ACCOUNTS of chemical research

Article

pubs.acs.org/accounts

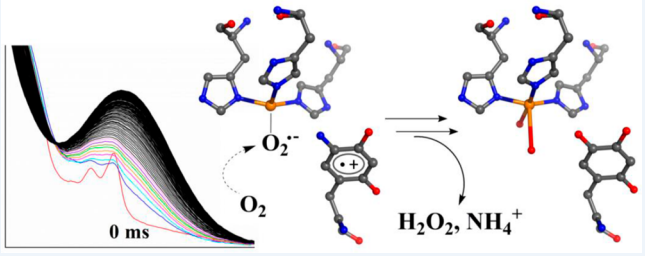
Inhibition and Oxygen Activation in Copper Amine Oxidases

Eric M. Shepard*,‡ and David M. Dooley*,†

‡Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana 59717, United States

†Office of the President, University of Rhode Island, Kingston, Rhode Island 02881, United States

CONSPECTUS: Copper-containing amine oxidases (CuAOs) use both copper and 2,4,5-trihydroxyphenylalanine quinone (TPQ) to catalyze the oxidative deamination of primary amines. The CuAO active site is highly conserved and comprised of TPQ and a mononuclear type II copper center that exhibits five-coordinate, distorted square pyramidal coordination geometry with histidine ligands and equatorially and axially bound water in the oxidized, resting state. The active site is buried within the protein, and CuAOs from various sources display remarkable diversity with respect to the composition of the active site channel and cofactor accessibility. Structural and mechanistic factors that influence substrate preference and inhibitor sensitivity and selectivity have been defined. This Account summarizes the strategies used to design selective CuAO inhibitors based on active site channel characteristics, leading to either enhanced steric fits or the trapping of reactive electrophilic products. These findings provide a framework to support the future development of candidate molecules aimed at minimizing the negative side effects associated with drugs containing amine functionalities. This is vital given the existence of human diamine oxidase and vascular adhesion protein-1, which have distinct amine substrate preferences and are associated with different metabolic processes. Inhibition of these enzymes by antifungal or antiprotozoal agents, as well as classic monoamine oxidase (MAO) inhibitors, may contribute to the adverse side effects associated with drug treatment. These observations provide a rationale for the limited clinical value associated with certain amine-containing pharmaceuticals and emphasize the need for more selective AO inhibitors.



This Account also discusses the novel roles of copper and TPQ in the chemistry of O_2 activation and substrate oxidation. Reduced CuAOs exist in a redox equilibrium between the Cu(II)−TPQ_{AMQ} (aminoquinol) and Cu(I)−TPQ_{SQ} (semiquinone). Elucidating the roles of Cu(I), TPQ_{SQ}, and TPQ_{AMQ} in O_2 activation, for example, distinguishing inner-sphere versus outer-sphere electron transfer mechanisms, has been actively investigated since the discovery of TPQ_{SQ} in 1991 and has only recently been clarified. Kinetics and spectroscopic studies encompassing metal substitution, stopped-flow and temperature-jump relaxation methods, and oxygen kinetic isotope experiments have provided strong support for an inner-sphere electron transfer step from Cu(I) to O_2. Data for two enzymes support a mechanism wherein O_2 prebinds to a three-coordinate Cu(I) site, yielding a $[Cu^{II}(\eta^1\text{-}O_2^{-1})]^+$ intermediate, with H_2O_2 generated from ensuing rate-determining proton coupled electron transfer from TPQ_{SQ}. While kinetics data from the cobalt-substituted yeast enzyme indicated that O_2 is reduced through an outer-sphere process involving TPQ_{AMQ}, new findings with a bacterial CuAO demonstrate that both the Cu(II) and Co(II) forms of the enzyme operate via parallel mechanisms involving metal−superoxide intermediates. Structural observations of a coordinated TPQ_{SQ}−Cu(I) complex in two CuAOs supports previous indications that Cu(II)/(I) ligand substitution chemistry may be mechanistically relevant. Substantial evidence indicates that rapid and reversible inner-sphere reduction of O_2 at a three-coordinate Cu(I) site occurs, but the existence of a coordinated semiquinone in some AOs suggests that, in these enzymes, an outer-sphere reaction between O_2 and TPQ_{SQ} may also be possible, since this is expected to be energetically favorable compared with outer-sphere electron transfer from TPQ_{AMQ} to O_2.

INTRODUCTION

Copper-containing amine oxidases (CuAOs) participate in the breakdown and regulation of biologically active amines and have critical roles in many organisms.[1,2] These enzymes reduce O_2 and produce H_2O_2, which is involved in signaling.[3] CuAOs employ both copper and 2,4,5-trihydroxyphenylalanine quinone (TPQ); TPQ is derived from a conserved tyrosine in a self-processing, post-translational event requiring only copper and molecular oxygen.[4] Consequently, copper plays an essential role in the biogenesis of TPQ and is also involved in the catalytic cycle. Structures of several CuAOs have been solved by X-ray crystallography and show that the dimeric enzymes contain a single active site per monomer comprised of the TPQ cofactor and a copper ion situated ∼10−12 Å from the protein surface (Figure 1; see ref 4 and references therein). The Cu(II) ion displays five-coordinate, distorted square pyramidal coordination geometry with three equatorial histidine ligands and equatorially and axially bound water molecules (Figure 1B);[4,5] the water molecules provide flexibility during redox

Received: December 23, 2014
Published: April 21, 2015

DOI: 10.1021/ar500460z
Acc. Chem. Res. 2015, 48, 1218−1226

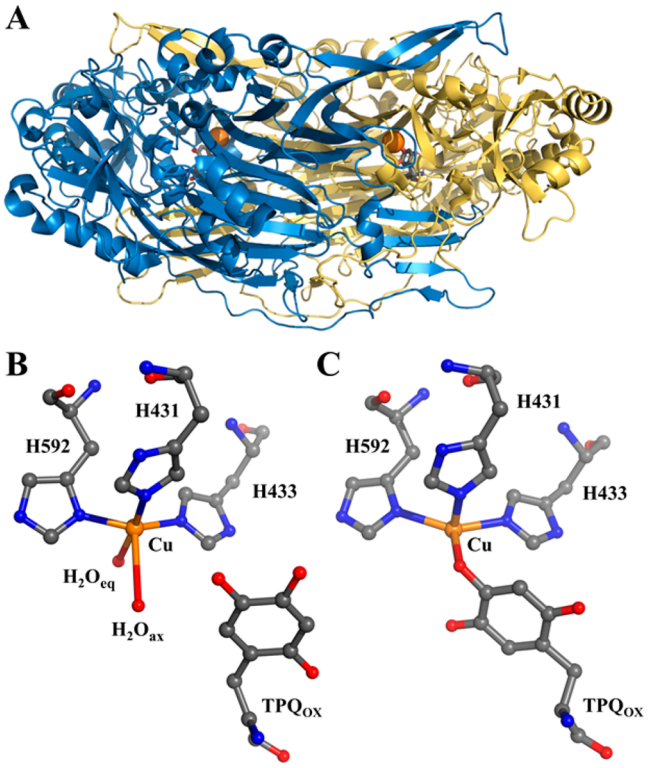


Figure 1. Human diamine oxidase structure with monomeric subunits shown in blue and gold (A).[27] AGAO active site architecture with TPQ "off"-copper (B) and "on"-copper (C).[7]

cycling, allowing the copper ion to change coordination number without substantially altering the protein structure.[6] TPQ can coordinate to the Cu(II); both "off"-copper (Figure 1B) and "on"-copper (Figure 1C) states have been characterized[7−9] and shown to exist in equilibrium in solution.[10] In recent years, the understanding of the structure, mechanism, and biological roles of CuAOs has advanced substantially, especially with regard to the role of copper and the molecular interactions controlling substrate and inhibitor binding. This Account highlights recent findings regarding selective inhibition and the chemistry of O_2 activation.

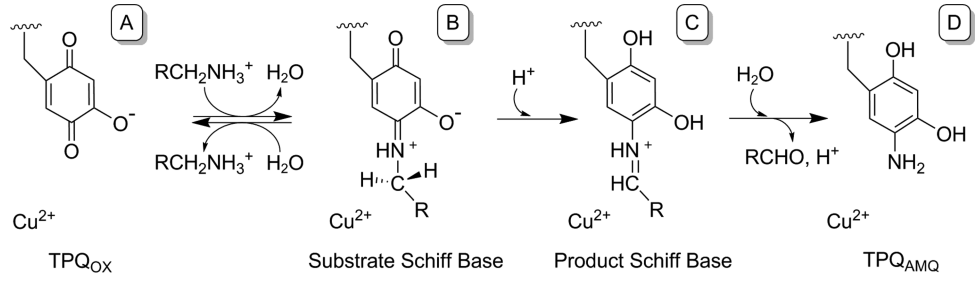
REDUCTIVE HALF-REACTION AND MECHANISM BASED INHIBITION

TPQ oxidizes a wide range of primary amines: short- to long-chain aliphatic mono- and diamines, including multiple arylalkylamines.[4,11,12] Catalysis proceeds through a ping-pong mechanism: TPQ is reduced by substrate to generate product aldehyde in the reductive half-reaction (Scheme 1); the reduced TPQ is then reoxidized by O_2 in the oxidative half-reaction (Scheme 2).[7,8]

During the reductive half-reaction, Cu(II)−TPQ_{ox} (Scheme 1A) reacts with a primary amine to form a quinoneimine "substrate Schiff base" (Scheme 1B, TPQ_{SSB}). The decisive step is the conversion of TPQ_{SSB} to a quinolaldimine "product Schiff base" (Scheme 1C, TPQ_{PSB}), facilitated by α-carbon proton abstraction by a conserved aspartate.[13] The aldehyde product is then released via hydrolysis, generating the reduced aminoquinol (Cu(II)−TPQ_{AMQ}, Scheme 1D). Analogs, such as 2-hydrazinopyridine (2-HP), benzylhydrazine, and tranylcypromine, have been used to structurally model enzyme−substrate complexes (Scheme 1B) and indicate that TPQ must be in an "off"-copper conformation with the C5 carbonyl of TPQ positioned toward the conserved aspartate that facilitates stereospecific proton abstraction.[8,14−16] The ECAO-2HP structure illustrates how Tyr369 positions the cofactor by hydrogen bonding to TPQ-O4 and how the active site "gate" Tyr381 rotates permitting access to TPQ.[8]

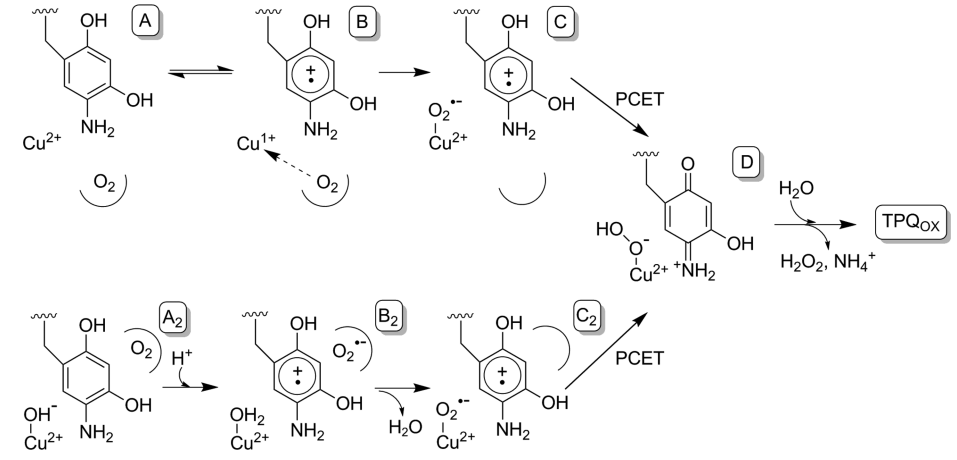
CuAO structures reveal variations among active site channel dimensions, accessibility, and composition; these differences influence substrate amine preference for a given enzyme. Consequently, these characteristics may be exploited to develop selective mechanism-based inhibitors.[12,17,18] One productive approach incorporates functionality to generate an electrophile upon the imine shift accompanying Cα proton abstraction (Scheme 1, B → C). Two strategies involve introduction of either unsaturation at the β position or a halogen.[19] The former produces an electrophilic α,β-unsaturated aldehyde (Figure 2A), whereas the latter yields an S_N2-activated α-haloaldehyde. Four compounds designed in this manner were subsequently screened against bacterial, yeast, plant, and mammalian CuAOs.[17] Striking differences were observed in the reactivity of 4-(2-naphthyloxy)-2-butyn-1-amine (NOBA) toward a plant (pea seedlings, PSAO) and a bacterial (*Arthrobacter globiformis*, AGAO) amine oxidase. NOBA completely inactivated AGAO at stoichiometric levels but behaved as a substrate for PSAO.[17] The AGAO−inhibitor adducts were discovered to form via nucleophilic attack of the TPQ_{AMQ} amino group (Scheme 1D) at the C3 position of the electrophilic α,β-unsaturated propargyl aldehyde products of both NOBA (Figure 2A) and 4-(4-methylphenoxy)-2-butyn-1-amine (MOBA).[18] AGAO structures with 4-aryloxy adducts revealed extensive contacts with amino acids comprising a hydrophobic pocket, with the more potent compounds displaying superior steric fits (Figure 2B).[18] The hydrophobic pocket in AGAO traps the reactive α,β-unsaturated aldehyde, enabling it to react with TPQ_{AMQ}; a similar mode of inhibition is operative for aryl substituted 2,3-butadienamine analogs.[20] Importantly, for CuAO enzymes which lack similar hydrophobic pockets, inactivation is less

Scheme 1. Proposed Reductive Half-Reaction Mechanism



DOI: 10.1021/ar500460z
Acc. Chem. Res. 2015, 48, 1218−1226

Scheme 2. Inner-Sphere (A → C) and Outer-Sphere (A2 → C2) Pathways for Electron Transfer during the Oxidative Half-Reaction



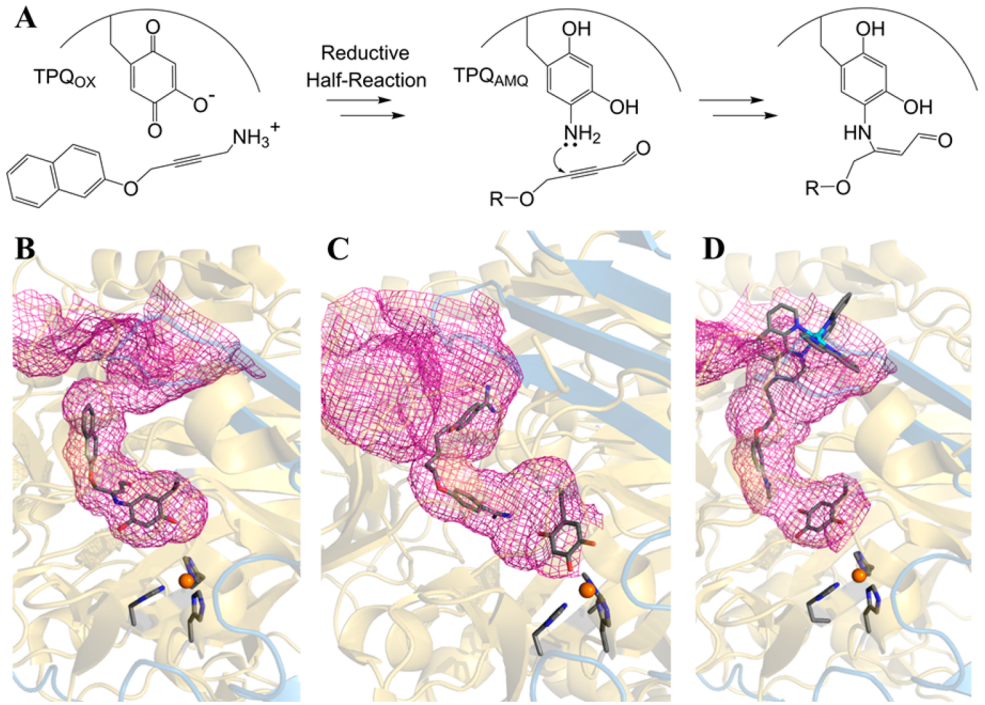


Figure 2. Amine oxidase inhibition. TPQ_{AMQ}-4-(2-naphthyloxy)-2-butenal production in AGAO following oxidation of NOBA (R = 2-naphthyl) (A) and the associated crystal structure (B).[18] Noncovalent inhibition highlighted by pentamidine binding in hDAO (C)[27] and ruthenium(II) molecular wire binding in AGAO (D).[28] A solvent radius setting of 2.0 (PyMOL)[64] was utilized to generate active site channels (magenta mesh).

frequent.[17] In such cases, inhibition may only arise when the unsaturated aldehyde contacts another protein-based nucleophile.[18]

INHIBITION BY PHARMACEUTICALS

Pharmaceuticals with amine functionality may target CuAOs *in vivo*, thereby disrupting the normal balance of amine metabolism and physiology. The presence of monoamine oxidase flavoenzymes (MAOs), which also oxidize amines to aldehydes, raises the possibility of cross-reactivity between CuAOs and MAOs with naturally occurring biogenic amines and with drugs containing amine functionalities. MAOs are a therapeutic target for inhibition in patients suffering from depression, and the MAO-directed inhibitor tranylcypromine (TCP) has traditionally been used to treat depression, but side effects associated with treatment limit its clinical value.[21] *In vitro* studies demonstrate that TCP acts as a general inhibitor of CuAOs by a mechanism that involves substrate Schiff base formation (Scheme 1B).[15,16,22] The crystal structure of the AGAO−TCP complex reveals that the active-site gate Tyr381 stabilizes the inhibitor adduct via formation of a side to face π-stacking interaction with the cyclopropyl ring.[15,16] Remarkably, TCP strongly inactivates mammalian plasma CuAO (vascular adhesion protein, VAP-1) but does not inhibit human diamine oxidase (hDAO);[22] inhibition of human VAP-1 might contribute to the drug's deleterious side effects and appropriate screening of candidate antidepressants against human CuAOs may be useful in drug development.[23]

The ability to design selective AO inhibitors would be highly beneficial in terms of minimizing side effects that arise from

DOI: 10.1021/ar500460z
Acc. Chem. Res. 2015, 48, 1218−1226

cross-reactivity. A- and B-type MAOs oxidize amines by a mechanism that does not involve an imine shift,[24] thereby providing a further level of discrimination from CuAOs. Accordingly, a series of 3-pyrroline compounds capable of effecting transamination-specific irreversible modification were demonstrated to inactivate only the TPQ-dependent CuAOs, while acting as pure substrates for MAO-B.[25,26] The remarkable selectivity toward CuAOs by these inhibitors arises from the reactivity of TPQ toward secondary amines, with inhibited enzyme containing a reduced pyrrolylated cofactor.[25,26]

The consequences associated with *in vivo* CuAO inhibition are emphasized by the antiprotozoal drugs berenil (1,3-bis(4′-amidinophenyl)triazene) and pentamidine (1,5-bis(4-amidinophenoxy)pentane), which are, respectively, used in the treatment of trypanosomiasis in animals and *Pneumocystis carinii* pneumonia infections in humans. These compounds inhibit hDAO and VAP-1 with K_i values in the low nanomolar to low micromolar range; drug regimes associated with these compounds cause severe side effects (see discussion in ref 27). The *in vivo* inhibition of hDAO is particularly detrimental because this enzyme oxidizes several biogenic amines, including histamine, 1-methylhistamine, agmatine, putrescine, cadaverine, and spermidine, and is therefore expected to be involved in numerous metabolic pathways.[11] Remarkably, the potent inhibition of hDAO by berenil and pentamidine is not associated with TPQ derivatization but occurs through noncovalent contacts in the active site channel (Figure 2C).[27] These observations parallel the results of a molecular design strategy to develop potent and specific reversible CuAO inhibitors based upon substrate channel binding, much like a "cork in a bottle". Characterization of the inhibition and binding of ruthenium(II) and rhenium(I) molecular wires to AGAO established that selected complexes bind in the active site channel and are very effective inhibitors (Figure 2D).[28,29]

Collectively, the results document the success of strategies used to develop potent and selective AO inhibitors. The availability of the hDAO and human VAP-1 crystal structures provides a foundation for future studies aimed at the rational design of specific CuAO inhibitors as potential therapeutic agents, as well as probing the molecular basis for the affinity and selectivity of new pharmaceuticals toward enzymes from disparate species.[27,30,31]

OXIDATIVE HALF-REACTION AND THE ROLE OF COPPER

It is well established that Cu(II)−TPQ_{AMQ} exists in equilibrium with Cu(I)−TPQ_{SQ} (semiquinone) (Scheme 2, A ⇌ B). The magnitude of K_{eq} for this interconversion varies among enzymes from different sources.[32] Owing to this internal redox equilibrium, it has been difficult to discern whether Cu(I), TPQ_{SQ}, or TPQ_{AMQ} reacts directly with O_2. We and others have proposed that O_2 binds to the Cu(I) center of Cu(I)−TPQ_{SQ} and is reduced to $O_2^{\bullet-}$ by a non-rate-limiting inner-sphere electron transfer process.[32] The reaction of Cu(I) with O_2 is a reasonable expectation given the well documented reactivity of Cu(I) sites with O_2 in copper-containing metalloproteins[33−35] and three-coordinate Cu(I) model complexes.[36,37] In contrast, other studies suggested that O_2 binds in a hydrophobic pocket adjacent to TPQ and is reduced to $O_2^{\bullet-}$ by TPQ_{AMQ} via a rate-limiting outer-sphere electron transfer reaction (Scheme 2, A2 → B2).[14] Despite these fundamental mechanistic differences, both inner- and outer-sphere pathways converge at the Cu(II)−hydroperoxide, iminoquinone (TPQ_{IMQ}) intermediate (Scheme 2D).[38]

Coordinated water (Figure 1B) facilitates redox-related changes in coordination geometry, and this has been confirmed by X-ray absorption spectroscopic analysis of the Cu(I) state.[5] Nuclear magnetic relaxation dispersion measurements performed on CuAOs demonstrated the presence of a rapidly exchanging copper bound H_2O molecule,[39] and ESEEM studies revealed that azide displaces the equatorial H_2O ligand to copper.[40−42] Additional evidence for the structural flexibility of the copper center is provided in substrate reduced structures of ECAO and HPAO, which show tetrahedral copper coordination environments.[9,43]

The role of copper in catalysis has been examined through various techniques. Azide addition perturbs the internal Cu(II)/Cu(I) redox equilibrium in the substrate reduced state, and kinetics data demonstrates that for PSAO, azide is a weak noncompetitive inhibitor of the reductive half-reaction but is a more potent competitive inhibitor of the oxidative half-reaction.[41,44] The relatively good agreement between the K_i value for the oxidative half-reaction and the K_d value for the Cu(II)−N_3^- complex in reduced PSAO suggests that azide coordination to the TPQ_{AMQ}−Cu(II) state shifts the TPQ_{AMQ}−Cu(II) ⇌ TPQ_{SQ}−Cu(I) equilibrium toward the former species, thereby inhibiting electron transfer from TPQ_{AMQ} to Cu(II).[44] Competition between azide and O_2 arises from the combination of rapid electron transfer between TPQ_{AMQ} and Cu(II) and the fast, reversible ligand-substitution reaction of N_3^- for coordinated H_2O in a tetragonal Cu(II) complex.[39,41]

Structural studies of intermediates during the oxidative half-reactions in ECAO and HPAO have been quite informative and have shown the existence of deprotonated and protonated forms of the iminoquinone, as well as a dioxygen species bound side-on to copper that likely represents H_2O_2.[9,43] Oxygen binding has been probed indirectly through solving X-ray crystal structures under Xe gas;[45,46] only a single site was shared between AGAO, PPLO, PSAO, and BSAO, and this site is the closest to the Cu/TPQ center, being ∼7.4−7.7 Å from copper and ∼9.3−9.8 Å from TPQ. Plausible trajectories for O_2 toward Cu/TPQ would involve initial close approach to copper (Figure 3A);[45−47] support for this hypothesis is provided in a recent structure of AGAO that shows an O_2 molecule bound close to the conserved Xe site.[48] Collectively, the structures suggest that the β-sandwich domain, the amine substrate channel, and the interior lake are all suitable for transient O_2 binding with subsequent delivery to the active site.[45,48−50]

A critical requirement of the inner-sphere electron transfer mechanism is that the electron transfer rate (k_{ET}) from TPQ_{AMQ} to Cu(II) must exceed measured k_{cat}(amine) values under steady-state conditions. Temperature jump relaxation studies have shown that k_{ET} values at neutral pH in PSAO, APAO, and AGAO are ∼20000, 75, and 145 s^{-1}, respectively.[51−53] The faster k_{ET} in PSAO perhaps arises from a lower reorganization energy for the Cu(II)/Cu(I) redox couple.[5] Support for this hypothesis derives from the observation that PSAO is the only CuAO to display no increase in disorder of the Cu(I) site following dithionite reduction of the Cu(II) form, suggesting that a low reorganization energy exists.[5] Despite differences in the magnitude of k_{ET}, the values are greater than the intramolecular turnover rate constants (k_{cat}) for respective preferred amine substrates at all measured pH values.[51−53]

DOI: 10.1021/ar500460z
Acc. Chem. Res. 2015, 48, 1218−1226

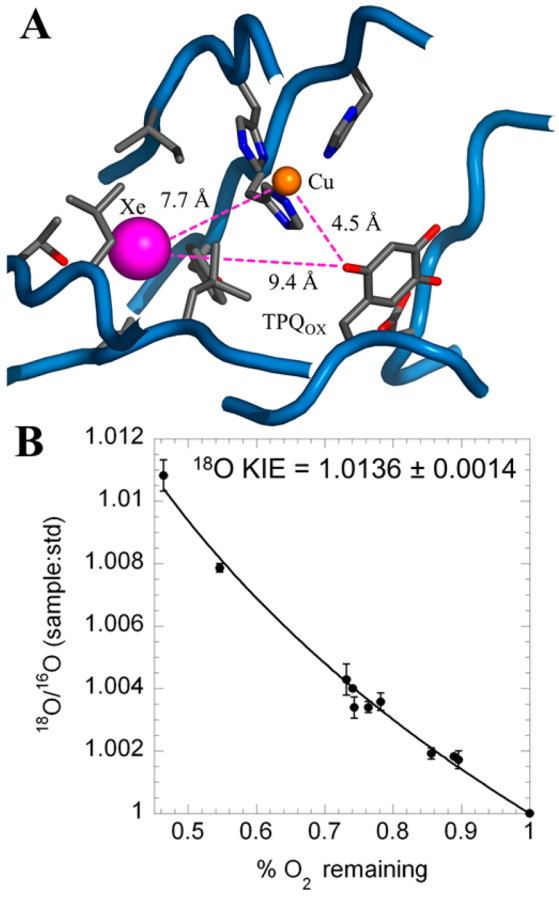


Figure 3. Oxygen binding and reactivity in PSAO. The consensus Xe (magenta sphere) binding pocket with associated trajectories to copper and TPQ (A).[45] Oxygen isotope fractionation (B), as reprinted with permission from ref 59. Copyright 2008 American Chemical Society.

The magnitude of the k_{ET} value between TPQ_{AMQ} and Cu(II) in AGAO (145 s^{-1}, Figure 4A)[53] permits direct examination by stopped-flow methods of the spectroscopic changes associated with the TPQ_{AMQ} and TPQ_{SQ} species upon exposure to O_2. Reoxidation rates under single turnover conditions of substrate-reduced AGAO are independent of O_2 concentration, demonstrating that the oxidative half-reaction is not limited by O_2 (Figure 4B,C).[38] The visible absorbance features of TPQ_{SQ} (λ_{max} = 436, 466 nm) disappear in the first few milliseconds following the introduction of 700 μM O_2 (Figure 4B), whereas these bands persist longer in experiments performed with 150 μM O_2 (Figure 4C). This behavior suggests that the TPQ_{SQ}–Cu(I) species is the reactive intermediate, with the rapid decay of the TPQ_{SQ} absorbance features at 700 μM O_2 consistent with the fast reaction of O_2 with the available TPQ_{SQ}–Cu(I) during the dead time of the stopped-flow instrument.[38]

Experiments in H_2O and D_2O exhibit similar reoxidation rate constants, although studies in D_2O show a KIE associated with formation of TPQ_{OX} from TPQ_{IMQ} (Scheme 2).[38] Difference spectra reveal a 310 nm feature that disappears during the initial phase of the half-reaction; this is consistent with conversion of TPQ_{AMQ}–Cu(II) to TPQ_{SQ}–Cu(I),[38] which then reacts with O_2 to produce Cu(II)–OOH^-.[38,54] Experiments in D_2O and basic pH(D) values exhibit buildup of a ~400 nm feature, assigned as arising from the Cu–hydroperoxide intermediate.[38,54]

Importantly, the distinct absorbance features of TPQ_{AMQ} and TPQ_{SQ} allowed spectral deconvolution of the substrate-reduced enzyme into component contributions, thus permitting TPQ_{AMQ} and TPQ_{SQ} reactivity to be probed individually by global fitting analysis.[38] Utilization of a three-state model shows that the experimental absorbance changes are only reproduced when the direct reaction of TPQ_{SQ}–Cu(I) is invoked (Figure 4D). Modeling an outer-sphere mechanism involving the TPQ_{AMQ}–Cu(II) does not reproduce the experimental spectra (Figure 4E,F).[38]

Results from metal substitution experiments provided evidence for both mechanisms of O_2 reduction (Scheme 2). For example, Co(II)-substituted HPAO displays a k_{cat} similar to the wild-type enzyme at pH 7.0.[14,55] Kinetic differences between the Co(II)-substituted enzyme and the Cu(II)-enzyme were attributed to a substantially greater $K_M(O_2)$, associated with an increase in the pK_a for the axial H_2O ligand.[14,55] These observations provide experimental support for an outer-sphere mechanism involving electron transfer to noncoordinated O_2 from TPQ_{AMQ}, with Cu(II) serving as a binding site for reduced oxygen species (Scheme 2, A2 → C2).

In contrast to the results with HPAO, Co(II)- and Ni(II)-substituted AGAO exhibit k_{cat} values that are only ~1 s^{-1}, relative to 110 s^{-1} for Cu(II)-AGAO.[56] The lower catalytic activities of Co(II)- and Ni(II)-AGAO are ascribed to impaired efficiency of the oxidative half-reaction, wherein the rate-determining step changes from TPQ_{IMQ} hydrolysis in Cu(II)-AGAO to O_2 reduction.[38,54,56] Similar to the results with AGAO, Co(II)-substituted ECAO shows a substantially depressed k_{cat}(amine) value with no alterations in the magnitude of $K_M(O_2)$, and no significant rate enhancement is observed under saturating O_2.[57] Moreover, Co(II)-substituted PSAO displays a diminished k_{cat} value toward O_2 reduction (4.7% relative to the native Cu(II)-enzyme), consistent with an inner-sphere mechanism for the wild-type enzyme.[58]

The most compelling evidence for an inner-sphere mechanism in PSAO comes from competitive oxygen kinetic isotope experiments ($[k_{cat}/K_M(^{16,16}O_2)]/[k_{cat}/K_M(^{16,18}O_2)]$).[59] Importantly, the rate-limiting step in PSAO catalysis is dependent upon the substrate amine; TPQ_{SQ} spectral features are absent in the steady-state for either benzylamine or putrescine substrates, indicating that no rate-limiting ET steps during cofactor reoxidation exist.[59] The $[k_{cat}/K_M(^{16,16}O_2)]/[k_{cat}/K_M(^{16,18}O_2)]$ ratio during PSAO catalysis yields a KIE value of 1.0136 ± 0.0014 that is invariable over a wide range of temperature and pH values; the lack of evidence for an alteration in the step limiting $k_{cat}/K_M(O_2)$ over a wide experimental range argues against the existence of kinetic complexity (Figure 3B).[59] DFT analysis demonstrates that the ^{18}O KIEs are consistent with the pre-equilibrium binding of O_2 to Cu(I), forming a $[Cu^{II}(\eta^1\text{-}O_2^{-1})]^+$ intermediate, with subsequent rate-determining electron transfer from TPQ_{SQ} to O_2.[59] The magnitude of ^{18}O KIE values associated with outer-sphere processes typically range between 1.025 and 1.030, and the repressed values measured for PSAO are consistent with an inner-sphere process.[35,60] An outer-sphere mechanism has been argued to exist in HPAO and BSAO despite the ^{18}O KIE values in these enzymes (1.0101 ± 0.0029 and 1.0097 ± 0.0010),[55,61] largely due to the presumed existence of kinetic complexity linked either to partially rate-limiting O_2 binding in a non-metal hydrophobic pocket or $O_2^{\bullet-}$ binding to Cu(II) (Scheme 2, A2

DOI: 10.1021/ar500460z
Acc. Chem. Res. 2015, 48, 1218–1226

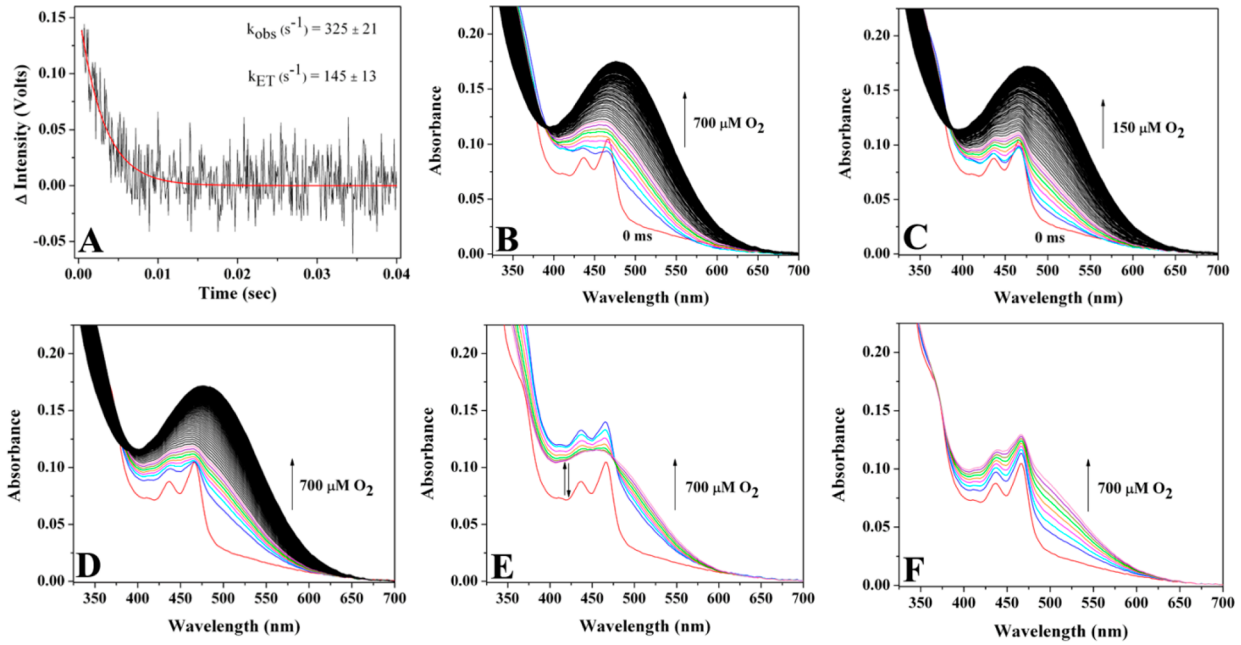


Figure 4. Kinetic and spectroscopic reactivity of reduced AGAO. Temperature jump relaxation profile illustrating the electron transfer rate from TPQ_{AMQ} to Cu(II) (A), as adapted from ref 53. Stopped-flow kinetics show the rapid disappearance of TPQ_{SQ} following exposure to O_2 (B and C). Panels D, E, and F depict calculated spectra from user-defined models with global analysis software (Pro-Kineticist II, Applied Photophysics) for the experimental data in panel B. Results with TPQ_{SQ}–Cu(I) modeled as the reactive intermediate (non-rate-limiting, D) and TPQ_{AMQ}–Cu(II) as the reactive intermediate in either non-rate-limiting (E) or rate-limiting (F) manner. Adapted from ref 38. Copyright 2008 American Chemical Society. The following color scheme applies to panels B and C (experimental) and D, E, and F (theoretical). The red line represents the experimentally obtained spectrum prior to O_2 introduction. Other colored spectra follow exposure to O_2 and correspond to 1.92 ms (blue), 3.2 ms (cyan), 4.48 ms (magenta), 5.76 ms (orange), 7.04 ms (green), 8.32 ms (dark yellow), 9.6 ms (violet), and 10.88 ms (light magenta), with all subsequent time point spectra shown in black (panels B, C, and D).

→ C2).[14,55,61,62] This putative complexity does not exist in PSAO, and a positive entropy of activation for $k_{cat}/K_M(O_2)$ suggests a multistep process where electron transfer follows prebinding of O_2 to copper.[59] This may be explained by release of bound H_2O from a metal center, and EXAFS studies with PSAO among other CuAOs demonstrated that copper undergoes a decrease in coordination number from five to three upon reduction with the release of copper-bound H_2O.[5] This could provide a generic mechanism whereby CuAOs exploit copper's reactivity and bind O_2 to propagate inner-sphere electron transfer.[59]

The data summarized above provides a convincing argument for an operative inner-sphere process in both AGAO and PSAO. How, then, do these enzymes display activity, albeit at considerably reduced rates, when copper is replaced with cobalt?[56,58] Metal replacement could block the preferred inner-sphere pathway forcing these enzymes to access a catalytically inferior outer-sphere process.[58] Alternatively, an inner-sphere mechanism may always be operative, and in the case of metal substitution, the Co(II) would initially reduce O_2 to yield Co(III)–O_2^{-1}.[58] Experimental support for this hypothesis has recently been provided by comparison of Cu(II)-AGAO to Co(II)-AGAO.[63] The ^{18}O KIE values for these enzymes (1.0164 ± 0.0014 and 1.0179 ± 0.0023), coupled with similar deuterium KIEs on $k_{cat}/K_M(O_2)$, indicate that these enzymes operate with analogous mechanisms. This is remarkable given the drastically increased $K_M(O_2)$ in the cobalt-substituted enzyme; kinetic, spectroscopic, and computational analyses suggest that the more negative Co(III)/Co(II) redox potential influences reversible O_2 binding and reduction at the reduced metal center such that $[Co^{III}(\eta^1\text{-}O_2^{-1})]$–$TPQ_{SQ}$ is destabilized relative to $[Cu^{II}(\eta^1\text{-}O_2^{-1})]$–$TPQ_{SQ}$.[63] The deuterium KIE on $k_{cat}/K_M(O_2)$ is characteristic of rate-limiting PCET and is associated with η^1-hydroperoxide formation wherein the metal–η^1-superoxide removes H^+/e^- from TPQ_{SQ}. This long-range PCET occurs at a distance of ∼6 Å and is promoted by the link between the O(2) on TPQ_{SQ} and the metal-coordinated η^1-superoxide; the η^1-hydroperoxide is anchored via a H_2O molecule that H-bonds to the conserved tyrosine, which in turn H-bonds with the 4-OH group of TPQ_{SQ}.[63]

Recent X-ray structures of AGAO and HPAO have revealed for the first time the presence of the TPQ_{SQ}–Cu(I) species; the structures show that the copper center exhibits tetrahedral geometry with TPQ_{SQ} as an axial ligand.[9,48] Cu(I) complexes display facile ligand-substitution chemistry, and the conformational flexibility of TPQ is well documented (Figure 1),[7,9,10] consistent with a catalytic intermediate composed of unbound TPQ_{SQ} and three-coordinate Cu(I) to which O_2 reversibly binds and is reduced.[38,59,63] The observation of the Cu(I)–TPQ_{SQ} complex has led to the suggestion that second sphere residues may modulate Cu(I)–TPQ_{SQ} reactivity with O_2 depending on whether TPQ_{SQ} is coordinated to Cu(I), with coordination presumably blocking oxygen's access to copper.[9] Regardless, outer-sphere electron transfer could be the preferred mechanistic channel for O_2 reduction by Cu(I)–TPQ_{SQ}, because the thermodynamic barrier for superoxide formation would be lower for reduction by TPQ_{SQ} relative to TPQ_{AMQ}. Further experimental assessments of these hypotheses are needed, along with examination of metal substituted HPAO's ability to utilize a Co(III)/Co(II) couple.

DOI: 10.1021/ar500460z
Acc. Chem. Res. 2015, 48, 1218–1226

SUMMARY

This Account has summarized several salient experiments regarding two contemporary aspects of the biological inorganic chemistry of copper-containing amine oxidases: (1) understanding the factors that govern substrate and inhibitor selectivity and efficacy in these enzymes; (2) probing the O_2 reduction mechanism, for which an inner-sphere electron transfer process from a three-coordinate Cu(I) center appears to be operative for at least AGAO and PSAO. CuAOs have provided an exceptional opportunity for discerning how a relatively simple mononuclear copper site can facilitate two very different reactions, the six-electron redox biogenesis of TPQ from tyrosine and the two-electron oxidation of an aminoquinol by O_2.

AUTHOR INFORMATION

Corresponding Authors

*Voice, 406-994-6162; fax, 406-994-5407; e-mail, eshepard@montana.edu.
*Voice, 401-874-2444; fax, 401-874-7149; e-mail, davedooley@ds.uri.edu.

Notes

The authors declare no competing financial interest.

Biographies

Eric M. Shepard graduated from Rocky Mountain College with a B.Sc. degree in chemistry (1999). He received his Ph.D. (2006) in biochemistry from Montana State University in the laboratory of David Dooley, where he was supported by a NSF IGERT fellowship. He is currently a Senior Research Scientist in the laboratory of Joan Broderick where he studies [FeFe]-hydrogenase H-cluster biosynthesis.

David M. Dooley graduated from the University of California, San Diego, with a B.A. in chemistry (1974) and received his Ph.D. (1979) in chemistry from Caltech in the laboratory of Harry Gray. He was Professor of Chemistry at Amherst College before becoming Head of the Department of Chemistry and Biochemistry at Montana State University in 1993. He then became Provost and Vice President for Academic Affairs at Montana State and now serves as the President for the University of Rhode Island. His research interests encompass copper−oxygen chemistry in metalloproteins.

DEDICATION

This Account is dedicated to the lives of two preeminent scientists in the field of CuAO research, Lawrence M. Sayre and Hans C. Freeman.

REFERENCES

(1) Halcrow, M.; Knowles, P.; Phillips, S. Copper proteins in the transport and activation of dioxygen, and the reduction of inorganic molecules. In *Handbook on Metalloproteins*; Bertini, I., Sigel, A., Sigel, H., Eds.; Marcel Dekker, Inc.: New York, 2001.
(2) Knowles, P. F.; Dooley, D. M. Amine Oxidases. In *Metal Ions in Biological Systems*; Sigel, H., Sigel, A., Eds.; Marcel Dekker, Inc.: New York, 1994; Vol. *30*.
(3) Rhee, S. G. Cell signaling. H_2O_2, a necessary evil for cell signaling. *Science* **2006**, *312*, 1882−1883.
(4) Rokhsana, D.; Shepard, E. M.; Brown, D. E.; Dooley, D. M. Amine Oxidase and Galactose Oxidase. In *Copper Oxygen Chemistry*; Karlin, K. D., Itoh, S., Eds.; John Wiley & Sons: Hoboken, NJ, 2011; Vol. *4*, Chapter 3.
(5) Dooley, D. M.; Scott, R. A.; Knowles, P. F.; Colangelo, C. M.; McGuirl, M. A.; Brown, D. E. Structures of the Cu(I) and Cu(II) forms of amine oxidases from X-ray absorption spectroscopy. *J. Am. Chem. Soc.* **1998**, *120*, 2599−2605.
(6) Jameson, R. F. Coordination Chemistry of Copper with Regard to Biological Systems. In *Properties of Copper*; Sigel, H., Ed.; Marcel Dekker, Inc.: New York, 2000; Vol. *12*.
(7) Wilce, M. C.; Dooley, D. M.; Freeman, H. C.; Guss, J. M.; Matsunami, H.; McIntire, W. S.; Ruggiero, C. E.; Tanizawa, K.; Yamaguchi, H. Crystal structures of the copper-containing amine oxidase from *Arthrobacter globiformis* in the holo and apo forms: implications for the biogenesis of topaquinone. *Biochemistry* **1997**, *36*, 16116−16133.
(8) Wilmot, C. M.; Murray, J. M.; Alton, G.; Parsons, M. R.; Convery, M. A.; Blakeley, V.; Corner, A. S.; Palcic, M. M.; Knowles, P. F.; McPherson, M. J.; Phillips, S. E. Catalytic mechanism of the quinoenzyme amine oxidase from *Escherichia coli*: Exploring the reductive half-reaction. *Biochemistry* **1997**, *36*, 1608−1620.
(9) Johnson, B. J.; Yukl, E. T.; Klema, V. J.; Klinman, J. P.; Wilmot, C. M. Structural snapshots from the oxidative half-reaction of a copper amine oxidase: Implications for O_2 activation. *J. Biol. Chem.* **2013**, *288*, 28409−28417.
(10) Green, E. L.; Nakamura, N.; Dooley, D. M.; Klinman, J. P.; Sanders-Loehr, J. Rates of oxygen and hydrogen exchange as indicators of TPQ cofactor orientation in amine oxidases. *Biochemistry* **2002**, *41*, 687−696.
(11) Elmore, B. O.; Bollinger, J. A.; Dooley, D. M. Human kidney diamine oxidase: Heterologous expression, purification, and characterization. *J. Biol. Inorg. Chem.* **2002**, *7*, 565−579.
(12) Chang, C. M.; Klema, V. J.; Johnson, B. J.; Mure, M.; Klinman, J. P.; Wilmot, C. M. Kinetic and structural analysis of substrate specificity in two copper amine oxidases from *Hansenula polymorpha*. *Biochemistry* **2010**, *49*, 2540−2550.
(13) Murray, J. M.; Saysell, C. G.; Wilmot, C. M.; Tambyrajah, W. S.; Jaeger, J.; Knowles, P. F.; Phillips, S. E.; McPherson, M. J. The active site base controls cofactor reactivity in *Escherichia coli* amine oxidase: X-ray crystallographic studies with mutational variants. *Biochemistry* **1999**, *38*, 8217−8227.
(14) Mure, M.; Mills, S. A.; Klinman, J. P. Catalytic mechanism of the topa quinone containing copper amine oxidases. *Biochemistry* **2002**, *41*, 9269−9278.
(15) Wilmot, C. M.; Saysell, C. G.; Blessington, A.; Conn, D. A.; Kurtis, C. R.; McPherson, M. J.; Knowles, P. F.; Phillips, S. E. Medical implications from the crystal structure of a copper-containing amine oxidase complexed with the antidepressant drug tranylcypromine. *FEBS Lett.* **2004**, *576*, 301−305.
(16) Langley, D. B.; Trambaiolo, D. M.; Duff, A. P.; Dooley, D. M.; Freeman, H. C.; Guss, J. M. Complexes of the copper-containing amine oxidase from *Arthrobacter globiformis* with the inhibitors benzylhydrazine and tranylcypromine. *Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.* **2008**, *64*, 577−583.
(17) Shepard, E. M.; Smith, J.; Elmore, B. O.; Kuchar, J. A.; Sayre, L. M.; Dooley, D. M. Towards the development of selective amine oxidase inhibitors. Mechanism-based inhibition of six copper containing amine oxidases. *Eur. J. Biochem.* **2002**, *269*, 3645−3658.
(18) O'Connell, K. M.; Langley, D. B.; Shepard, E. M.; Duff, A. P.; Jeon, H. B.; Sun, G.; Freeman, H. C.; Guss, J. M.; Sayre, L. M.; Dooley, D. M. Differential inhibition of six copper amine oxidases by a family of 4-(aryloxy)-2-butynamines: Evidence for a new mode of inactivation. *Biochemistry* **2004**, *43*, 10965−10978.
(19) Abeles, R. H.; Maycock, A. L. Suicide enzyme inactivators. *Acc. Chem. Res.* **1976**, *9*, 313−319.
(20) Ernberg, K.; Zhong, B.; Ko, K.; Miller, L.; Nguyen, Y. H.; Sayre, L. M.; Guss, J. M.; Lee, I. Structural and enzyme activity studies demonstrate that aryl substituted 2,3-butadienamine analogs inactivate *Arthrobacter globiformis* amine oxidase (AGAO) by chemical derivatization of the 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor. *Biochim. Biophys. Acta* **2011**, *1814*, 638−646.

DOI: 10.1021/ar500460z
Acc. Chem. Res. 2015, 48, 1218−1226

(21) Youdim, M. B.; Bakhle, Y. S. Monoamine oxidase: Isoforms and inhibitors in Parkinson's disease and depressive illness. *Br. J. Pharmacol.* **2006**, *147*, S287−296.
(22) Shepard, E. M.; Heggem, H.; Juda, G. A.; Dooley, D. M. Inhibition of six copper-containing amine oxidases by the antidepressant drug tranylcypromine. *Biochim. Biophys. Acta* **2003**, *1647*, 252−259.
(23) Knowles, P.; Kurtis, C.; Murray, J.; Saysell, C.; Tambyrajah, W.; Wilmot, C.; McPherson, M.; Phillips, S.; Dooley, D.; Brown, D.; Rogers, M.; Mure, M. Hydrazine and amphetamine binding to amine oxidases: Old drugs with new prospects. *J. Neural Transm.* **2007**, *114*, 743−746.
(24) Edmondson, D. E.; Binda, C.; Wang, J.; Upadhyay, A. K.; Mattevi, A. Molecular and mechanistic properties of the membrane-bound mitochondrial monoamine oxidases. *Biochemistry* **2009**, *48*, 4220−4230.
(25) Lee, Y.; Ling, K. Q.; Lu, X.; Silverman, R. B.; Shepard, E. M.; Dooley, D. M.; Sayre, L. M. 3-Pyrrolines are mechanism-based inactivators of the quinone-dependent amine oxidases but only substrates of the flavin-dependent amine oxidases. *J. Am. Chem. Soc.* **2002**, *124*, 12135−12143.
(26) Zhang, Y.; Ran, C.; Zhou, G.; Sayre, L. M. Highly potent 3-pyrroline mechanism-based inhibitors of bovine plasma amine oxidase and mass spectrometric confirmation of cofactor derivatization. *Bioorg. Med. Chem.* **2007**, *15*, 1868−1877.
(27) McGrath, A. P.; Hilmer, K. M.; Collyer, C. A.; Shepard, E. M.; Elmore, B. O.; Brown, D. E.; Dooley, D. M.; Guss, J. M. Structure and inhibition of human diamine oxidase. *Biochemistry* **2009**, *48*, 9810−9822.
(28) Contakes, S. M.; Juda, G. A.; Langley, D. B.; Halpern-Manners, N. W.; Duff, A. P.; Dunn, A. R.; Gray, H. B.; Dooley, D. M.; Guss, J. M.; Freeman, H. C. Reversible inhibition of copper amine oxidase activity by channel-blocking ruthenium(II) and rhenium(I) molecular wires. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13451−13456.
(29) Langley, D. B.; Brown, D. E.; Cheruzel, L. E.; Contakes, S. M.; Duff, A. P.; Hilmer, K. M.; Dooley, D. M.; Gray, H. B.; Guss, J. M.; Freeman, H. C. Enantiomer-specific binding of ruthenium(II) molecular wires by the amine oxidase of *Arthrobacter globiformis*. *J. Am. Chem. Soc.* **2008**, *130*, 8069−8078.
(30) Foot, J. S.; Yow, T. T.; Schilter, H.; Buson, A.; Deodhar, M.; Findlay, A. D.; Guo, L.; McDonald, I. A.; Turner, C. I.; Zhou, W.; Jarolimek, W. PXS-4681A, a potent and selective mechanism-based inhibitor of SSAO/VAP-1 with anti-inflammatory effects in vivo. *J. Pharmacol. Exp. Ther.* **2013**, *347*, 365−374.
(31) Bligt-Linden, E.; Pihlavisto, M.; Szatmari, I.; Otwinowski, Z.; Smith, D. J.; Lazar, L.; Fulop, F.; Salminen, T. A. Novel pyridazinone inhibitors for vascular adhesion protein-1 (VAP-1): old target-new inhibition mode. *J. Med. Chem.* **2013**, *56*, 9837−9848.
(32) Dooley, D. M.; McGuirl, M. A.; Brown, D. E.; Turowski, P. N.; McIntire, W. S.; Knowles, P. F. A Cu(I)-semiquinone state in substrate-reduced amine oxidases. *Nature* **1991**, *349*, 262−264.
(33) Murthy, N. N.; Karlin, K. D. Biomimetic copper-dioxygen chemistry. In *Mechanistic Bioinorganic Chemistry*; Thorp, H. H., Pecoraro, V. L., Eds.; American Chemical Society: Washington D.C., 1995; Vol. *246*; pp 165−193.
(34) Whittaker, J. W. Oxygen reactions of the copper oxidases. *Essays Biochem.* **1999**, *34*, 155−172.
(35) Roth, J. P. Advances in studying bioinorganic reaction mechanisms: isotopic probes of activated oxygen intermediates in metalloenzymes. *Curr. Opin. Chem. Biol.* **2007**, *11*, 142−150.
(36) Karlin, K. D.; Kaderli, S.; Zuberbuehler, A. D. Kinetics and thermodynamics of copper(I)/dioxygen interaction. *Acc. Chem. Res.* **1997**, *30*, 139−147.
(37) Cramer, C. J.; Tolman, W. B. Mononuclear Cu−O_2 complexes: Geometries, spectroscopic properties, electronic structures, and reactivity. *Acc. Chem. Res.* **2007**, *40*, 601−608.
(38) Shepard, E. M.; Okonski, K. M.; Dooley, D. M. Kinetics and spectroscopic evidence that the Cu(I)-semiquinone intermediate reduces molecular oxygen in the oxidative half-reaction of *Arthrobacter globiformis* amine oxidase. *Biochemistry* **2008**, *47*, 13907−13920.
(39) Dooley, D. M.; McGuirl, M. A.; Cote, C. E.; Knowles, P. F.; Singh, I.; Spiller, M.; Brown, R. D. I.; Koenig, S. H. Coordination chemistry of copper-containing amine oxidases: Nuclear magnetic relaxation dispersion studies of copper binding, solvent-water exchange, substrate and inhibitor binding, and protein aggregation. *J. Am. Chem. Soc.* **1991**, *113*, 754−761.
(40) Dooley, D. M.; Golnik, K. C. Spectroscopic and kinetics studies of the inhibition of pig kidney diamine oxidase by anions. *J. Biol. Chem.* **1983**, *258*, 4245−4248.
(41) Dooley, D. M.; Cote, C. E. Copper(II) coordination chemistry in bovine plasma amine oxidase: Azide and thiocyanate binding. *Inorg. Chem.* **1985**, *24*, 3996−4000.
(42) McCracken, J.; Peisach, J.; Dooley, D. M. Cu(II) coordination of amine oxidases: Pulsed EPR studies of histidine imidazole, water, and exogenous ligand coordination. *J. Am. Chem. Soc.* **1987**, *109*, 4064−4072.
(43) Wilmot, C. M.; Hajdu, J.; McPherson, M. J.; Knowles, P. F.; Phillips, S. E. Visualization of dioxygen bound to copper during enzyme catalysis. *Science* **1999**, *286*, 1724−1728.
(44) Juda, G. A.; Shepard, E. M.; Elmore, B. O.; Dooley, D. M. A comparative study of the binding and inhibition of four copper-containing amine oxidases by azide: Implications for the role of copper during the oxidative half-reaction. *Biochemistry* **2006**, *45*, 8788−8800.
(45) Duff, A. P.; Trambaiolo, D. M.; Cohen, A. E.; Ellis, P. J.; Juda, G. A.; Shepard, E. M.; Langley, D. B.; Dooley, D. M.; Freeman, H. C.; Guss, J. M. Using xenon as a probe for dioxygen-binding sites in copper amine oxidases. *J. Mol. Biol.* **2004**, *344*, 599−607.
(46) Lunelli, M.; Di Paolo, M. L.; Biadene, M.; Calderone, V.; Battistutta, R.; Scarpa, M.; Rigo, A.; Zanotti, G. Crystal structure of amine oxidase from bovine serum. *J. Mol. Biol.* **2005**, *346*, 991−1004.
(47) Medda, R.; Mura, A.; Longu, S.; Anedda, R.; Padiglia, A.; Casu, M.; Floris, G. An unexpected formation of the spectroscopic Cu(I)-semiquinone radical by xenon-induced self-catalysis of a copper quinoprotein. *Biochimie* **2006**, *88*, 827−835.
(48) Murakawa, T.; Hayashi, H.; Sunami, T.; Kurihara, K.; Tamada, T.; Kuroki, R.; Suzuki, M.; Tanizawa, K.; Okajima, T. High-resolution crystal structure of copper amine oxidase from *Arthrobacter globiformis*: Assignment of bound diatomic molecules as O_2. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2013**, *69*, 2483−2494.
(49) Johnson, B. J.; Cohen, J.; Welford, R. W.; Pearson, A. R.; Schulten, K.; Klinman, J. P.; Wilmot, C. M. Exploring molecular oxygen pathways in *Hansenula polymorpha* copper-containing amine oxidase. *J. Biol. Chem.* **2007**, *282*, 17767−17776.
(50) Pirrat, P.; Smith, M. A.; Pearson, A. R.; McPherson, M. J.; Phillips, S. E. Structure of a xenon derivative of *Escherichia coli* copper amine oxidase: Confirmation of the proposed oxygen-entry pathway. *Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.* **2008**, *64*, 1105−1109.
(51) Dooley, D. M.; Brown, D. E. Intramolecular electron transfer in the oxidation of amines by methylamine oxidase from *Arthrobacter* P1. *J. Biol. Inorg. Chem.* **1996**, *1*, 205−209.
(52) Turowski, P. N.; McGuirl, M. A.; Dooley, D. M. Intramolecular electron transfer rate between active-site copper and topa quinone in pea seedling amine oxidase. *J. Biol. Chem.* **1993**, *268*, 17680−17682.
(53) Shepard, E. M.; Dooley, D. M. Intramolecular electron transfer rate between active-site copper and TPQ in *Arthrobacter globiformis* amine oxidase. *J. Biol. Inorg. Chem.* **2006**, *11*, 1039−1048.
(54) Hirota, S.; Iwamoto, T.; Kishishita, S.; Okajima, T.; Yamauchi, O.; Tanizawa, K. Spectroscopic observation of intermediates formed during the oxidative half-reaction of copper/topa quinone-containing phenylethylamine oxidase. *Biochemistry* **2001**, *40*, 15789−15796.
(55) Mills, S. A.; Goto, Y.; Su, Q.; Plastino, J.; Klinman, J. P. Mechanistic comparison of the cobalt-substituted and wild-type copper amine oxidase from *Hansenula polymorpha*. *Biochemistry* **2002**, *41*, 10577−10584.
(56) Kishishita, S.; Okajima, T.; Kim, M.; Yamaguchi, H.; Hirota, S.; Suzuki, S.; Kuroda, S.; Tanizawa, K.; Mure, M. Role of copper ion in

DOI: 10.1021/ar500460z
Acc. Chem. Res. 2015, 48, 1218−1226

bacterial copper amine oxidase: Spectroscopic and crystallographic studies of metal-substituted enzymes. *J. Am. Chem. Soc.* **2003**, *125*, 1041−1055.
(57) Smith, M. A.; Pirrat, P.; Pearson, A. R.; Kurtis, C. R.; Trinh, C. H.; Gaule, T. G.; Knowles, P. F.; Phillips, S. E.; McPherson, M. J. Exploring the roles of the metal ions in *Escherichia coli* copper amine oxidase. *Biochemistry* **2010**, *49*, 1268−1280.
(58) Mills, S. A.; Brown, D. E.; Dang, K.; Sommer, D.; Bitsimis, A.; Nguyen, J.; Dooley, D. M. Cobalt substitution supports an inner-sphere electron transfer mechanism for oxygen reduction in pea seedling amine oxidase. *J. Biol. Inorg. Chem.* **2012**, *17*, 507−515.
(59) Mukherjee, A.; Smirnov, V. V.; Lanci, M. P.; Brown, D. E.; Shepard, E. M.; Dooley, D. M.; Roth, J. P. Inner-sphere mechanism for molecular oxygen reduction catalyzed by copper amine oxidases. *J. Am. Chem. Soc.* **2008**, *130*, 9459−9473.
(60) Roth, J. P. Oxygen isotope effects as probes of electron transfer mechanisms and structures of activated O_2. *Acc. Chem. Res.* **2009**, *42*, 399−408.
(61) Su, Q.; Klinman, J. P. Probing the mechanism of proton coupled electron transfer to dioxygen: The oxidative half-reaction of bovine serum amine oxidase. *Biochemistry* **1998**, *37*, 12513−12525.
(62) Welford, R. W.; Lam, A.; Mirica, L. M.; Klinman, J. P. Partial conversion of *Hansenula polymorpha* amine oxidase into a "plant" amine oxidase: implications for copper chemistry and mechanism. *Biochemistry* **2007**, *46*, 10817−10827.
(63) Liu, Y.; Mukherjee, A.; Nahumi, N.; Ozbil, M.; Brown, D.; Angeles-Boza, A. M.; Dooley, D. M.; Prabhakar, R.; Roth, J. P. Experimental and computational evidence of metal-O_2 activation and rate-limiting proton-coupled electron transfer in a copper amine oxidase. *J. Phys. Chem. B* **2013**, *117*, 218−229.
(64) DeLano, W. L. PyMOL; Schrödinger, LLC, New York, 2002.

DOI: 10.1021/ar500460z
Acc. Chem. Res. 2015, 48, 1218−1226