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Electron flow through biological molecules: Does hole hopping protect proteins from oxidative damage?

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Abstract

Biological electron transfers often occur between metal-containing cofactors that are separated by very large molecular distances. Employing photosensitizer-modified iron and copper proteins, we have shown that single-step electron tunneling can occur on nanosecond to microsecond timescales at distances between 15 and 20 angstroms. We also have shown that charge transport can occur over even longer distances by hole hopping (multistep tunneling) through intervening tyrosines and tryptophans. In this Perspective, we advance the hypothesis that such hole hopping through Tyr/Trp chains could protect oxygenase, dioxygenase, and peroxidase enzymes from oxidative damage. In support of this view, by examining the structures of P450 (CYP102A) and 2OG-Fe (TauD) enzymes, we have identified candidate Tyr/Trp chains that could transfer holes from uncoupled high-potential intermediates to reductants in contact with protein surface sites.

BACKGROUND

Many vital biological transformations involve the incorporation of one (monooxygenases) or two (dioxygenases) O-atoms from molecular oxygen into organic substrates. Enzymes that utilize oxygen must coordinate the delivery of four protons and four electrons to O₂ in order to prevent the formation of harmful molecular oxidants (O₂⁻, HO₂[•], H₂O₂, HO[•]), collectively known as reactive oxygen species (ROS). It is our view that the risks posed by reactive intermediates are so great that oxygen-utilizing enzymes have protection mechanisms to help them avoid inactivation when the primary electron/proton transfer mechanism is disrupted.

The mechanism of O₂ reduction by cytochrome *c* oxidase illustrates some of the challenges facing these enzymes (Wikström, 2012; Yu et al., 2012; Yu et al., 2011). Reaction of the fully four-electron reduced enzyme (Cu_A^{II,I}, Fe^{II}-heme *a*, Fe^{II}-heme *a*₃, Cu_B^I) with O₂ generates an intermediate designated as P_R. When the two-electron reduced, mixed valence enzyme (Cu_A^{II,II}, Fe^{III}-heme *a*, Fe^{II}-heme *a*₃, Cu_B^I) reacts with O₂, the P_M intermediate is formed. The O-O bond has been cleaved in both P_R and P_M to produce Fe^{IV}(O)-heme *a*₃ and Cu_B^{II} in the binuclear site. The difference between P_R and P_M is in the source of the fourth electron: P_M is thought to have a Tyr²⁴⁴ radical (bovine numbering), whereas the fourth electron in P_R is provided by Fe^{II}-heme *a*. When P_M is prepared using H₂O₂, the hole on

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(TyrO[•])²⁴⁴ is believed to migrate through (Trp^{•+})²³⁶ to (TyrO[•])¹²⁹; the latter residue is suggested to participate in proton pumping (Yu et al., 2012). The key point is that Tyr²⁴⁴ is available to fill the gap when the fourth electron required for O₂ reduction cannot be supplied by Fe^{II}-heme *a* (Wikström, 2012; Yu et al., 2012).

In many oxygenases, including the cytochromes P450 (P450) and the 2-oxo-glutarate dependent nonheme iron oxygenases (2OG-Fe), the four electrons required for O₂ reduction have different origins (Figure 1). Typically, two electrons are delivered from a reductase (P450) or co-substrate (2OG), and the remaining two electrons are provided by the organic substrate (Denisov et al., 2005; Hausinger, 2004; Whitehouse, Bell & Wong, 2012). In the consensus mechanism for iron oxygenases, the first two electrons induce O-O bond cleavage, producing a powerfully oxidizing ferryl species. The ferryl complex abstracts a hydrogen atom from the substrate and HO[•] rebound leads to hydroxylated product (Denisov et al., 2005; Hausinger, 2004; Whitehouse et al., 2012). For enzymes with broad substrate specificities, or when operating in the presence of xenobiotic compounds, the fidelity of substrate oxidation is less than 100%, with potentially damaging consequences (Chen et al., 2008; De Matteis et al., 2012; Denisov et al., 2007a; Grinkova et al., 2013; Saban, Flagg & Knapp, 2011; Staudt, Lichtenb. F & Ullrich, 1974). This circumstance is manifested as an increased molar ratio of O₂ consumption to substrate hydroxylation (uncoupling). We think it likely that organisms have evolved protection mechanisms to guard against deactivation of oxygenase enzymes in the event of uncoupled O₂ consumption. In particular, we suggest that radical transfer pathways are employed to deliver strongly oxidizing holes ($E^{\circ} \sim 1$ V vs. NHE) from ferryl complexes in active sites to less fragile regions of oxygenases.

In this Perspective we will advance the hypothesis that there are potentially protective radical chains in P450 and 2OG-Fe: but first we will review what we know about the factors controlling hopping through aromatic amino acids in multistep electron tunneling constructs designed in azurin, a prototypal cupredoxin.

RADICAL TRANSFER PATHWAYS IN AZURIN

Azurin is a robust cupredoxin (128 residues) that is amenable to site-directed mutagenesis and surface-labeling with photosensitizers (Farver & Pecht, 2011; Gray & Winkler, 2010; Reece & Nocera, 2009; Wilson, Yu & Lu, 2013). Oxidized radicals of Trp and Tyr are substantially stronger acids than their neutral precursors (Trp, $pK_a > 14$; Trp^{•+}, $pK_a = 4$; TyrOH, $pK_a = 10$; TyrOH^{•+}, $pK_a = -1$) (Aubert et al., 2000; Bonin et al., 2010; Costentin et al., 2009; Harriman, 1987; Jovanic, Harriman & Simic, 1986); management of the acidic proton is a critically important factor controlling radical formation with these amino acids. Proton management is particularly challenging for buried amino acids and, thus far, we have not succeeded in detecting buried Trp or Tyr radicals as ET intermediates. Our kinetics data indicate that surface exposed Trp^{•+} and NO₂TyrO[•] radicals can, in appropriate constructs, accelerate Cu^I oxidation by distant Re- and Ru-diimine complexes (Shih et al., 2008; Warren et al., 2013a).

Multistep ET through Trp and Tyr radicals in azurin

We have used *Pseudomonas aeruginosa* azurin as a test bed for mechanistic investigations of Trp and Tyr radical formation in protein ET reactions (Blanco-Rodriguez et al., 2011; Shih et al., 2008; Takematsu et al., 2013; Warren et al., 2012; Warren et al., 2013a). Our initial investigation revealed that Cu^I oxidation by a photoexcited Re^I-diimine complex (Re^I(CO)₃(4,7-dimethyl-1,10-phenanthroline)) covalently bound at His¹²⁴ on a His¹²⁴Gly¹²³Trp¹²²Met¹²¹ β-strand (ReHis¹²⁴Trp¹²²Cu^I-azurin) occurs in a few nanoseconds, fully two orders of magnitude faster than documented for single-step electron tunneling at a 19-Å donor-acceptor distance, owing to a two-step hopping mechanism involving a Trp^{•+} radical intermediate (Shih et al., 2008).

Our work on multistep ET in sensitizer-modified azurin is informed by semiclassical ET theory (Marcus & Sutin, 1985). Given a particular spatial arrangement of redox cofactors, we can predict driving-force dependences of the relative time constants for single-step ($\tau_{ss} = 1/k_{ss}$) and multistep (τ_{hop}) electron transport (Warren et al., 2012). Alternatively, given the redox and reorganization energetics, we can predict the hopping propensity for different cofactor arrangements (Warren et al., 2013a). We considered three Ru(2,2'-bipyridine)₂(imidazole)(His^X)-labeled azurins (RuHis¹⁰⁷, RuHis¹²⁴, and RuHis¹²⁶) and examined the hopping advantage (τ_{ss}/τ_{hop}) for a protein with a generalized intermediate (Int) situated between a diimine-Ru^{III} oxidant and Cu^I (Warren et al., 2013a). In all cases, the greatest hopping advantage occurs in systems where the Int-Ru^{III} distance is up to 5 Å shorter than the Int-Cu^I distance. The hopping advantage increases as systems orient nearer a linear Donor-Int-Acceptor configuration, owing to minimized intermediate tunneling distances. The smallest predicted hopping advantage is in RuHis¹²⁴ azurin, which has the shortest Ru-Cu distance of the three proteins. The hopping advantage is nearly lost as G° for the first step (Ru^{III} ← Int) rises above +0.15 eV. Isoergic initial steps provide a wide distribution of arrangements, where advantages as great as 10⁴ are possible (for a fixed donor-acceptor distance of 23.7 or 25.4 Å). A slightly exergonic Int → Ru^{III} step provides an even larger distribution of arrangements for productive hopping, which will be the case as long as the driving force for the first step is not more favorable than that for overall transfer.

We tested these predictions experimentally in three Ru-His labeled azurins using nitrotyrosinate (NO₂TyrO⁻) as a redox intermediate (RuHis¹⁰⁷(NO₂TyrOH)¹⁰⁹; RuHis¹²⁴(NO₂TyrOH)¹²²; and RuHis¹²⁶(NO₂TyrOH)¹²²; $E^\circ(\text{NO}_2\text{TyrO}^{\bullet-}) \approx 1.02$ V vs. NHE) (Figure 2) (Warren et al., 2013a). The first two systems have cofactor placements that are close to the predicted optimum; the last system has a larger first-step distance, which is predicted to decrease the hopping advantage. The phenol pK_a of 3-nitrotyrosine (7.2) permitted us to work at near-neutral pH, rather than high pH (>10) required for hopping with tyrosinate. ET via nitrotyrosinate avoids the complexities associated with the proton-coupled redox reactions of tyrosine. We found specific rates of Cu^I oxidation more than 10 times greater than those of single-step ET in the corresponding azurins lacking NO₂TyrOH, confirming that NO₂TyrO⁻ accelerates long-range ET. The results are in excellent agreement with hopping maps developed using semiclassical ET theory and parameters derived from our body of protein ET measurements (Gray & Winkler, 2010; Warren et al., 2012; Warren et al., 2013a).

POTENTIAL RADICAL TRANSFER PATHWAYS IN CYTOCHROME P450

The cytochromes P450 are members of a superfamily of heme oxygenases that perform two broad functional roles: xenobiotic metabolism and biosynthesis (Denisov et al., 2005; Johnson & Stout, 2013; Nebert, Wikvall & Miller, 2013; Orr et al., 2012; Whitehouse et al., 2012). The oxygenation chemistry catalyzed by some P450 enzymes is tightly coupled to substrate hydroxylation: one mole of product is produced for each mole of O₂ consumed. In many enzymes, however, particularly the eukaryotic proteins with broad substrate specificities, hydroxylation is much less efficiently coupled to O₂ consumption (frequently less than 10%) (Denisov et al., 2007a; Grinkova et al., 2013; Staudt et al., 1974). When the enzyme does not transfer an O-atom to substrate, it can produce reactive oxygen species (O₂⁻, H₂O₂) or a second H₂O molecule (Puntarulo & Cederbaum, 1998). The production of ROS can lead to rapid degradation of the enzyme and other harmful chemistry. In the case of oxidase chemistry (formation of 2H₂O from O₂), two reducing equivalents must be delivered by sources other than the substrate. When a CYP enzyme binds a refractory substrate, ferryl formation is likely to proceed, but substrate hydroxylation is inhibited. Under these circumstances, chains of redox-active Tyr, Trp, Cys, and/or Met residues can direct the oxidizing hole to the protein periphery where it can react with intracellular antioxidants such as glutathione.

Enzymes from the 2OG-Fe superfamily use 2-oxoglutarate as a 2-electron donating co-substrate, Fe²⁺ as a cofactor, and O₂ to effect the hydroxylation of organic substrates (Figure 1). The 2OG-Fe enzymes exhibit a wide array of biological functions including collagen biosynthesis, lysyl hydroxylation of RNA splicing proteins, DNA repair, RNA modification, chromatin regulation, epidermal growth factor-like domain modification, hypoxia sensing, and fatty acid metabolism (Mantri et al., 2012; Rose et al., 2011). The 2OG-Fe oxygenase enzymes have conserved double-stranded β-helix folds with octahedral Fe binding sites comprised of the HXD/E...H triad providing two His imidazole ligands and one monodentate carboxylate ligand. The remaining three coordination sites in the resting enzyme are occupied by O-donors from 2OG and a water ligand.

Several 2OG-Fe enzymes have been reported to undergo autocatalyzed oxidative modifications of aromatic amino acids. In the taurine-2OG dioxygenase that catalyzes the conversion of taurine to bisulfite, EPR data indicate the transient formation of a Tyr⁷³-based radical that converts to an Fe^{III}-catecholate (Mantri et al., 2012). In 2,4-dichlorophenoxyacetate oxygenase (TfdA) and factor-inhibiting hypoxia-inducible factor (FIH) there is evidence for Trp hydroxylation when substrate is unavailable (Mantri et al., 2012). These aromatic amino acid oxidations lead to inactivation of the enzyme. As with P450, we suggest that radical chains of Trp, Tyr, Cys, and/or Met residues in 2OG-Fe hydroxylases protect the enzymes from damage in the event of slow or unsuccessful substrate hydroxylation by diverting the powerfully oxidizing hole from Fe^{IV}(O) to the protein surface, where it can react with intracellular reductants (*e.g.*, glutathione). This diversion of oxidizing equivalents would extend the functional lifetime of an enzyme.

When considering the many remarkable transformations catalyzed by natural enzymes, it is easy to be left with the impression that these macromolecules are perfect catalysts that, after

millions of years of tinkering, have solved the riddle of simultaneously maximizing speed, selectivity, and specificity. Upon closer inspection, however, heme and nonheme oxygenases are far from perfect catalysts, yet manage to accomplish their primary functions. Indeed, in many oxygenases, the coupling between oxygen consumption and substrate hydroxylation is extremely low. The most abundant P450 in human liver, CYP3A4, is a case in point (Denisov et al., 2007b; Grinkova et al., 2013). For enzyme incorporated into nanodiscs (Grinkova, Denisov & Sligar, 2010), the coupling of substrate hydroxylation to NADH consumption was 16% for testosterone as a substrate, 10% for bromocriptine, and 2% for tamoxifen (Grinkova et al., 2013). It is fair to say that, although the primary CYP3A4 function may be substrate hydroxylation, the primary enzyme activity is distributed more or less equally between H₂O₂ and H₂O production (Grinkova et al., 2013). Indeed, it would not be inaccurate to characterize CYP3A4 as a flawed oxidase that occasionally oxygenates organic substrates. More importantly, unless the enzyme were protected from damage in the event of uncoupled turnover, CYP3A4 would function not as a catalyst but as a stoichiometric reagent. A similar situation exists for uncoupled turnover in the 2OG-Fe enzymes.

The active sites of heme and nonheme oxygenases often are deeply buried within a polypeptide matrix. Consequently, powerfully oxidizing active site holes cannot efficiently migrate in single-step tunneling reactions to the enzyme surface for reduction by external reagents (Winkler & Gray, 2014a; Winkler & Gray, 2014b). We have shown that multistep tunneling reactions can be hundreds to thousands of times faster than their single-step counterparts (Shih et al., 2008; Warren et al., 2012; Warren et al., 2013a; Warren, Winkler & Gray, 2013b). Radical transfer pathways composed of Tyr, Trp, Cys, and Met residues are ideally suited to deliver active-site oxygenase holes to enzyme surfaces when reaction with substrate is disrupted.

A biologically useful Fe-oxygenase protection mechanism requires that a fine balance be struck between substrate reaction and hole migration to the surface. Overly efficient hole migration would lower enzyme hydroxylation activity, while a sluggish pathway would be ineffective at protecting the enzyme. Active-site hole scavenging in P450 by the natural reductase may be possible, but the timing of this reaction would be extremely variable, owing to fluctuations in reductase concentration. In the 2OG-Fe enzymes, there is no reductase that could protect the enzyme. An intraprotein radical transfer mechanism can be tuned to provide the proper balance between enzyme protection and substrate reaction. We suggest that the first step in the hole-migration pathway is the critical determinant of ferryl survival time. Once a radical forms on the first residue in the pathway (the gateway residue), further migration to the surface is rapid. In the potential pathways that we have identified, the distance from the active site to the first pathway residue is often longer than subsequent steps. In addition to the longer distance, proton coupling and enzyme conformational changes could contribute to limiting the rate of the first step in the transfer chain.

CYP102A1

CYP102A1 from *Bacillus megaterium* (also known as P450 BM3) is a rare example of a bacterial Class II cytochrome P450 enzyme in which both reductase and heme domains are

contained within a single polypeptide chain (Miura & Fulco, 1974; Narhi & Fulco, 1986). The enzyme catalyzes the remarkably rapid hydroxylation of long-chain fatty acids using NAD(P)H and O₂, without the presence of any other proteins or cofactors (Narhi & Fulco, 1986). The full-length enzyme (CYP102A1_{HR}) has been expressed in *E. coli*, as have independent heme (CYP102A1_H) and reductase (CYP102A1_R) domains (Boddupalli, Estabrook & Peterson, 1990; Boddupalli et al., 1992; Li, Darwish & Poulos, 1991; Narhi, Wen & Fulco, 1988; Oster, Boddupalli & Peterson, 1991). The individual domains, as well as an assembly between the heme domain and a flavin-containing reductase domain, have been structurally characterized (Girvan et al., 2007; Sevrioukova et al., 2000; Warman et al., 2005). The soluble, 119 kDa CYP102A1_H enzyme serves as a convenient model system for the more complex membrane-bound enzyme assemblies (Whitehouse et al., 2012).

Uncoupled substrate, O₂, and NAD(P)H consumption in P450 catalysis is a well-recognized and relatively common phenomenon (De Matteis et al., 2012; De Matteis et al., 2002; Denisov et al., 2007a; Grinkova et al., 2013; Puntarulo & Cederbaum, 1998; Staudt et al., 1974). If two reducing equivalents are not delivered to O₂ by the substrate, then alternative sources are necessary to avoid ROS production and/or enzyme degradation. In some cases, the extra equivalents can be delivered by NAD(P)H, leading to NAD(P)H:O₂ molar consumption ratios greater than 1 (De Matteis et al., 2012). Exogenous reductants such as bilirubin and uroporphyrinogen have been shown to contribute reducing equivalents during NAD(P)H/O₂ CYP102A1 turnover in the presence of halogenated (perfluorolaurate) substrates (De Matteis et al., 2012). Although it is possible that an active site hole could tunnel to the protein surface in a single step, a multistep radical transfer mechanism would be far more efficient. There are two attractive radical transfer pathways from the CYP102A1 heme to the protein surface (Figure 3) (Girvan et al., 2007). Pathway I is comprised of heme-Trp⁹⁶-Trp⁹⁰-Tyr³³⁴; pathway II is heme-Cys¹⁵⁶-Tyr¹¹⁵-Met¹¹²-Tyr³⁰⁵.

CYP102A1 radical transfer pathway I

The shortest direct distance between aromatic atoms of CYP102A1 Trp⁹⁶ and the heme is 7.3 Å and Trp(Nε)⁹⁶ is hydrogen bonded to the heme propionate (Girvan et al., 2007). Sequence alignment (UniProtKB) in the P450 family suggests that Trp is conserved at this position in >75% of the members of this group. Interestingly, of the 698 sequences with Trp at this position, all but 5 derive from eukaryotic sources, whereas about half of the proteins with His at this position derive from bacterial or archaeal sources. In this regard, it is noteworthy that archaeal CYP119 does not have a Trp residue at this site and is the only P450 in which Cmpd-1 has been characterized (Park et al., 2002; Rittle & Green, 2010). The strong conservation of the Trp⁹⁶ residue has been noted previously (Munro et al., 1994). To the best of our knowledge, no role other than structural has been reported for this highly conserved Trp residue in P450 (Whitehouse et al., 2012).

We suggest that Trp⁹⁶ is the gateway residue for hole transfer from the heme to the protein surface during uncoupled turnover. Studies of the reactions of substrate-free P450_{cam} (CYP101) with peracids revealed that a second intermediate (Cmpd-ES) forms as a result of electron transfer from a Tyr residue to Cmpd-1 (Schünemann et al., 2004; Spolidak, Dawson & Ballou, 2005; Spolidak, Dawson & Ballou, 2006; Spolidak, Dawson & Ballou, 2008). A

Cmpd-ES intermediate has been detected in CYP102A1 and Trp⁹⁶ has been implicated as one of the residues hosting the oxidized radical (Raner et al., 2006). Addition of NADPH to Cmpd-ES of the CYP102_{HR} holoenzyme regenerates the ferric resting state; and formation of these radicals may play a protective role during uncoupled P450 catalysis (Spolitak et al., 2006). A combined computational/experimental investigation of CYP102A1 implicated buried Trp⁹⁶, Trp⁹⁰, His⁹², and Tyr³³⁴ residues as components of an ET pathway that could deliver reducing equivalents to Cmpd-1 from the protein surface (Vidal-Limon et al., 2013). The shortest aromatic contacts in this chain are: Trp⁹⁶-Trp⁹⁰, 8.4 Å; Trp⁹⁰-Tyr³³⁴, 4.4 Å (Girvan et al., 2007). The environment around Tyr³³⁴ appears well-suited for radical formation: the phenol hydroxyl group is hydrogen-bonded to both a carboxylate (Asp⁶⁸) and a water molecule (HOH¹²¹⁵).

Our prior studies of P450 ET reactions are consistent with involvement of Trp⁹⁶ in a radical transfer pathway to the heme (Ener et al., 2010). We have found that Ru^{II}(bpy)₂(phen^{•-}-Cys⁹⁷) can deliver an electron across 24 Å to the Fe^{III}-heme in 20 μs, and Ru^{III}(bpy)₂(phen-Cys⁹⁷)CYP102A1_H can oxidize the heme to a porphyrin radical in under 2 μs (Ener et al., 2010). The latter reaction is particularly rapid given the low driving force (<200 meV) expected for the transformation. We have prepared a Trp⁹⁶His mutant and found that Ru^{III}(bpy)₂(phen-Cys⁹⁷)(His⁹⁶)CYP102A1_H does not promote photochemical heme oxidation to Cmpd-2. Electron transfer to the Fe^{III}-heme from Ru^{II}(bpy)₂(phen^{•-}-Cys⁹⁷)(His⁹⁶), however, is unaffected by the Trp⁹⁶His mutation.

CYP102A1 radical transfer pathway II

The second potential radical transfer pathway in CYP102A1, heme-Cys¹⁵⁶-Tyr¹¹⁵-Met¹¹²-Tyr³⁰⁵, does not appear as favorable as pathway I, due largely to a long distance between the heme and the first step in the path. The distance from Cys(Sγ)¹⁵⁶ to the closest heme aromatic carbon atom (10.8 Å) is slightly longer than the shortest aromatic-aromatic contact between the heme and Tyr¹¹⁵ (10.2 Å). If a radical is formed on Tyr¹¹⁵, hole transport to the surface Tyr³⁰⁵ via Met(Sδ)¹¹² could provide a secondary protection route.

POTENTIAL RADICAL TRANSFER PATHWAYS IN 2OG-Fe OXYGENASES

TauD

The 2-oxoglutarate nonheme iron oxygenases catalyze substrate hydroxylation reactions in a fashion that is reminiscent of the cytochromes P450, but with some critical distinctions (Figure 1). The consensus mechanism for catalysis involves Fe²⁺ binding to the apo-enzyme followed by 2OG incorporation. Substrate binding induces loss of the water ligand from Fe²⁺, creating a vacant coordination site for O₂ binding. Oxidation of 2OG produces CO₂, succinate, and an Fe^{IV}(O) center that is thought to hydroxylate substrate via the usual H-atom abstraction, hydroxyl rebound cycle (Mantri et al., 2012; Rose et al., 2011). The 2OG-Fe hydroxylases differ from the P450 enzymes in that substrate hydroxylation proceeds from the Fe^{IV}(O) oxidation level (equivalent to P450 Cmpd-2). The *E. coli* 2OG-Fe enzyme TauD is synthesized under conditions of sulfur deprivation (Hausinger, 2004); large quantities of TauD have been prepared by over expression in *E. coli* BL21(DE3)(pME4141) cells (Eichhorn et al., 1997; Ryle, Padmakumar & Hausinger, 1999). The enzyme catalyzes the

hydroxylation of taurine (2-aminoethanesulfonate), producing an unstable species that decomposes into sulfite and aminoacetaldehyde (Hausinger, 2004). In the absence of taurine, the enzyme will slowly consume O₂ and become inactivated: protein analysis indicates hydroxylation of Tyr⁷³ (Koehtop et al., 2006; Ryle et al., 2003). Although deuterated substrates coupling between oxygen consumption and substrate hydroxylation is diminished, 2OG oxidation is not, suggesting that Fe^{IV}(O) continues to be formed in the presence of refractory substrates; and bis-Tris buffer, a potential reducing agent, decreases coupling between O₂ activation and C-H hydroxylation (McCusker & Klinman, 2009). We suggest that when Fe^{IV}(O) is unable to effect substrate hydroxylation, the oxidizing hole is directed to the protein surface where it can be reduced by external reagents.

TauD radical transfer pathways

We have identified two possible radical transfer pathways in the structure of TauD: the most attractive pathway from Fe to the surface has four Trp residues: Fe-Trp²⁴⁸-Trp¹²⁸-Trp²⁴⁰-Trp²³⁸, relevant distances are: Fe-Trp²⁴⁸, 4.8 Å; Trp²⁴⁸-Trp¹²⁸, 3.1 Å; Trp¹²⁸-Trp²⁴⁰, 3.7 Å; Trp²⁴⁰-Trp²³⁸, 3.7 Å (Figure 4) (O'Brien et al., 2003). The structure of this Trp chain compares favorably to that identified in *E. coli* DNA photolyase (4-5 Å separations) (Byrdin et al., 2003; Lukacs et al., 2006). The photolyase chain has just three Trp residues, and hole migration from FADH^{*} to Trp³⁰⁶ at the protein surface is complete in less than 10 ns (Byrdin et al., 2003; Lukacs et al., 2006). We anticipate that a hole injected by Fe^{IV}(O)-TauD into Trp²⁴⁸ should migrate to Trp²³⁸ at the surface in less than 1 μs. A secondary radical transfer pathway in TauD [Fe-Tyr⁷³-Tyr¹⁶⁴-(Trp¹⁷⁴, Tyr¹⁶²)] is of particular interest because hydroxylated Tyr⁷³ has been found during turnover in the absence of taurine (Koehtop et al., 2006; Ryle et al., 2003). Both Trp¹⁷⁴ and Tyr¹⁶² are well-exposed at the enzyme surface and both (or just one) of these residues could be involved in a radical transfer pathway. Relevant distances are: Fe-Tyr⁷³, 6.5 Å; Tyr⁷³-Tyr¹⁶⁴, 5.0 Å; Tyr¹⁶⁴-Trp¹⁷⁴, 4.2 Å; Tyr¹⁶⁴-Tyr¹⁶², 7.6 Å; Trp¹⁷⁴-Tyr¹⁶², 8.8 Å (O'Brien et al., 2003).

OUTLOOK

Functional radical transfer pathways have been identified in several enzymes, including ribonucleotide reductase (Argirevic et al., 2012; Holder et al., 2012; Offenbacher et al., 2013a; Offenbacher et al., 2013b; Sjöberg, 1997; Stubbe et al., 2003; Stubbe & van der Donk, 1998; Worsdorfer et al., 2013; Yokoyama et al., 2011), photosystem II (Boussac et al., 2013; Keough et al., 2013; Sjöholm et al., 2012), DNA photolyase (Aubert et al., 1999; Aubert et al., 2000; Byrdin et al., 2003; Kodali, Siddiqui & Stanley, 2009; Li, Heelis & Sancar, 1991; Lukacs et al., 2006; Sancar, 2003; Taylor, 1994; Woiczikowski et al., 2011), and MauG (Davidson & Liu, 2012; Davidson & Wilmot, 2013; Geng et al., 2013; Yukl et al., 2013). If radical transfer pathways do indeed provide protection mechanisms for enzymes operating at high electrochemical potentials, then it is likely that they will be found in many more redox-active enzymes. A survey of oxidoreductases in the protein data bank reveals that nearly 80% of structurally characterized peroxidases, oxygenases, and dioxygenases (enzyme classes EC 1.11, 1.13, and 1.14; 587 structures with sequence identity less than 90%) contain chains of 2 or more redox-active residues (Tyr, Trp, heme, Fe, Cu) separated by no more than 5 Å (Figure 5). The fraction increases to almost 90% if

the cutoff distance is increased to 8 Å. We think it very likely that hole hopping through these types of radical transfer chains greatly reduces the production of reactive oxygen species that destroy enzymes and other molecules in living cells.

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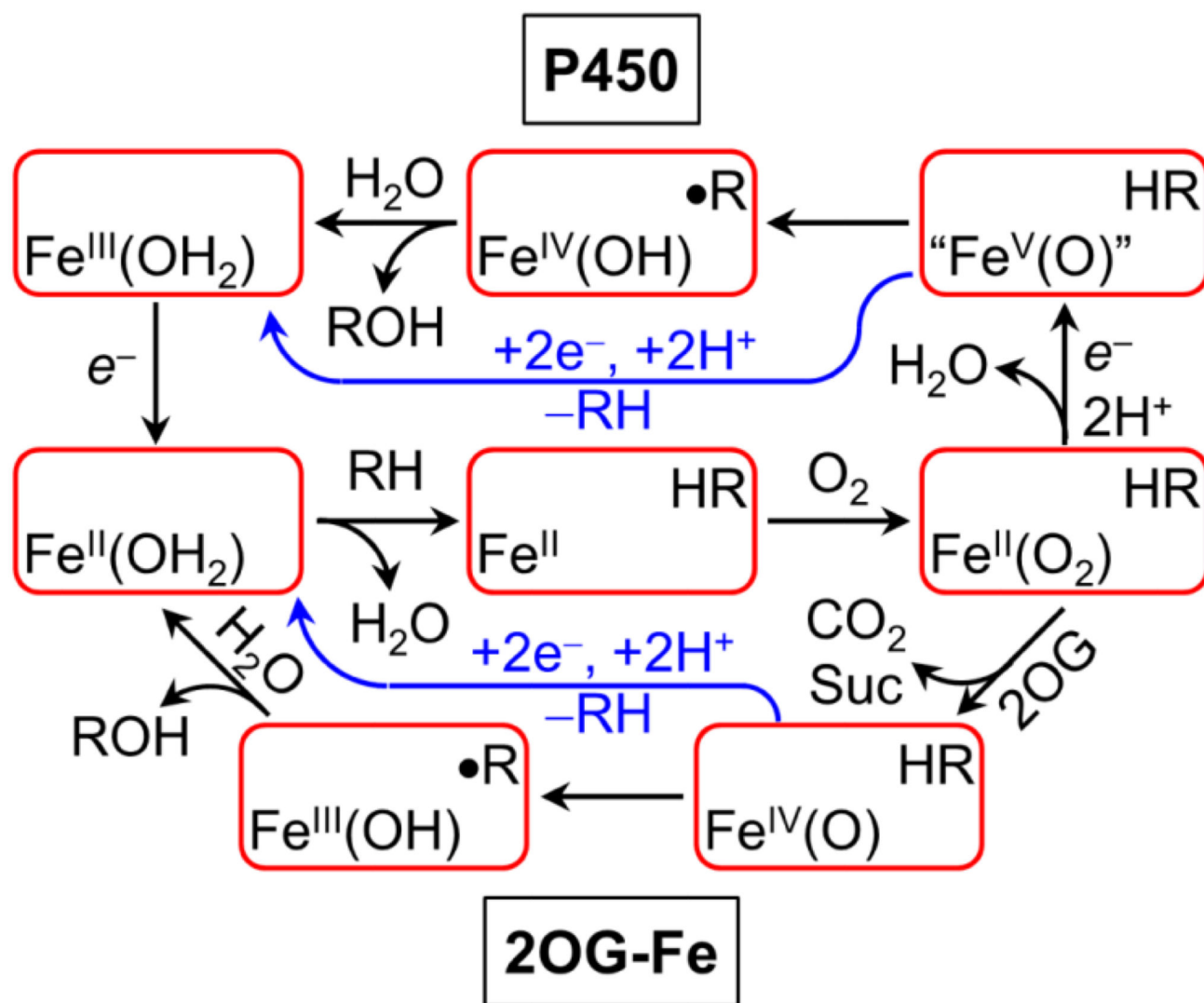


Figure 1. Schematic representation of the catalytic mechanisms of P450 and 2OG-Fe oxygenases: RH, substrate; 2OG, 2-oxoglutarate; Suc, succinate. Black arrows indicate the functional substrate hydroxylation pathways. Blue arrows indicate oxidase uncoupling pathways.

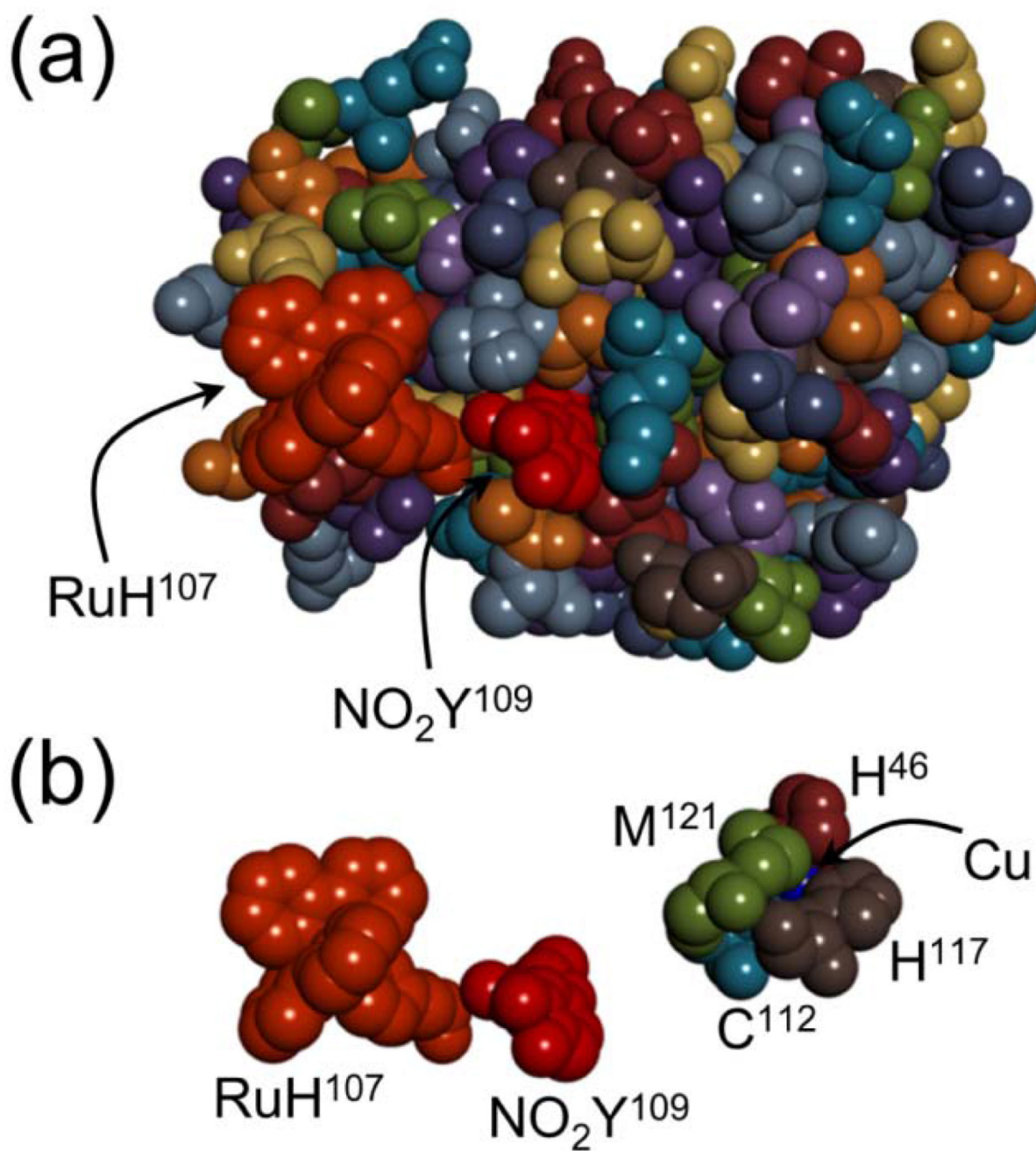


Figure 2.
(a) Space-filling structural model of RuHis¹⁰⁷NO₂TyrOH¹⁰⁹Cu-azurin. (b) Space filling models of the residues comprising the hole hopping pathway from Cu to RuHis¹⁰⁷.

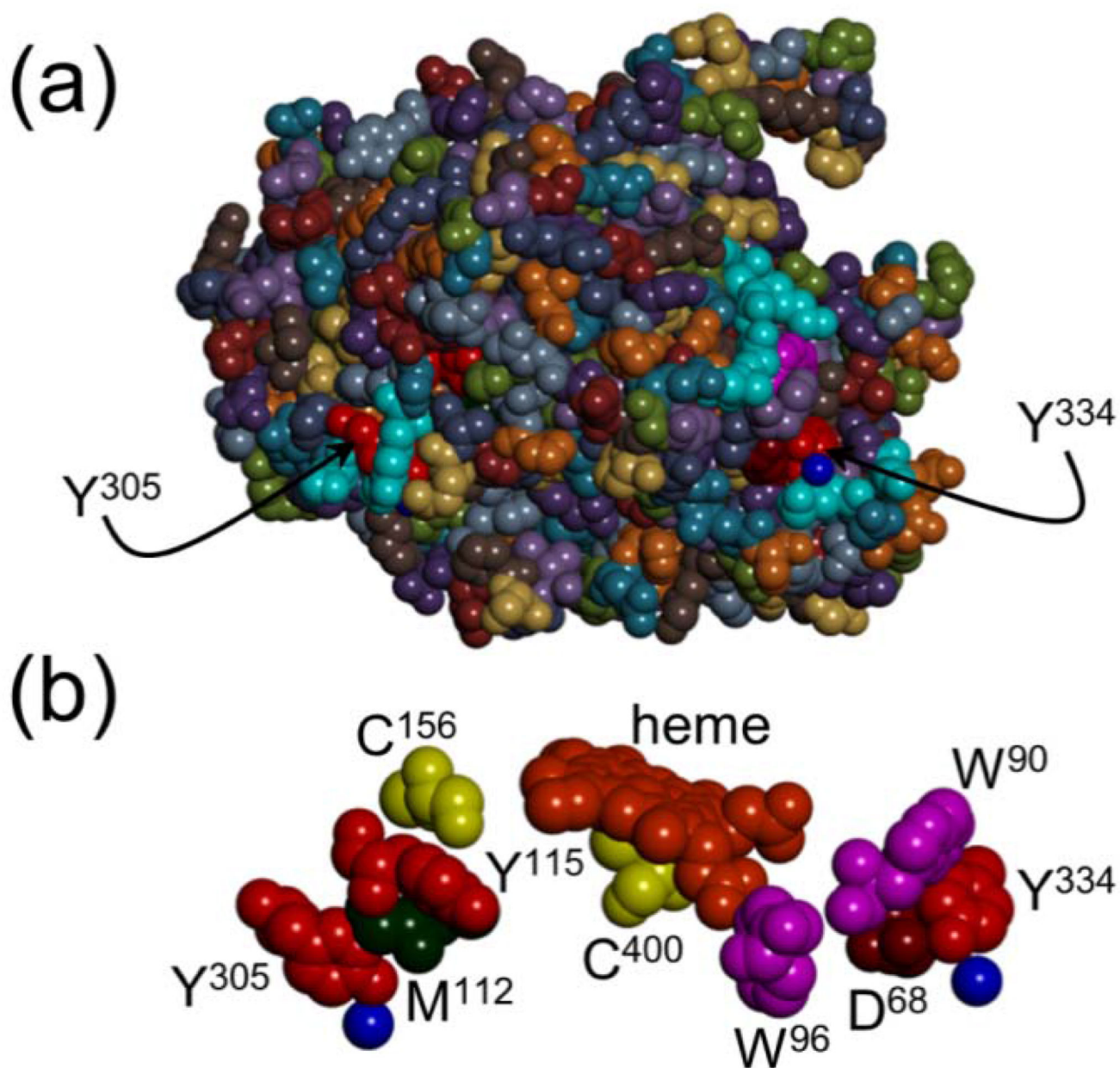


Figure 3.
(a) Space-filling structural model of the heme domain of CYP102A1 (PDB #2IJ2) highlighting the surface locations of terminal residues in pathways I (Tyr³³⁴) and II (Tyr³⁰⁵).
(b) Space-filling model of the residues comprising CYP102A1 radical transfer pathways I and II. Blue spheres represent structurally resolved water molecules.

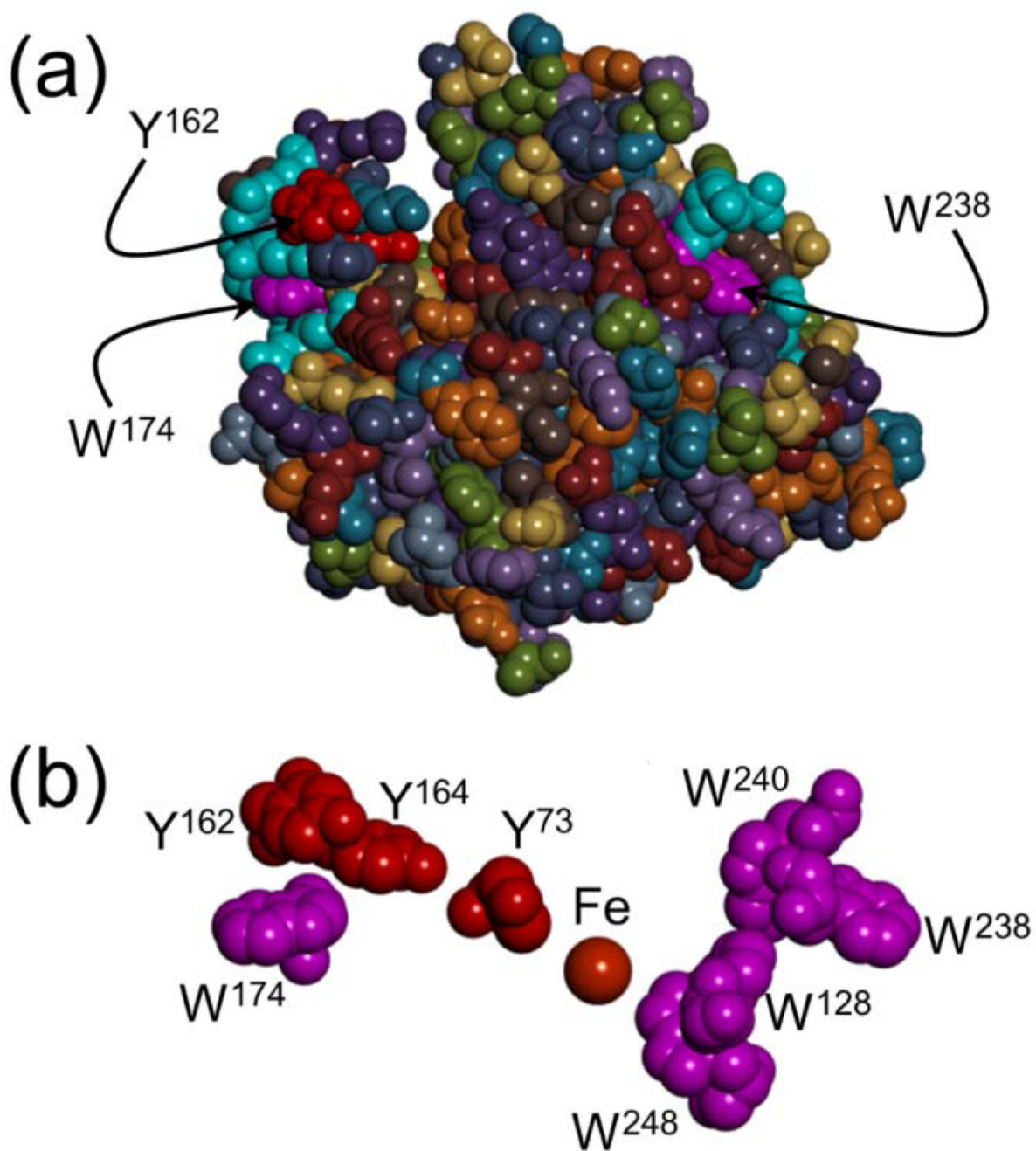


Figure 4. (a) Space-filling structural model of *E. coli* TauD (PDB #1OS7) highlighting the surface locations of terminal residues in postulated radical transfer pathways (Trp²³⁸, Trp¹⁷⁴, Tyr¹⁶²). (b) Space-filling model of the residues comprising TauD radical transfer pathways.

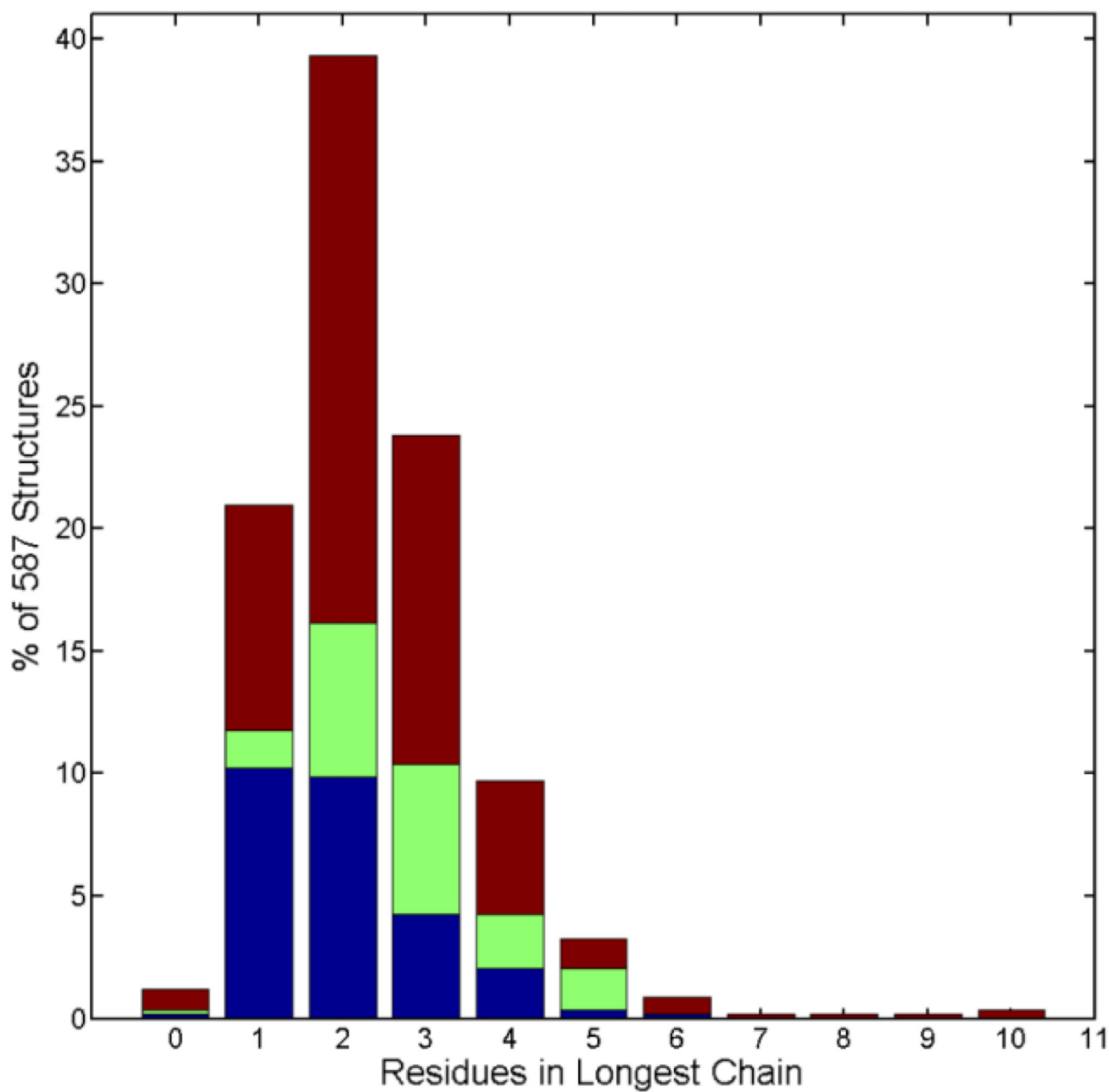


Figure 5. Distribution of radical transfer chain lengths among structurally characterized oxidoreductases from enzyme sub-classes EC 1.11 (peroxidases, blue), 1.13 (oxygenases, green) and 1.14 (dioxygenases, red). Radical transfer chains are defined to be composed of Tyr, Trp, heme, Fe, and Cu residues. Tyr residues were included only if a carboxylate (Asp, Glu) oxygen atom, an imidazole (His) nitrogen atom, or a water molecule was within 4 Å of the Tyr hydroxyl oxygen atom.