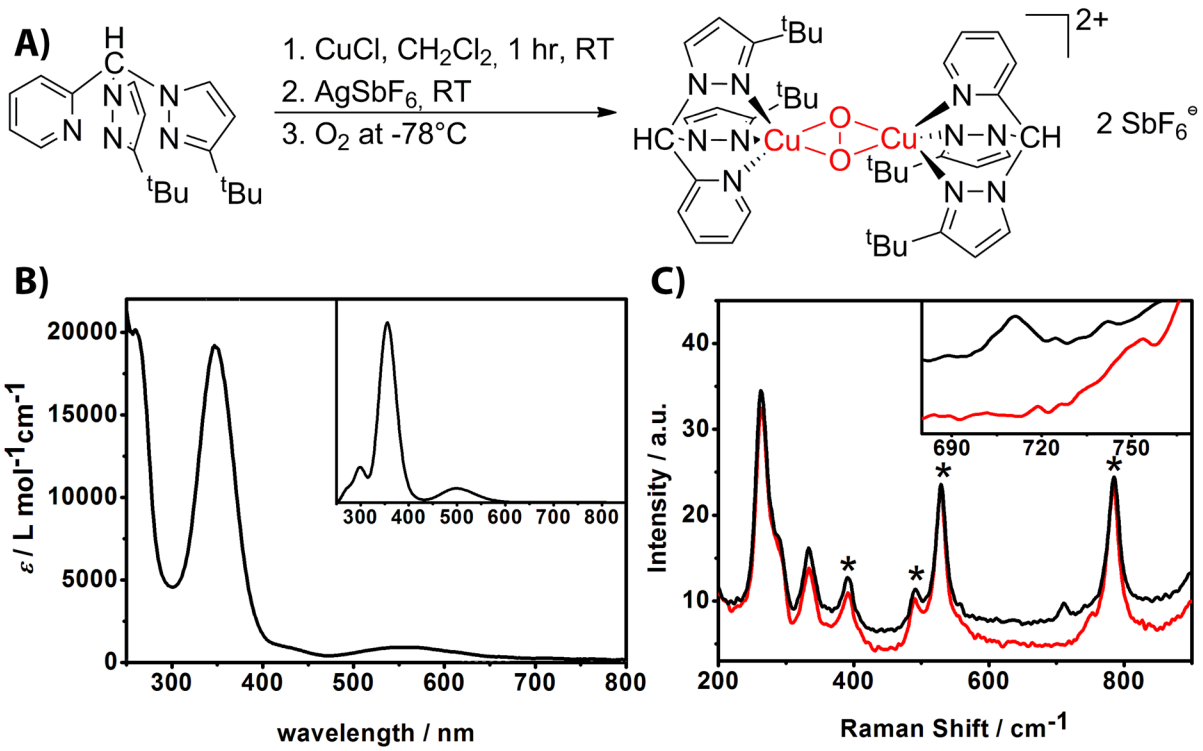


Figure 1.

A) Preparation of the side-on peroxide species 1. B) Absorption spectra of 1; (inset) TD-DFT predicted optical spectrum of 1. C) Resonance Raman spectra of 1 in acetone with 412 nm excitation (red: $^{16}O_2$, black: $^{18}O_2$, asterisks (*): solvent peaks); (inset) isotopic shift of 750 cm^{-1} feature.

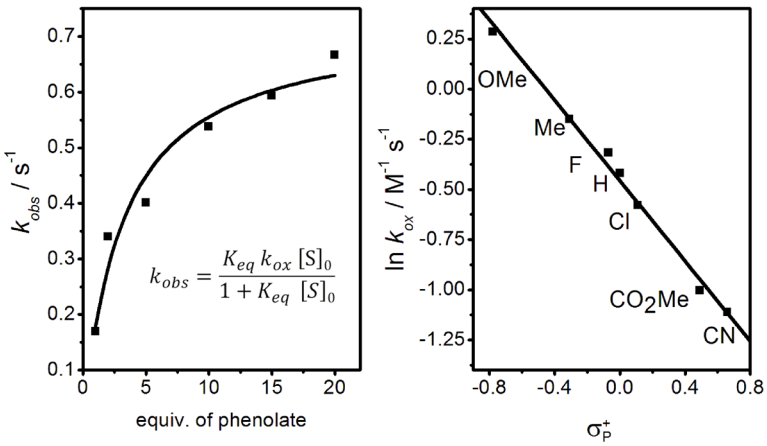


Figure 2.

Reactivity at −78°C in CH_2Cl_2. Left: Substrate-binding kinetics of the stoichiometric hydroxylation reaction with 4-fluorophenolate. Right: Hammett plot for the stoichiometric hydroxylation reaction with 1 – 20 equiv of various *p*-substituted phenolates.

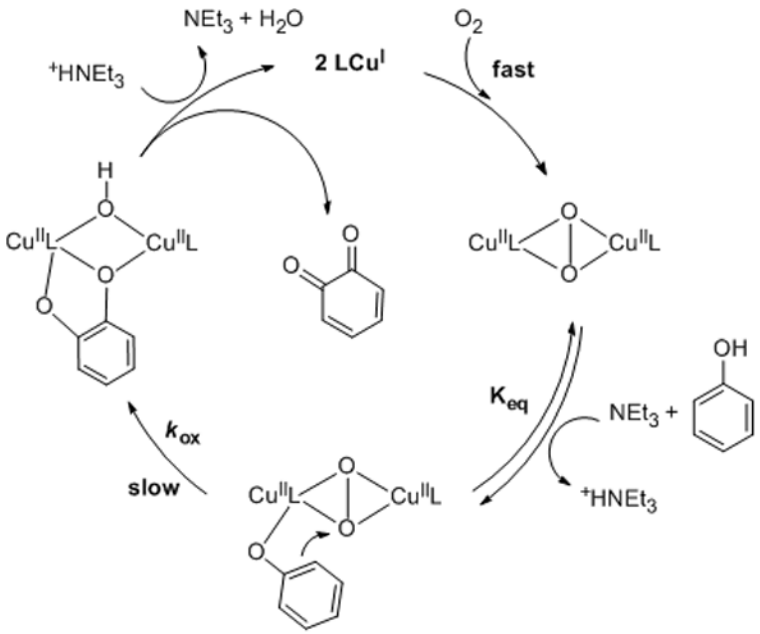
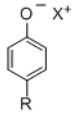


Figure 3.
Proposed catalytic mechanism of phenol oxidation by 1 in the presence of triethylamine.

Table 1

A) Reaction of Phenolic Substrates and 1 under a N_2 atmosphere at −78°C; B) Catalytic Oxidation of Phenols to Quinones.

A)	R	X^+	Equiv[a]	Time	% catechol[b]
	OMe	Na^+	5	5 min	>95
	Me	Na^+	5	5 min	>95
	Cl	Na^+	5	5 min	>95
	H	Na^+	5	5 min	>95
	F	Na^+	5	5 min	>95
	COOMe	Na^+	5	60 min	>95
	CN	Na^+	5	60 min	>95
	NO_2	Na^+	5	60 min	>90
	estrone[a]	Na^+	5	5 min	>90
	8-hydroxyquinoline[a]	Na^+	5	5 min	>90
	N-acetyl-tyrosine-ethylester	Na^+	5	5 min	>90
	OMe	$HNEt_3^+$	5	10 min	>95
	Me	$HNEt_3^+$	5	10 min	>95
	OMe	Na^+	1	7 days	>90
	OMe	$HNEt_3^+$	1	10 days	>90
	estrone	Na^+	1	8 days	>90
	8-hydroxyquinoline	Na^+	1	8 days	>90
	N-acetyl-tyrosine-ethylester	Na^+	1	5 days	>90

B) Substrates	Time	Turn-overs[a]
p-methoxyphenol	1 h	10
N-acetyl-tyrosine-ethylester	16 h	15
estrone	6 h	4
8-hydroxyquinoline	16 h	8

All reactions performed in CH_2Cl_2 at 1 mM [Cu] and quenched with acid at −78°C after the given reaction time.

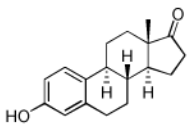
[a]Equiv relative to **1**.

[b] Yields based on the concentrations of **1**. No quinone or biphenol products were observed.

All reactions performed in CH_2Cl_2 at 25°C under 1 atm of O_2 with 25 equiv of substrate, 50 equiv NEt_3 and [Cu] = 1.0 mM.

[a] Turnover numbers based on the concentration of **1**.

estrone



8-hydroxyquinoline

N-acetyl-tyrosine-ethylester