Supporting information

An engineered glutamate in biosynthetic models of heme-copper oxidases drives complete product selectivity by tuning the hydrogen bonding network

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Tables

Table S1. Oxygen affinities of myoglobin mutants

Protein	k_{on} (μM^{-1} s^{-1})	k_{off} (s^{-1})	K_d (k_{off}/k_{on}; μM)
wtMb[1]	8.8	8.3	0.94
Cu$_B$Mb	0.041 ± 0.002	9 ± 1	220 ± 30
I107E-Cu$_B$Mb	0.19 ± 0.01	4 ± 4	20 ± 20
F33Y-Cu$_B$Mb[2]	0.021 ± 0.002	14 ± 1	700 ± 80
I107E-F33Y-Cu$_B$Mb	0.19 ± 0.01	26 ± 4	140 ± 20

Table S2. Data collection and refinement statistics for oxy-F33Y Cu_BMb structure.

Sample	I107E Cu_BMb (7KYR)	I107E F33Y Cu_BMb (7L3U)
Data Collection		
Source	NSLS x29	APS 21-ID-G
Date	2014-08-23	2013-06-04
Detector	ADSC Q315r-906	MAR/Rayonix MX300 CCD
Wavelength (Å)	1.075	0.9786
Data Reduction		
Software	HKL2000 v2.3.8 (Linux)	HKL2000 v2.3.1 (Linux)
Spacegroup	$P2_1$	$P2_12_12_1$
Unit cell (a x b x c; Å)	34.02 x 31.72 x 71.28	39.67 x 47.87 x 77. 88
(β; °)	102.4	--
Resolution range (highest shell)	50-1.70 (1.76-1.70)	50-1.48 (1.51-1.47)
Overall B-factor, ML (Å^2)	23.28	18.84
Overall B-factor, Wilson (Å^2)	25.02	20.18
Unique reflection	16343 (1600)	25784 (1268)
Completeness (%)	99.8 (98.5)	99.8 (99.9)
Redundancy	7.6 (5.8)	3.8 (3.7)
$<I/\sigma(I)>$	11.5	12.0
CC½	(0.843)	--
$R_{\text{merge/symm}}$	0.073 (0.635)	0.047 (0.534)
R_{pim}	0.025 (0.285)	--
$R_{\text{meas/rim}}$	0.077 (0.698)	--
Refinement		
Software	Phenix v1. 19rc7_4070 (Win)	Phenix v1.19rc7_4070 (Win)
Resolution range (highest shell)	34.82-1.708 (1.735-1.708)	20.48-1.467 (1.499-1.469)
Reflections	31359 (1171)	48282 (2646)
Completeness (%)	99.1 (83.3)	98.84 (86.53)
Free set (%; random)	10	4.74
R_{work}	0.166 (0.271)	0.201 (0.318)
R_{free}	0.210 (0.331)	0.234 (0.397)
Molecules in ASU	1	1
# of Atoms		
Protein	1234	1300
Heme	43	43
Solvent	128	145
Other	47	--
Average Isotropic B-factor	32.61	27.79
Protein	31.44	26.89
Heme	27.03	25.22
Solvent	42.11	36.62
Other	59.30	--
Standard Uncertainty (ML)	0.19	0.20
Bond RMSD (Å)	0.014	0.015
Angle RMSD (Å)	0.92	1.921
Ramachandran favored/disallowed (%)	98/0.00	96/0.00

Table S3. Data collection and refinement statistics for oxy-F33Y $\mathrm{Cu_B}$Mb structure.

Sample	Oxy-I107E-$\mathrm{Cu_B}$Mb (7L3Y)
Data Collection	
Source	SSRL 11-1
Date	2015-07-20
Detector	DECTRIS Pilatus 6M
Wavelength (Å)	0.98
Data Reduction	
Software	XDS v2015-03-01 Pointless v1.9.33 Aimless v0.5.12 Truncate v6.5.013 (Linux)
Spacegroup	$P2_12_12_1$
Unit cell (a x b x c; Å)	39.84 x 46.98 x 78.03
Resolution range (highest shell)	39-1.18 (1.20-1.18)
Overall B-factor, ML (Å^2)	7.19
Overall B-factor, Wilson (Å^2)	11.18
Unique reflection	47352 (1510)
Completeness (%)	97.0 (63.4)
Redundancy	3.9 (2.1)
$<I/\sigma(I)>$	31.7 (4.1)
CC½	(0.902)
$R_{merge/symm}$	0.024 (0.216)
R_{pim}	0.013 (0.169)
$R_{meas/rim}$	0.028 (0.275)
Refinement	
Software	Phenix v1.19rc7_4070 (Win)
Resolution range (highest shell)	39.02-1.18 (1.19-1.18)
Reflections	86347 (1303)
Completeness (%)	92.81 (40.7)
Free set (%; random)	4.77
R_{work}	0.1566 (0.2408)
R_{free}	0.1902 (0.3196)
Molecules in ASU	1
# of Atoms	
Protein	1282
Heme + Oxygen	45
Solvent	299
Average Isotropic B-factor	17.26
Protein	15.24
Heme + Oxygen	10.53
Solvent	26.96
Standard Uncertainty (ML)	0.11
Bond RMSD (Å)	0.013
Angle RMSD (Å)	1.397
Ramachandran favored/disallowed (%)	98/0.00

Figures

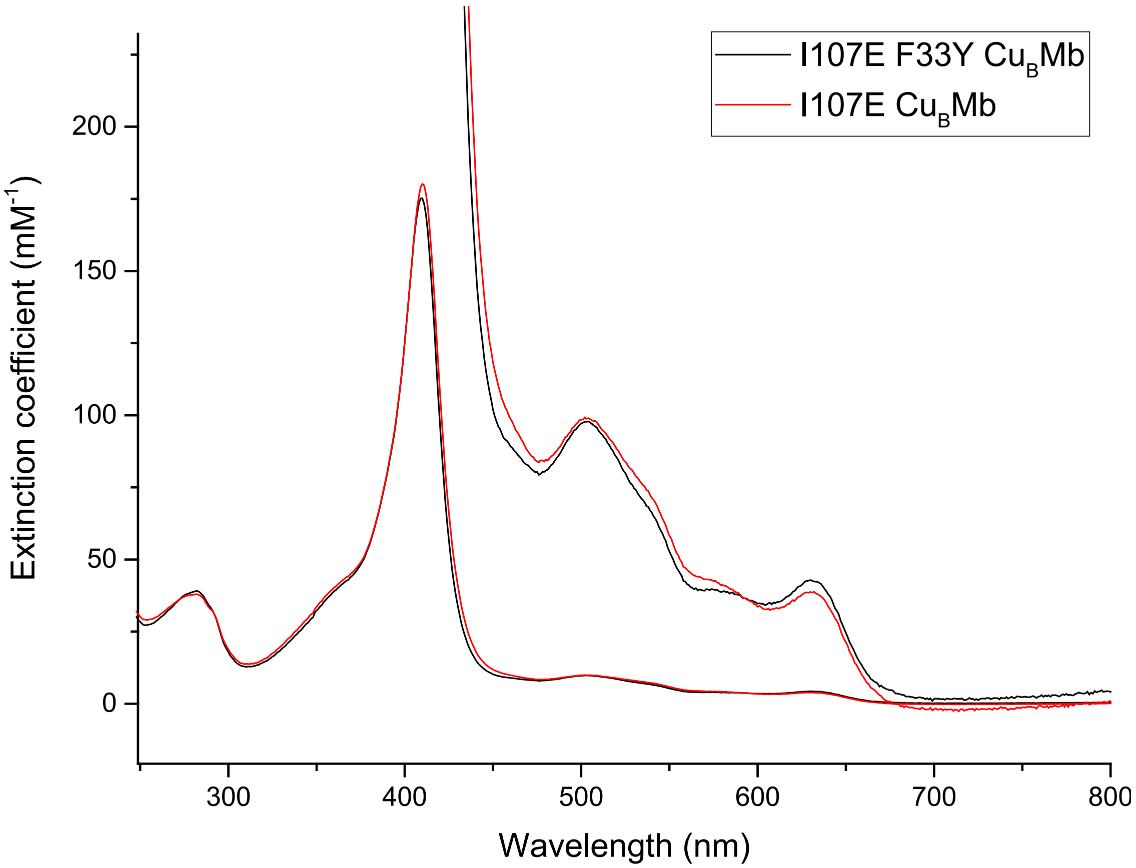

Figure S1. Resting state spectra of I107E-Cu_BMb and I107E/F33Y-Cu_BMb.

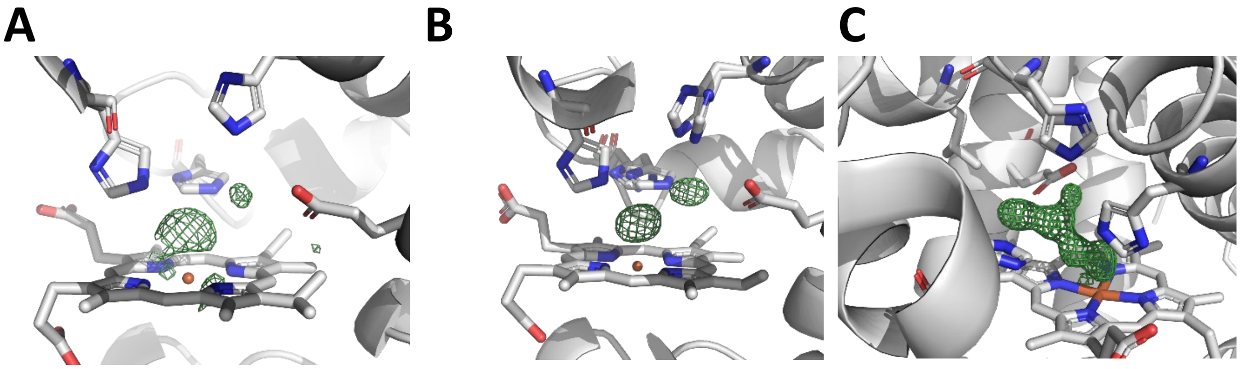

Figure S2. Omit maps of the distal pocket with the contents of the distal pocket omitted: (A) resting state I107E Cu_BMb contoured at 4-sigma; (B) resting state I107E F33Y Cu_BMb contoured at 4-sigma; (C) oxy-I107E Cu_BMb contoured at 3-sigma.

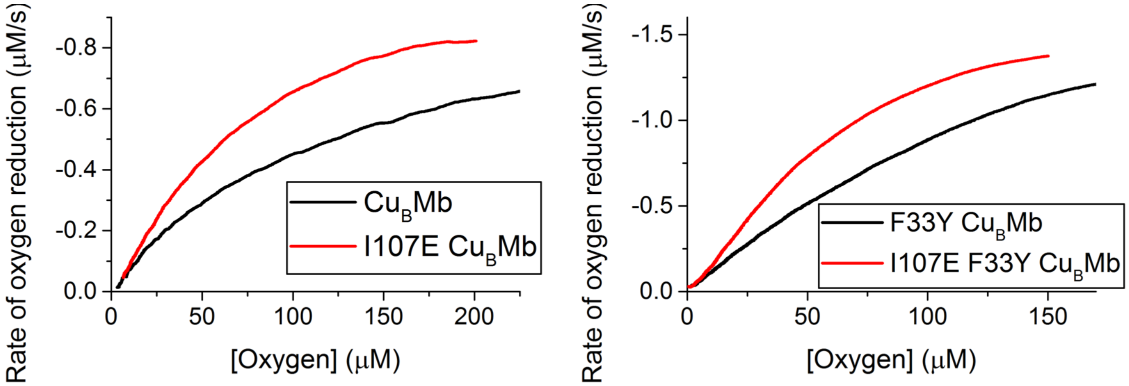

Figure S3. Oxygen consumption rate curves as a function of oxygen concentration.

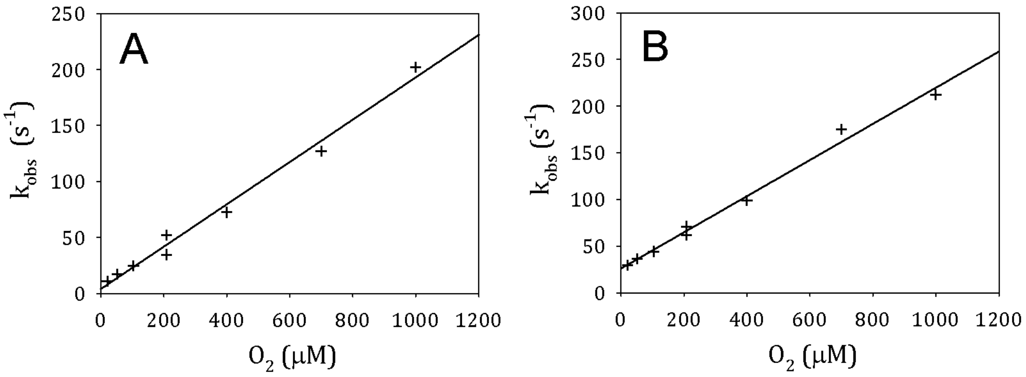

Figure S4. The rate constants for oxygen binding as a function of oxygen concentration for I107E-Cu$_B$Mb (A) and I107/F33Y-Cu$_B$Mb (B). The fitted lines were used to calculate the oxygen affinities (K_d) in Table S1.

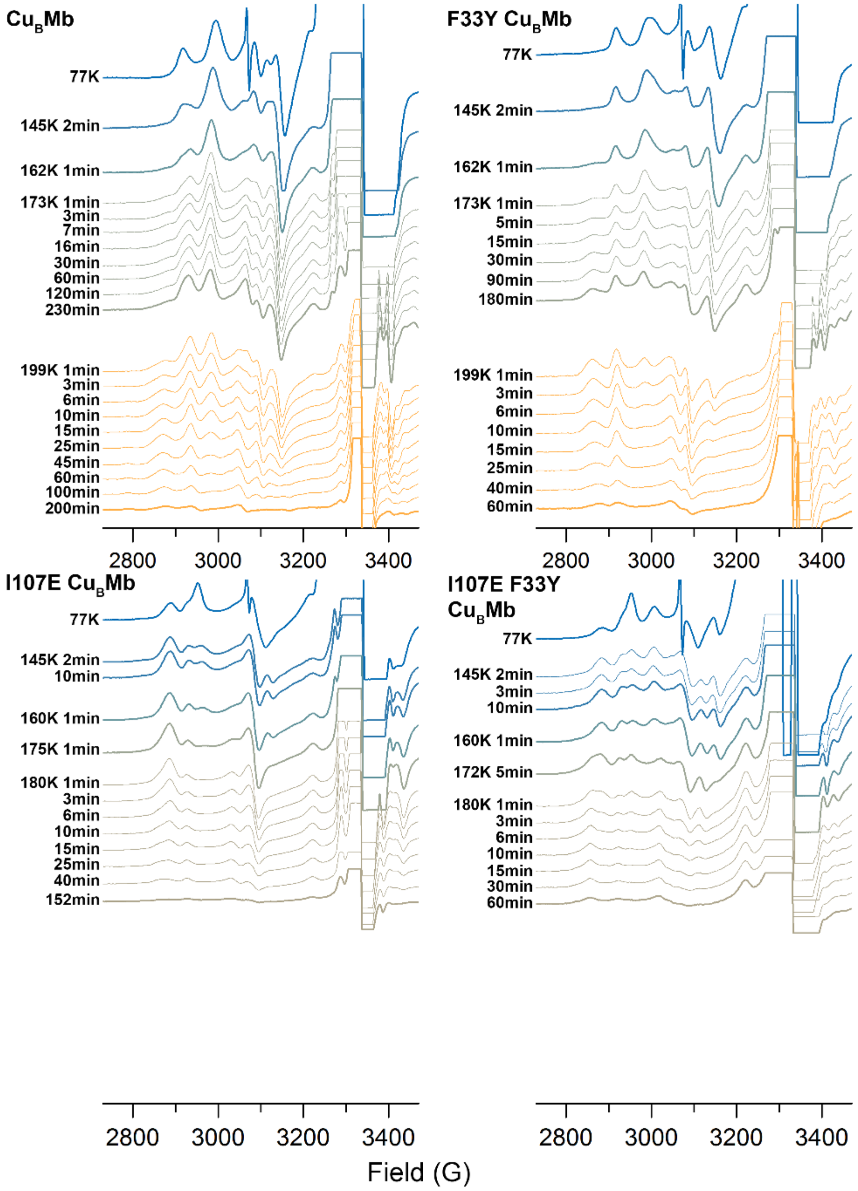

Figure S5. Full EPR spectral sets of annealing experiments

Figure S6. EPR spectrum of oxy-I107E/F33Y-Cu_BMb after cryoreduction-annealing-cryoreduction, showing ferric hydroxo signal.

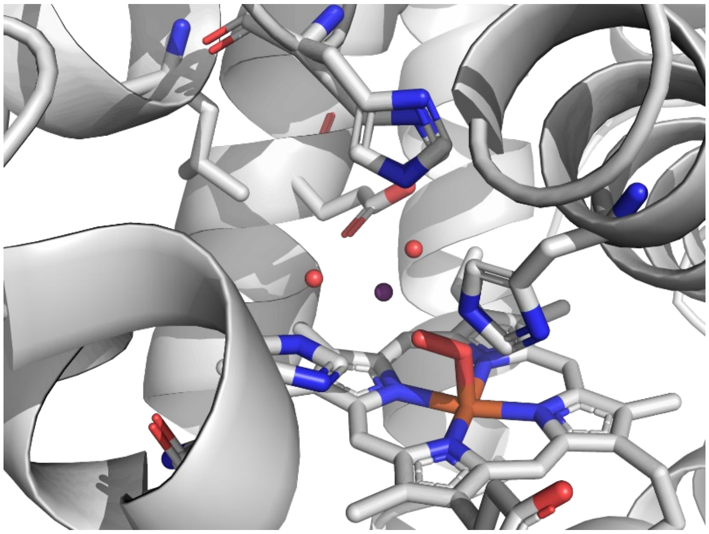

Figure S7. View of both configurations of the active site, with (red waters) and without (purple water) O_2 bound. Both configurations refine to ~50% occupancy, with B-factors of these atoms in the range of 10-15, similar to the surrounding protein.

References

(1) Carver, T. E.; Brantley, R. E.; Singleton, E. W.; Arduini, R. M.; Quillin, M. L.; Phillips, G. N.; Olson, J. S. A Novel Site-Directed Mutant of Myoglobin with an Unusually High O2 Affinity and Low Autooxidation Rate. *J. Biol. Chem.* **1992**, *267* (20), 14443–14450.

(2) Bhagi-Damodaran, A.; Kahle, M.; Shi, Y.; Zhang, Y.; Ädelroth, P.; Lu, Y. Insights Into How Heme Reduction Potentials Modulate Enzymatic Activities of a Myoglobin-Based Functional Oxidase. *Angew. Chem. Int. Ed.* **2017**, *56* (23), 6622–6626. https://doi.org/10.1002/anie.201701916.