Supporting information

Redesigning the Blue Copper Azurin into a Redox-active Mononuclear Nonheme Iron Protein: Preparation and Study of Fe(II)-M121E Azurin

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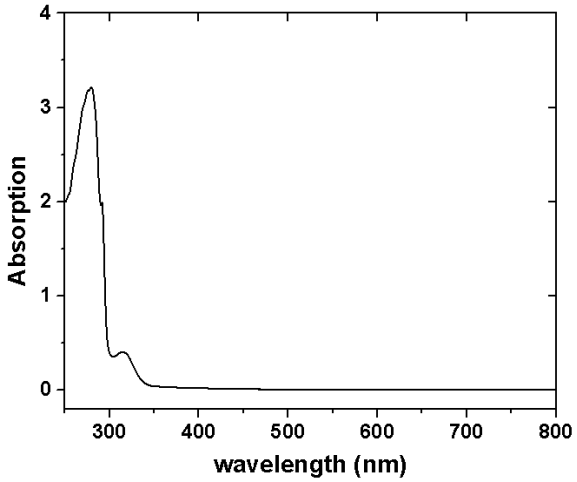

Figure S1. UV-Vis absorption spectra of Fe(II)-M121EAz monitored by Cary 5000.

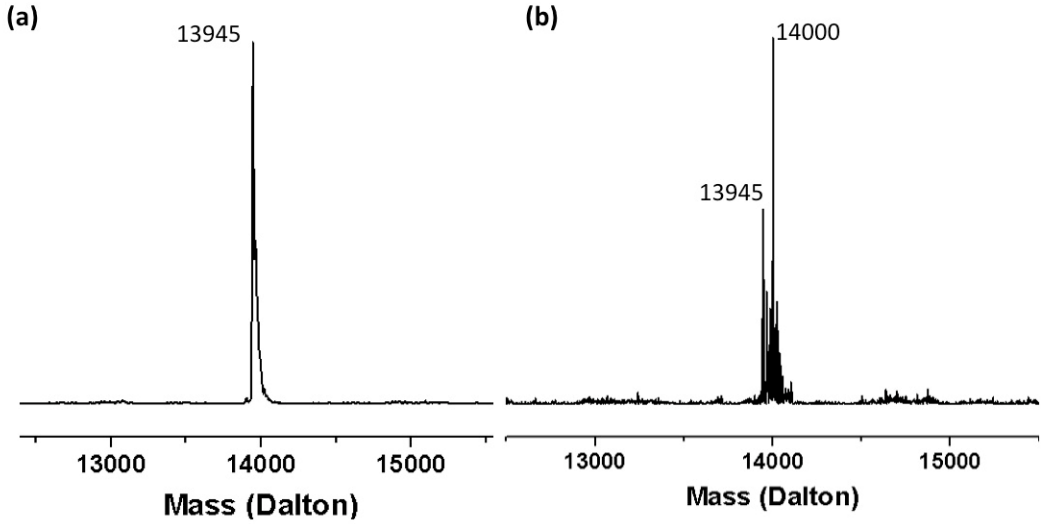

Figure S2. (a) ESI MS spectrum of apo M121EAz. (Calculated mass: 13943.7 Da); (b) Syringe-pump ESI MS spectrum of Fe(II)-M121EAz (Calculated mass: 13999.6 Da);

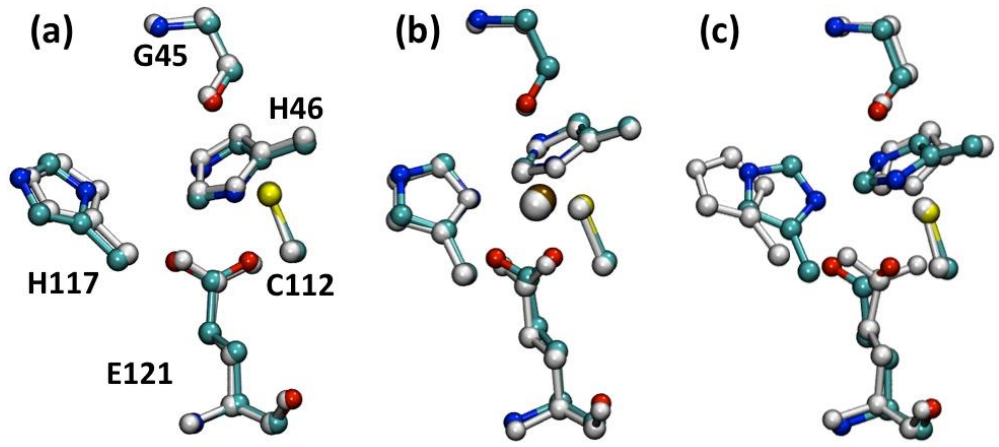

Figure S3. Subunit structure comparison of Fe(II)-M121EAz. Resolution: 2.0 Å. (a) Chain A (white) and chain C (colored). (b) Chain D (white) and chain B (colored). (c) Chain A (white) and chain B (colored).

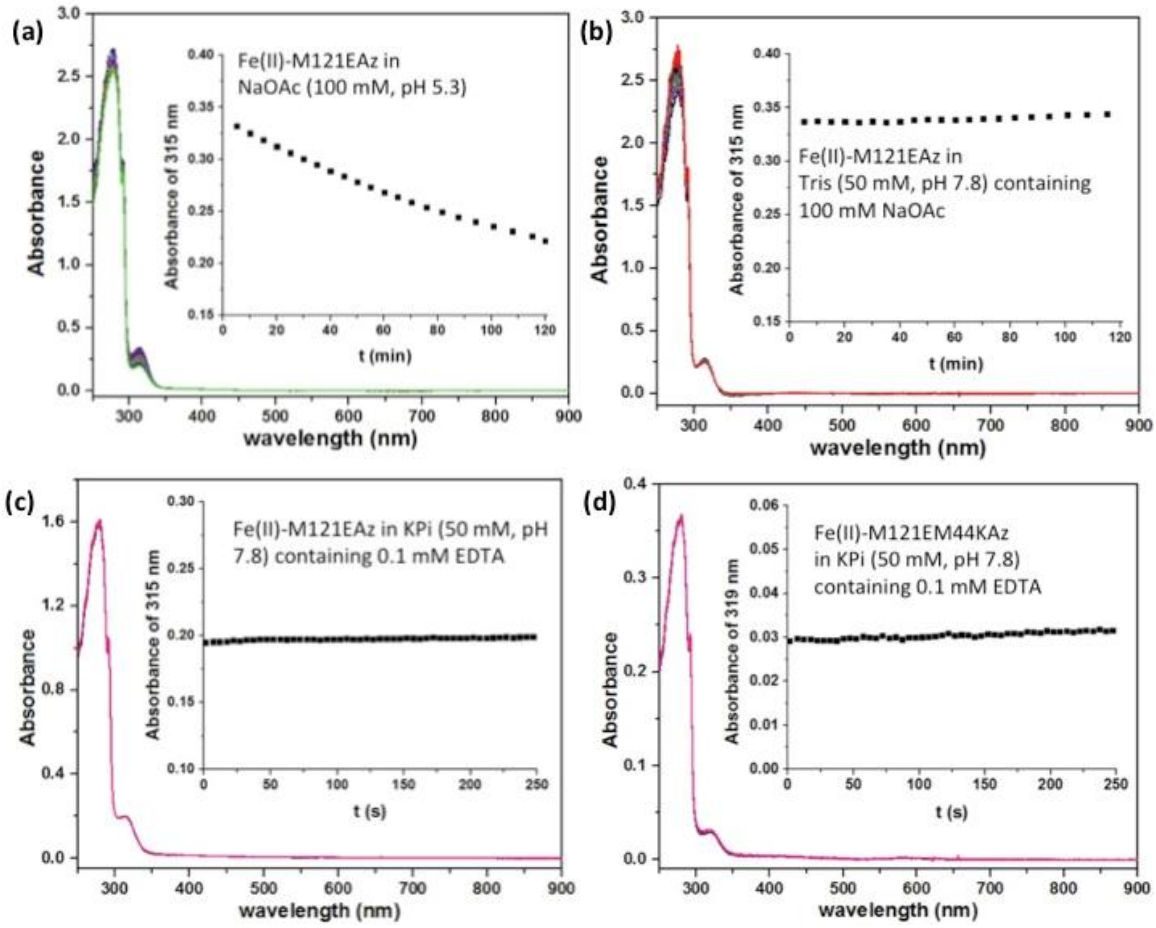

Figure S4. Stability test of Fe(II)-M121EAz and Fe(II)-M121EM44KAz.

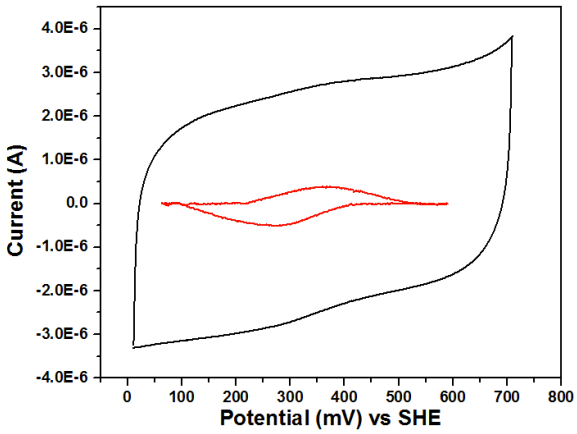

Figure. S5. Cyclic voltammogram of Fe-M121EAz at pH 7.0 on a pyrolytic graphite edge (PGE) electrode.

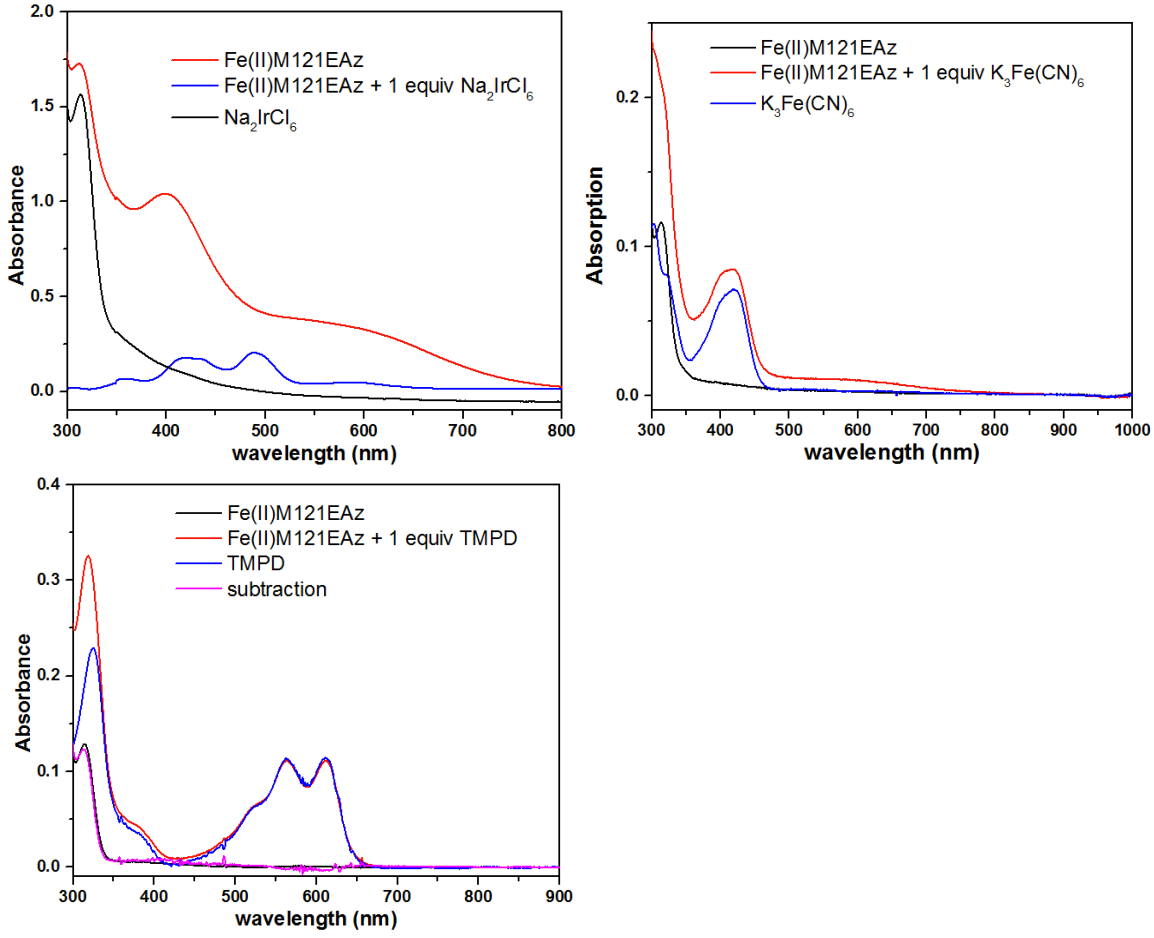

Figure. S6. Reaction mixture (red line) of Fe(II)-M121EAz (black line) with Na_2IrCl_6, $K_3Fe(CN)_6$ or TMPD (blue line in each graph) in MES (50 mM, pH 5.5) at room temperature.

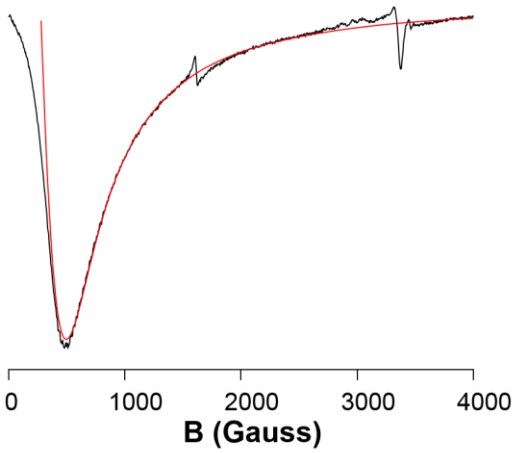

Figure S7. Perpendicular mode X-band EPR spectrum of Fe(II)-M121EAz recorded at 11.2 K. The black curve is the experimental spectrum, and the red curve is a simulation generated using the software SpinCount[1] for D = -3.47 cm^{-1} and E/D = 0.177; the parameter E/D was assumed to have a Gaussian distribution with $\sigma_{E/D}$ = 0.002 Conditions: microwave power; 2 mW, microwave frequency; 9.663 GHz, modulation amplitude; 1 mT.

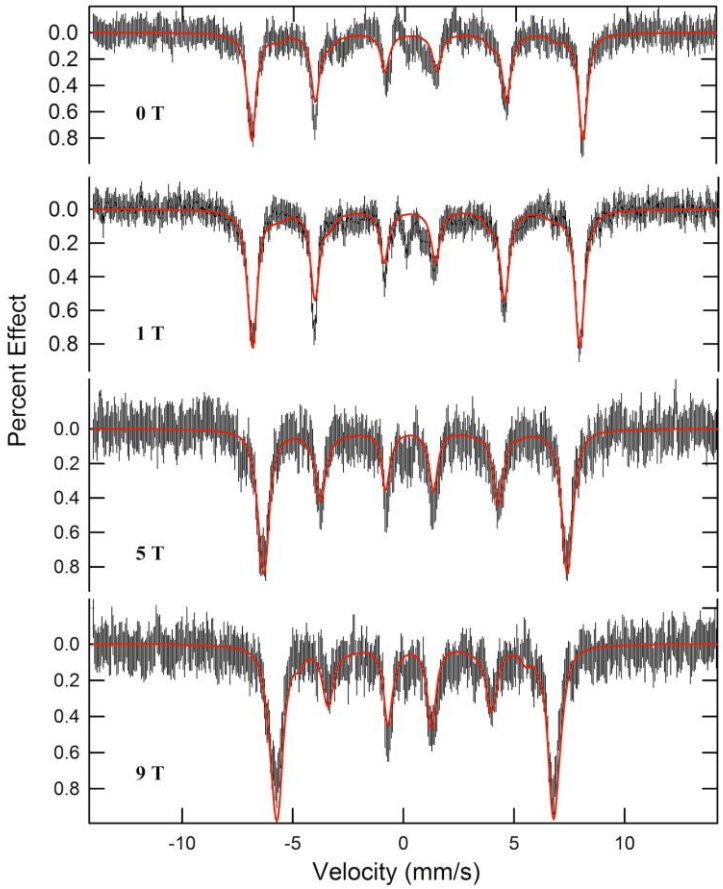

Figure S8. Variable field Mössbauer spectra of Fe(III)-M121EAz recorded at 4.2 K at fields indicated. The black hash-marked curves are the experimental spectra. Simulations, using the parameters of Table 1 in the main text, are shown as the solid red curves.

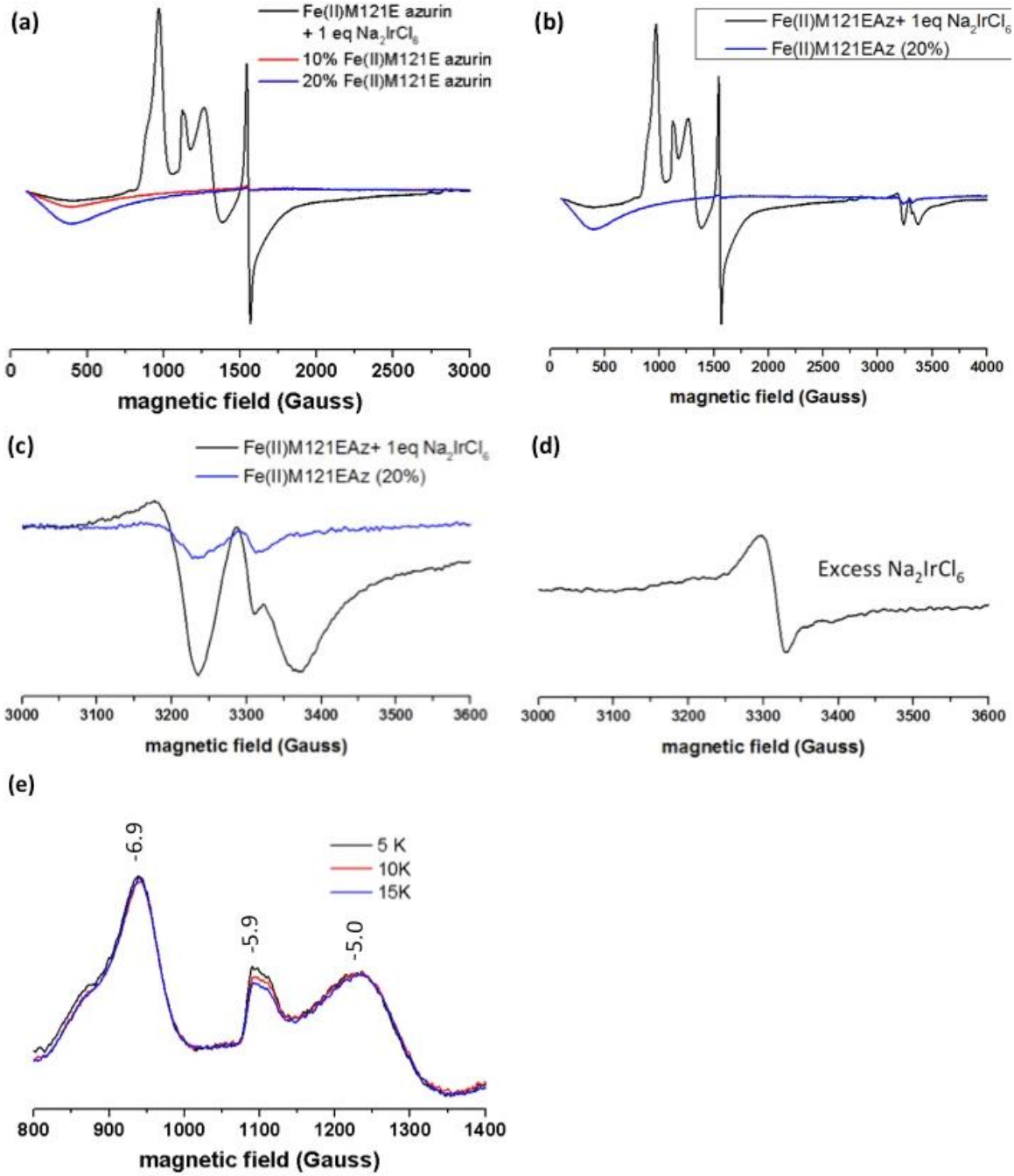

Figure S9. (a) Comparison of EPR spectra in MES (50 mM, pH 5.5) containing 20 % glycerol. *Black line*: reaction mixture of 4.2 mM Fe(II)-M121EAz with 1 equiv of Na_2IrCl_6 without PD-10 column.; *red line*: Fe(II)-M121EAz (0.42 mM); *blue line*: Fe(II)-M121EAz (0.85 mM). (Shapes of blue and red spectra were used to subtract Fe(II)M121EAz from raw data and estimate the amount of remaining ferrous protein after oxidation). (b) Full spectra of Fe(II)-M121EAz with 1 equiv of Na_2IrCl_6 in comparison to Fe(II)M121EAz. (c) g = 2 region of Fe(II)-M121EAz with 1 equiv of Na_2IrCl_6 in comparison to Fe(II)-M121EAz and (d) g = 2 region of Na_2IrCl_6. **(**e) EPR spectra of the reaction mixture of Fe(II)-M121EAz with 1 equiv of Na_2IrCl_6 after PD-10 column in MES (50 mM, pH 5.5) containing 20 % glycerol. Spectra collected from 5 K to 15 K, after subtraction of ferrous signal and rescaling. The change of the 5.9 signals indicates a negative D value.[2] Parameters: microwave power: 4 mW, microwave frequency: 9.21 GHz; modulation amplitude: 10 G.

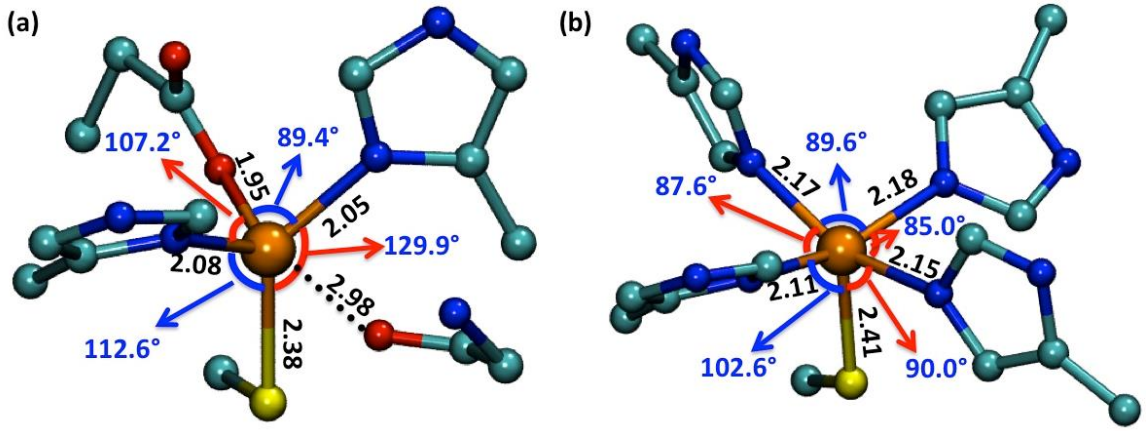

Figure S10. Structural comparison of (a) chain B of Fe(II)-M121EAz and (b) superoxide reductase (PDB ID: 2JI1). Distances (in Å) are shown in black fonts, and angles are shown in blue fonts. Color code: C, cyan; S, yellow; N, blue; O, red; Fe, orange.

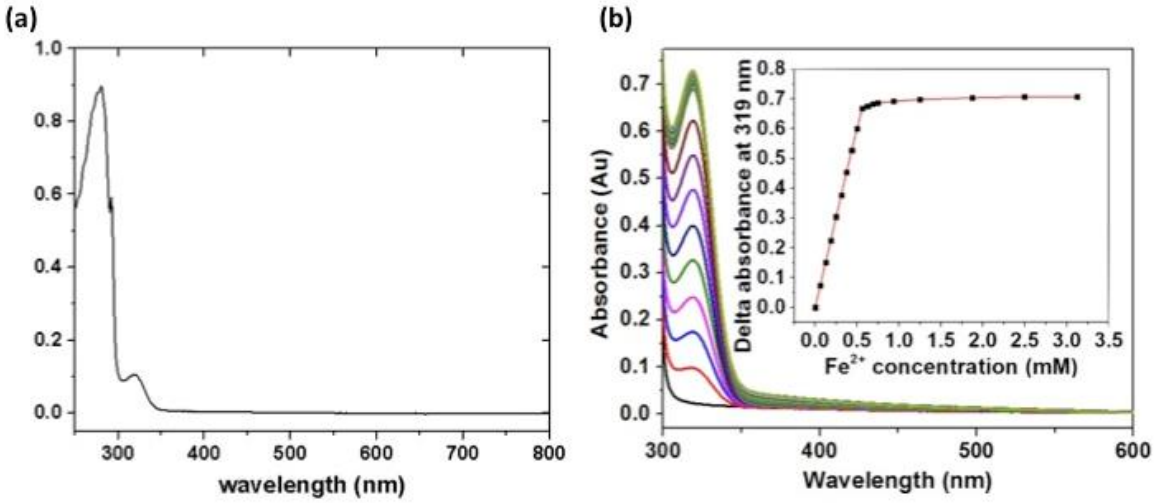

Figure S11. (a) UV-Vis absorption of Fe(II)-M121EM44KAz spectrum. (b) Development of absorbance at 319 nm after successive addition of 0.1-5 equiv of Fe^{2+} to apo-M121EM44KAz. (Inset) Titration curve of the absorbance maxima at 319 nm.

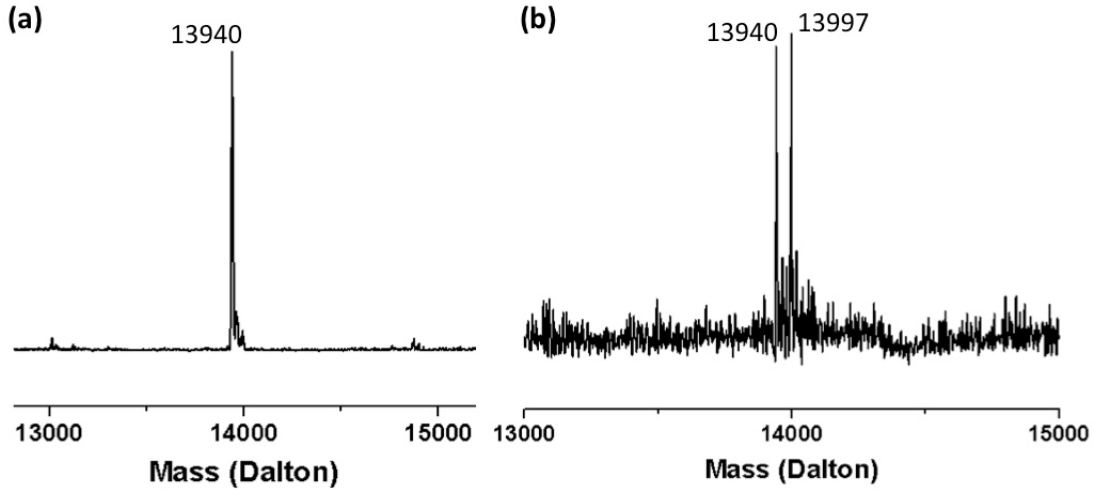

Figure S12. (a) ESI MS spectrum of apo-M121EM44KAz (calculated mass: 13940.7 Da); (b)Syringe-pump ESI MS spectrum of Fe(II)-M121EM44KAz (calculated mass: 13996.6 Da).

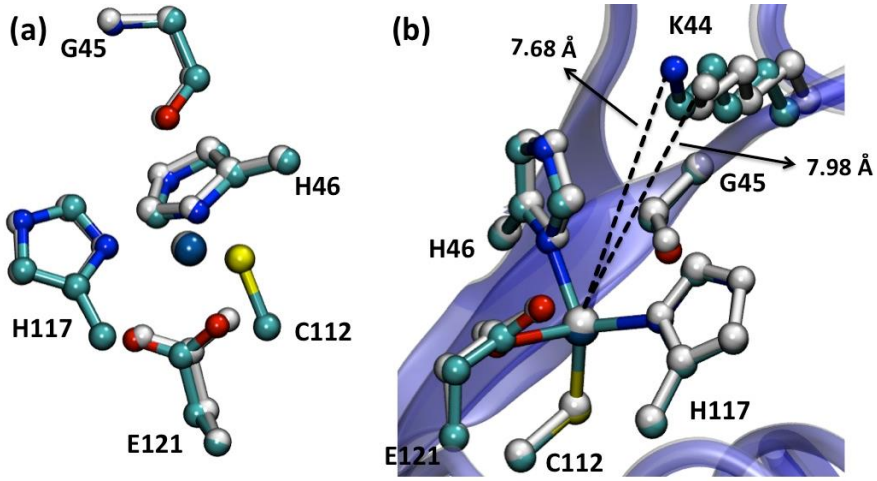

Figure S13. Subunit structure comparison of Cu(II)-M121EM44KAz. Resolution: 1.64 Å. (a) metal binding sites of Chain A (white) and chain B (colored). (b) K44 position in Chain A (white) and chain B (colored). Color code: C, cyan; S, yellow; N, blue; O, red; Cu, gray blue.

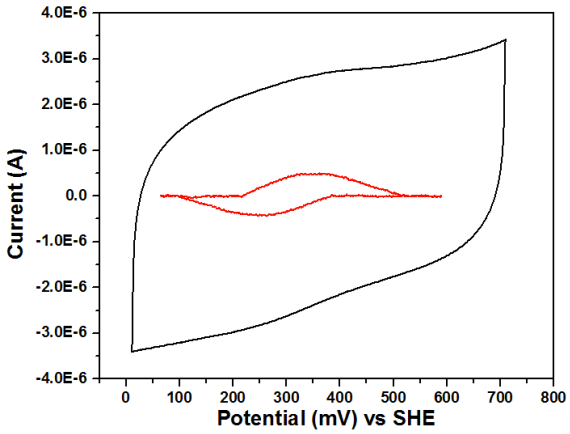

Figure. S14. Cyclic voltammogram of Fe(II)-M121EM44KAz at pH 7.0 on a pyrolytic graphite edge (PGE) electrode.

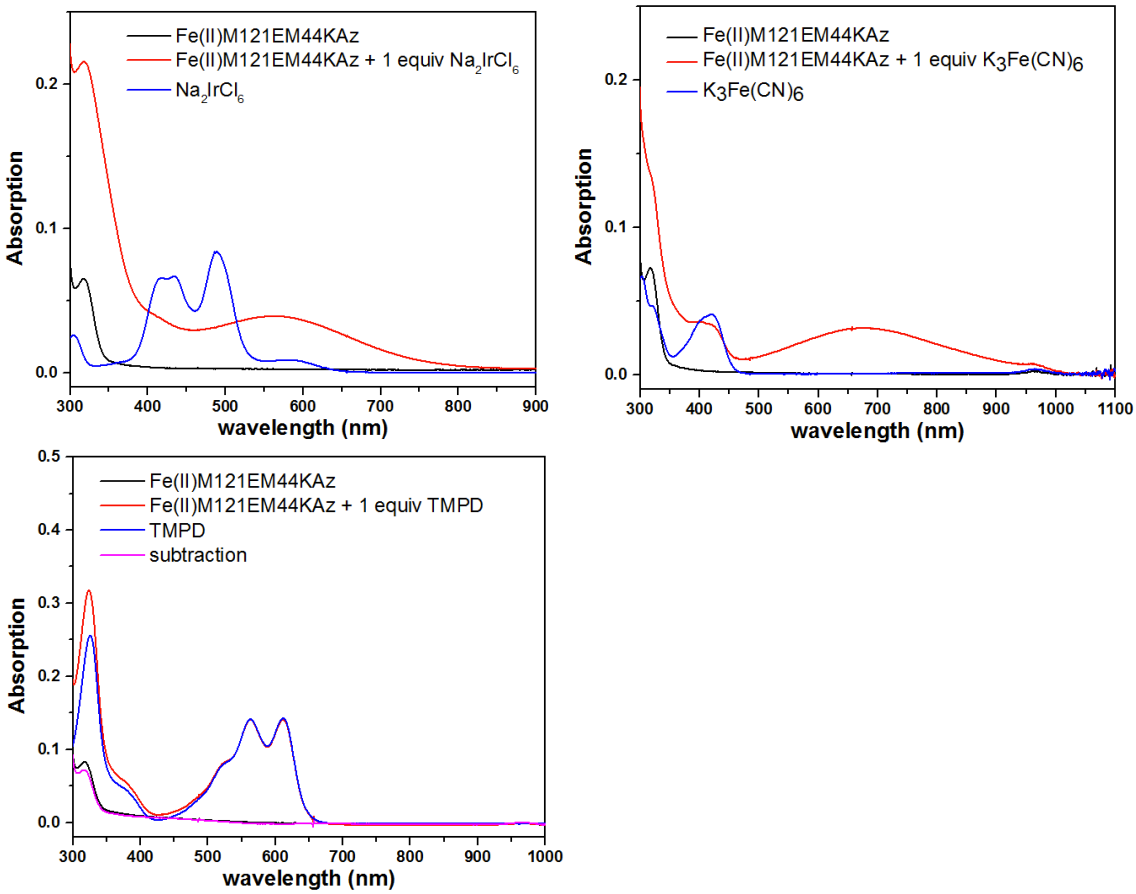

Figure. S15. Reaction mixture (red line) of Fe(II)-M121EM44KAz (black line) with Na_2IrCl_6, $K_3Fe(CN)_6$ or TMPD (blue line in each graph) in MES (50 mM, pH 5.5) at room temperature.

Table S1. Inductively Coupled Plasma (ICP) analysis of metals in Fe(II)-M121EAz*

Element	Found	Protein incorporation ratio
Cu	0.21ppm	0.09%
Fe	140.7ppm	70%
Zn	1.43ppm	0.62

* ε_{280nm}=8800 $M^{-1}cm^{-1}$ was used to determine the protein concentration.[3]

* Measured by inductively coupled plasma mass spectrometry (ICP-MS) using PerkinElmer-SCIEX ELAN DRCe ICP-MS.

Table S2. Crystallographic parameters for Fe(II)-M121EAz.[4]

Data Collection Statistics	
SpaceGroup	$P2_12_12_1$
Unit Cell	a = 56.853, b = 80.208, c = 109.04 α = 90°, β = 90°, γ = 90°
Wavelength (Å)	1.0750
Resolution (Å)	2.0
R_{sym} (%)	19.1
<I/σI>	13.3
Completeness (%)	99.6
Redundancy	13.2
Refinement Statistics	
Resolution (Å)	2.00 (46.38-2.00)
R-Factor (%)	20.46
R_{free} (%)	26.19
Protein atoms	3905
Water Molecules	373
Fe	2
Unique Reflections	32211
R.m.s.d.	
Bonds (Å)	0.0176
Angles (°)	1.9399

Table S3. Crystallographic parameters for Cu(II)-M121EM44KAz.[4]

Data Collection Statistics	
SpaceGroup	C121
Unit Cell	a = 55.285, b = 48.695, c = 95.068 α = 90°, β = 94.65°, γ = 90°
Wavelength (Å)	1.0750
Resolution (Å)	1.64
R_{sym} (%)	8.5
<I/σI>	14.7
Completeness (%)	98.4

Redundancy	13.2
Refinement Statistics	
Resolution (Å)	1.64 (47.38-1.64)
R-Factor (%)	18.12
R_{free} (%)	21.56
Protein atoms	1910
Water Molecules	205
Cu	6
Unique Reflections	30508
R.m.s.d.	
Bonds (Å)	0.007
Angles (˚)	0.98

References:

(1) Golombek, A. P.; Hendrich, M. P. *J. Magn. Reson.* **2003**, *165*, 33.

(2) Clay, M. D.; Jenney, F. E., Jr.; Hagedoorn, P. L.; George, G. N.; Adams, M. W. W.; Johnson, M. K. *J. Am. Chem. Soc.* **2002**, *124*, 788.

(3) Tennent, D. L.; McMillin, D. R. *J. Am. Chem. Soc.* **1979**, *101*, 2307.

(4) Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G. W.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S. *Acta Crystallogr., Sect. D Biol. Crystallogr.* **2011**, *D67*, 235.