SUPPLEMENTAL INFORMATION

EPR Fitting and Quantitation

The signal for the ferric species of N694C was simulated with an effective spin S' = 1/2 Hamiltonian (Figures 3B), with the parameters given in Table S1 (see Figure S1 for simulated spectra). Four rhombic doublets (centered at g ~ 4.3) and one axial doublet (g = 6) were required for the simulation.[a] The axial simulation parameters were used previously for WT sLO, while the rhombic simulations are distinct from that published for N694H/Fe^{III} and for N694G/Fe^{III}.[1, 2] It should be noted that for all of the rhombic signal simulations, it was difficult to match the variability of the g-strain of each g-component. Therefore, these simulations are approximations to the true signal shape. Four rhombic doublets were required because one would not adequately fit the broad base of the g ~ 4.3 signal. These four rhombic components are considered as a single species in the following discussion. The simulation areas were adjusted for the g_{ave} differences of the rhombic and axial signals as described by Aasa and Vangård and linewidth broadening was applied to each g-value.[3]

The simulated spectra were integrated over the entire field range to obtain the total EPR intensity from transitions within the middle Kramers doublet for the rhombic signal and the $M_S = \pm ½$ for the axial signal. Fe^{III}-EDTA was used as a standard for spin quantitation. The double integral was evaluated between 500 and 2500 G, just below the interfering signal from adventitious Cu^{II} at g of 2.0. Quantification of the signal intensity was dependent on the relative populations of the spin manifolds of each of the EPR active species. In order to account for the

[a] It should be noted that the feature with $g_{(eff)}$-values of 9.4, 1.45 and 0.9 is ascribed to the ground doublet of the rhombic S=5/2 spin system. Its simulated intensity is too high (Figure 3B) due to the fact that it covers a large field range (past accessible) and is dependent on the assumption that the D value of the rhombic N694C/Fe^{III} complex is the same as EDTA/Fe^{III}.

spin populations of the three M_s doublets, the ZFS parameters were estimated for each species from the temperature dependence of the EPR spectral intensity over the range 3.8 - 40 K under non-saturating conditions. The temperature dependence of the g ~ 4.3 signals were evaluated and their signal intensity versus temperature was approximately the same as Fe^{III}-EDTA (data not shown), indicating a |D| of approximately 1 cm^{-1} for the rhombic signal. This approximate zero-field splitting (ZFS) parameter was used to calculate the Boltzmann population distributions of the three doublets and the spin populations were corrected accordingly for the four signals at g ≈ 4.3. The g ~ 6 signal observed had significant spectral overlap with the other species and therefore its temperature dependence could not be obtained accurately from the EPR spectral data. Its D-value was set to +2 cm^{-1}, based on the fact that in WT sLO-1/Fe^{III} two axial signals are observed with D values of approximately 2 cm^{-1}.

1. Holman, T. R.; Zhou, J.; Solomon, E. I., *J. Am. Chem. Soc.* **1998,** 120, 12564-12572.
2. Segraves, E. N.; Chruszcz, M.; Neidig, M. L.; Ruddat, V.; Zhou, J.; Wecksler, A. T.; Minor, W.; Solomon, E. I.; Holman, T. R., *Biochemistry* **2006,** 45, 10233-10242.
3. Aasa, R.; Vangard, T., *J. Magn. Reson.* **1975,** 19, 308-315.

Table S1. Theoretical EPR simulation parameters

Component	E/D	g_x (Δg)[a]	g_y (Δg)[a]	g_z (Δg)[a]	Contribution (%)
Sim Rhombic 1	0.33	4.3 (0.05)	4.3 (0.05)	4.3 (0.05)	80 ± 8[b]
Sim Rhombic 2	0.31	4.4 (0.05)	4.3 (0.1)	4.1 (0.1)	
Sim Rhombic 3	0.29	4.5 (0.05)	4.2 (0.2)	4.0 (0.2)	
Sim Rhombic 4	0.29	4.5 (0.1)	4.2 (0.5)	4.0 (0.5)	
Sim Axial	0.0	6.0 (0.5)	6.0 (0.8)	2.0 (0.8)	20 ± 2

[a]Linewidths in g-values at half-height of the absorption curve are given in parentheses.
[b]Combined contribution of all four rhombic components.

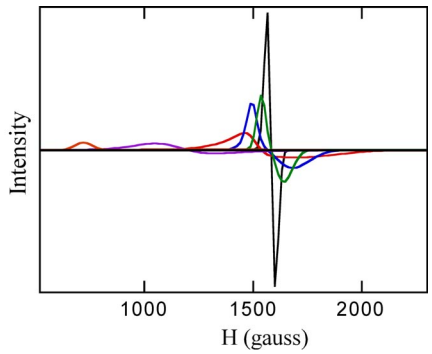

Figure S1. EPR Simulation Components for ferric N694C: sim rhombic 1 (black), sim rhombic 2 (red), sim rhombic 3 (blue), sim rhombic 4 (green), sim axial (purple) and the feature ascribed to the ground doublet of the rhombic spin system (orange).

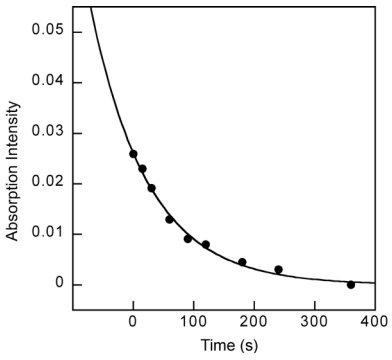

Figure S2. LA oxidation by N694C/Fe^{III}. Decay of the Cys→Fe^{III} CT band monitored by the decrease in absorption at 778 nm.

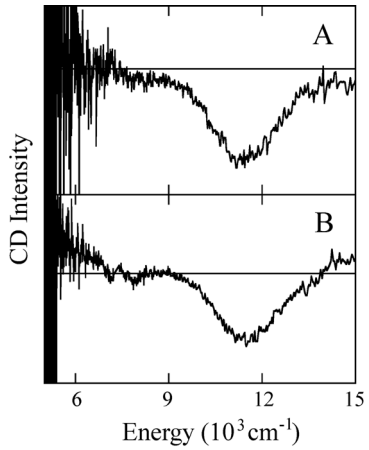

Figure S3. The 278 K CD spectrum of (A) N694C/Fe^{II} + 60% (v/v) glycerol and (B) N694C/Fe^{II} + oleic acid (saturating).