Supporting Information.

Preparation of complexes. $[Re(CO)_3(L)Cl]$ (Wrighton *et al.*, *J. Am. Chem. Soc.* 1974, *96*, 998-1003) and $[Re(CO)_3(L)(H_2O)]Otf$ (Sullivan *et al.*, *J. Chem. Soc., Chem. Commun.* 1984, 1244-1245) (L = phen or 4,7-Me_2phen) were prepared according to literature procedures.
The weakly luminescent $[Re(CO)_3(L)(H_2O)]^+$ species persist indefinitely in aqueous solution, and crystallize with well-defined stoichiometries; $[Re(CO)_3(phen)(H_2O)]Otf{\cdot}H_2O$ has been characterized by X-ray crystallography (Connick *et al.*, *Acta Cryst.* 1999, C55, 913-916).

Preparation and purification of azurin. (HisX)Az (X= 83, 107; Az =Azurin) was expressed (Piccioli *et al.*, *Inorg. Chem.* 1995, *34*, 737-742; Langen *et al.*, *Science* 1995, *268*, 1733-1735) and purified (Faham *et al.*, *Acta Cryst.* 1999, *D55*, 379-385) as previously described. (HisX)AzZn^{2+} was prepared by adding Zn^{2+} (*aq*) to cell extracts or by copper removal from $[Re(CO)_3(L)(HisX)]^+AzCu^+$, followed by reconstitution with Zn^{2+} (*aq*) (Blaszak *et al.*, *J. Biol. Chem.* 1983, *258*, 9886-9892).

Modification of azurin. $[Re(CO)_3(L)(HisX)]^+Az$ samples were prepared by *strictly* following the procedure described herein. In a typical synthesis 5-10 mg of (HisX)AzM (M = Cu^+, Cu^{2+}, Zn^{2+}) in < 100 μL (solvent: 25 mM HEPES pH 7.4) were mixed with approximately 1.5 mL of saturated aqueous $[Re(CO)_3(L)(H_2O)](Otf)$. [Note that after dilution the [HEPES] was ~ 1.5 mM and the pH ~7: *$[Re(CO)_3(L)L']$ (L' = Otf, Cl, P_i) neutral complexes are insoluble in water; thus, in general, labeling reactions must be conducted in low ionic strength solutions and in absence of Cl and P_i.*] The mixture was allowed to react for 4 to 7 days at 30-37 °C (the reaction of azurin with $[Re(CO)_3(L)(H_2O)]^+$ at room temperature is much slower). Some rhenium almost always precipitated during the course of the reaction. Labeling reactions were quenched by reducing the reaction mixture volume to ~100 μL, followed by gel filtration on PD-10 columns (Pharmacia); gel filtration columns were equilibrated with 20 mM NaP_i/750 mM NaCl/pH 7.0-7.4 buffer. $[Re(CO)_3(L)(H_2O)]^+$ reacts with protein surface residues other than histidines, and some binds non-covalently. The latter was effectively removed by allowing the protein solution to stand (after gel filtration) for at least 24 h in the high salt buffer (a yellow precipitate of $[Re(CO)_3(L)Cl]$ generally formed). Isolation of $[Re(CO)_3(L)(HisX)]^+AzM$ was achieved by means of two chromatographic steps: (a) separation of histidine-bound plus multiply-labeled rhenium-azurin was achieved by IMAC (Figure 1A, Di Bilio *et al.*, *J. Am. Chem. Soc.* 1998, *120*, 7551-7556); (b) $[Re(CO)_3(L)(HisX)]^+AzCu^{2+}$ was

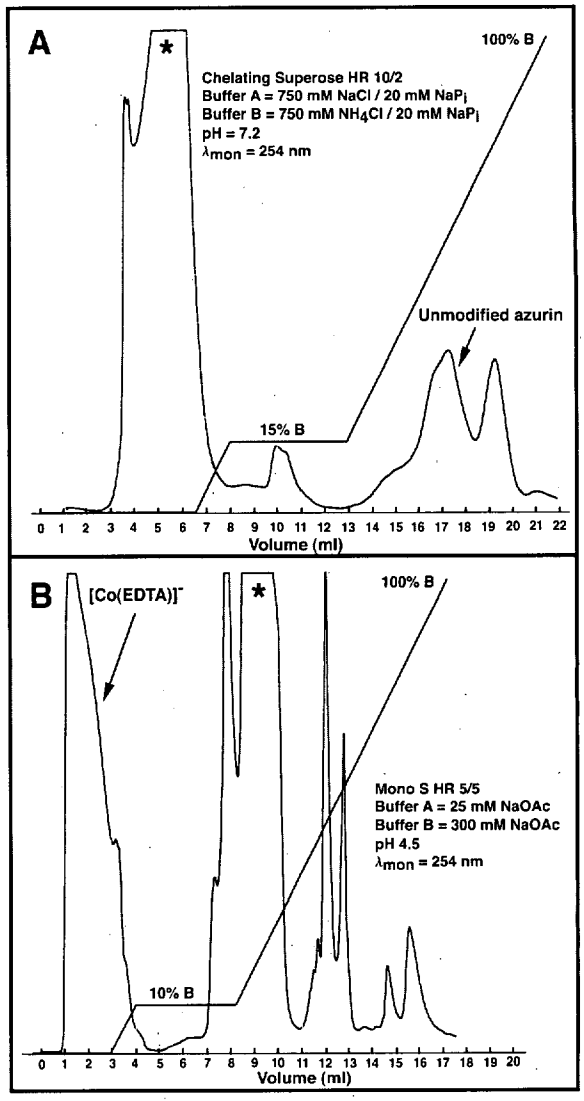

Figure 1. (A) Partial resolution by affinity chromatography of a mixture containing (His107)$AzCu^{2+}$ and Re-modified (His107)$AzCu^{2+}$: fractions 3-8 mL (starred peak) were combined, concentrated, de-salted, oxidized with excess $[Co(EDTA)]^-$, and separated by cation exchange chromatography (B); the starred peak (fractions 8-11 mL), which contained mainly $[Re(His107)]^+AzCu^{2+}$, was run a second time under the same conditions in order to achieve baseline separation (data not shown). Note, however, that some derivatives did not require a second cation exchange step.

isolated by cation exchange chromatography (Figure 1B). Unmodified azurin was recovered, re-purified, and re-used. Single rhenium modification was ascertained by electrospray mass spectrometry ($[Re(CO)_3(phen)(His83)]^+$Az, calc mass (apo form) 14395.32 amu, found 14395.4, 14456.3 (Cu^{2+} form); $[Re(CO)_3(phen)(His83Gln,Gln107His)]^+$Az, calc 14397.35, found 14395.7), and absorption spectroscopy (Figures 2 and 3). All modification procedures were conducted under normal room illumination.

Rhenium-tricarbonyl-diimine binding to histidine is irreversible.

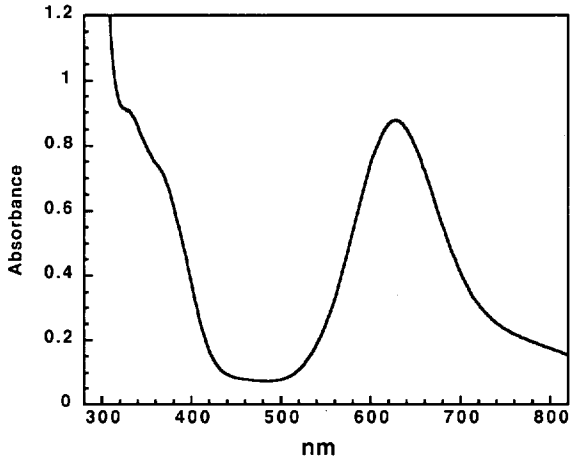

Figure 2. Absorption spectrum of $[Re(CO)_3(phen)(H83)]^+AzCu^{2+}$ in 25 mM acetate, pH 4.5.

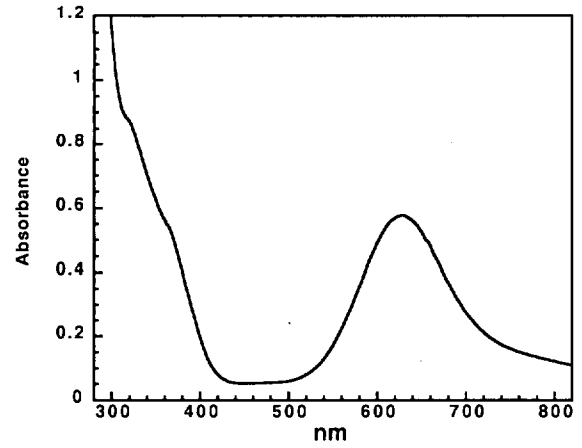

Figure 3. Absorption spectrum of $[Re(CO)_3(4,7\text{-dmphen})(His107)]^+AzCu^{2+}$ in 25 mM acetate , pH 4.5.

$[Re(CO)_3(phen)(im)]_2SO_4{\cdot}4H_2O$ is the model for $[Re(CO)_3(phen)(His)]^+$-proteins (Connick, et al., *Inorg. Chim. Acta* 1995, *240*, 169-173). Electronically excited $[Re(CO)_3(L)(im)]^+$ emits in aqueous solution at room temperature: L, $\lambda_{emission}$(nm), τ(ns); phen, 590, 120; 4,7-Me_2phen, 570, 360. Several $[Re(CO)_3(diimine)(His)]$ complexes have been characterized (Lin *et al.*, *Inorg. Chim. Acta* 1996, *242*, 179-183). The MLCT excited state of $[Re(CO)_3(L)(His)]^+$ is both a good oxidant and reductant ($E^\circ(Re^{+*/0}) \sim 1.4$ and $(Re^{2+/+*}) \sim -0.5$ V vs NHE). *$[Re(CO)_3(L)(HisX)]^+$Az reacts with several electron acceptors, e.g., MV^{2+}, $[Co(NH_3)_5Cl]^{2+}$, and $[Ru(NH_3)_6]^{3+}$.

Simulation of EPR spectra. The inhomogeneously broadened EPR spectra of irradiated $[Re(CO)_3(phen)(HisX)]^+AzZn^{2+}/[Co(NH_3)_5Cl]^{2+}$ (X = 83, 107) were simulated using the program WINEPR *Simfonia* (Version 1.25, Bruker Analytische Messtechnik GmbH); this software is based on the second-order perturbation solution of the standard spin Hamiltonian: $\mathcal{H} = HgS + SAI$. Simulations were performed by trial-and-error. The spin-Hamiltonian parameters estimated by simulation are collected in Tables 1 and 2. The experimental *vs* simulated spectra are shown in Figures 4 and 5. Note that the hyperfine coupling constants could not be assigned based on X-band data alone because of limited spectral resolution.

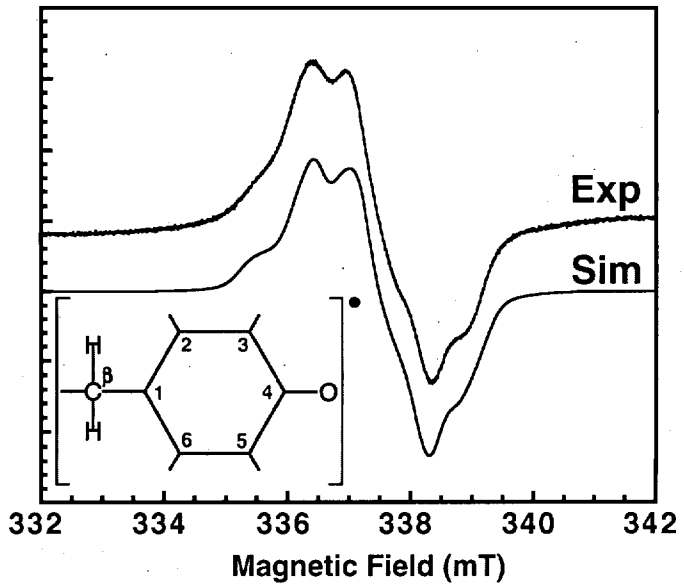

Figure 4. Experimental (upper trace) and simulated (lower trace, Table 1) EPR spectra of Tyr108•. Inset: neutral tyrosyl radical; carbons 1, 3, 5, and the oxygen atom are the sites of higher spin density. The simulation was carried out assuming that the two β protons are inequivalent. A hybrid lineshape 50% lorentzian + 50% gaussian, with an isotropic linewidth parameter of 3.8 gauss, was used in the simulation

	x	y	z
g	2.0080	2.0040	2.0018
A(H)	12.0	7.0	6.5
A(H)	3.0	3.0	3.0
A(2 x H)	7.5	5.0	7.5

Table 1. g tensor and absolute values of the hyperfine coupling constants (gauss) used in the simulation of the EPR spectrum of Tyr108$^{\bullet}$.

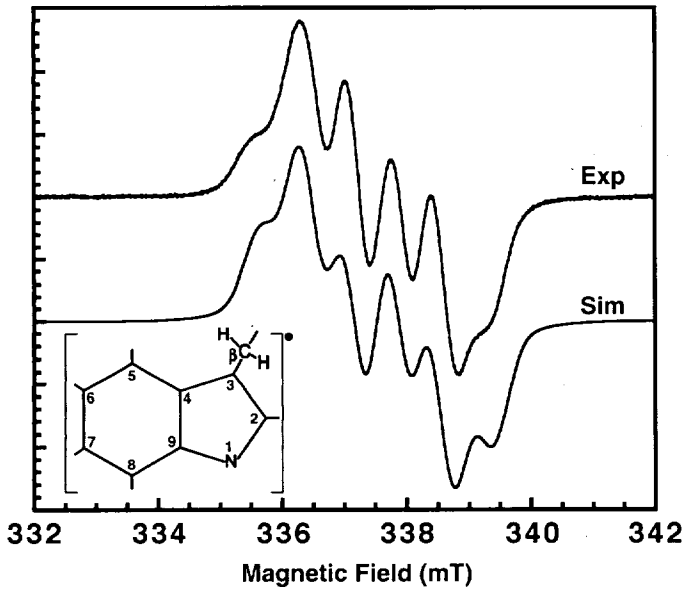

Figure 5. EPR spectrum of irradiated $[Re(CO)_3(phen)(His83)]^{+}AzZn^{2+}/[Co(NH_3)_5Cl]^{2+}$ (upper trace). Simulated spectrum (lower trace, Table 2) assuming a tryptophan radical. Inset: neutral tryptophan radical; carbons 3, 5, 7, and the indolyl nitrogen are the sites of higher spin. The "quartet' has been attributed to the interaction of the two (inequivalent) β protons with the large spin density on C(3) (Lendzian *et al.*, *J. Am. Chem. Soc.* 1996, *118*, 8111-8120. Gräslund *et al.*, *Annu. Rev. Biophys. Biomol.* 1996, *25*, 259-286). A hybrid lineshape 80% lorentzian + 20% gaussian, with an isotropic linewidth parameter of 4 gauss, was used in the simulation.

	x	y	z
g	2.0056	2.0056	2.0045
A(N)	2.0	8.0	2.0
A(H)	15.0	13.0	15.0
A(H)	6.9	7.8	6.9
A(2 x H)	2.0	2.0	2.0

Table 2. g tensor and absolute values of the hyperfine coupling constants (gauss) used in the simulation of the EPR spectrum assigned to Trp48$^{\bullet}$.

For calculations of spin densities and spectral assignments in various Tyr and Trp radicals see: (1) Lendzian, F.; Sahlin, M.; MacMillan, F.; Bittl, R.; Fiege, R.; Pötsch, S.; Sjöberg, B.-M.; Gräslund, A.; Lubitz, W.; Lassmann, G. *J. Am. Chem. Soc.* 1996, *118*, 8111-8120. (2) Gräslund, A.; Sahlin, M. *Annu. Rev. Biophys. Biomol.* 1996, *25*, 259-286.