Supporting Information

De Novo Design of a Single Chain Diphenylporphyrin Metalloprotein

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Figure S1. **Analytical Ultracentrigfugation**. A 300 µL sample of 150 µM PA_{SC} with 300 µM FeDPP(III) in 50 mM phosphate buffer, 100 mM NaCl at pH 7.5 was heated to 70 °C for 15 minutes, and allowed to equilibrate at room temperature, and centrifuged for 10 minutes at 13,200 rpm in an Eppendorf 5415D microcentrifuge to remove insoluble material. An aliquot of 110 µL was used for sedimentation equilibrium experiments that were performed at 25 °C using a Beckman XL-I analytical ultracentrifuge. The absorbance was monitored at 280 nm, and the sample was centrifuged at 25,000, 35,000, and 45,000 rpm. The data were analyzed using a modified global fitting routine in IGOR Pro (Wavemetric, Inc.). The data were well described by assuming a single molecular weight species. The protein maintains its calculated partial specific volume when the monomeric molecular weight is held constant. The protein sedimented as a single molecular species with a computed molecular weight of 14,150 D, which was in excellent agreement with that computed for the monomer, 13,932 D. The partial specific volume of 0.7137 was calculated using SEDNTERP (2).

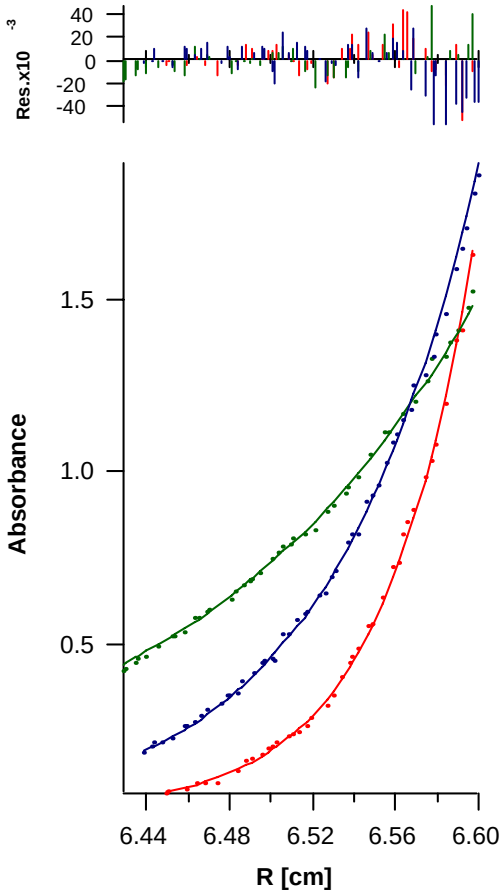


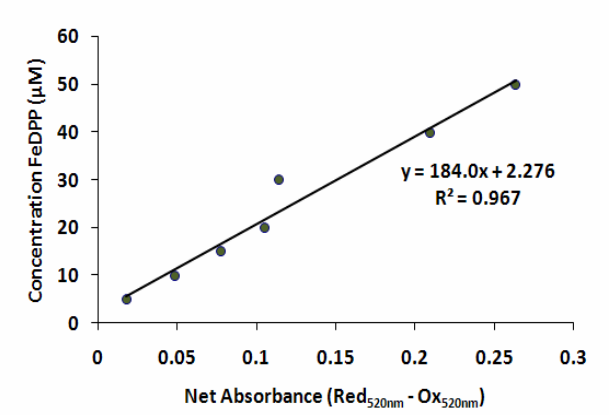
Figure S2. **Binding Stoichiometry of Fe-DPP in Cofactor/Protein Complex (Hemochrome Method).** To accurately determine the porphyrin/protein ratios in the monomeric peak fraction from SEC, we used the hemochrome assay to determine the cofactor concentration, and an HPLC assay to determine the protein concentration (1).

(a) **Quantifying the Porphyrin (Standard Curve).** A standard curve was obtained using 5 to 50 µM solutions of FeDPP(III) in DMSO, diluted with 300 µL 40% v/v pyridine in 200 mM NaOH and, finally, brought up to a final volume of 600 µL with water. The standards were fully oxidized with 25 µL 50 mM potassium ferricyanide and subsequently reduced with 4 mg solid sodium dithionite. The λ_{max} for Q-band oxidized was 565 nm, λ_{max} Q-band reduced was 520 nm. The $\Delta\varepsilon$ ($\varepsilon_{reduced} - \varepsilon_{oxidized}$ at 520 nm) was 1.84 10^{-4} $M^{-1}cm^{-1}$. The absorbance was obtained using a Cary 300 Bio UV-visible spectrophotometer.

(b) **Quantifying Porphyrin and Protein (SEC samples).** The isolated monomeric complex from size exclusion chromatography was concentrated to 300 µL using Amicon Ultra-4 10,000 MWCO centrifuge membrane filter (Millipore). A 150 µL aliquot of the concentrated sample was added to 25 µL potassium ferricyanide, 125 µL 50 mM phosphate buffer and 100 mM NaCL, and 300 µL 40% v/v pyridine in 200 mM NaOH. It was subsequently reduced with 4 mg solid sodium dithionite, equilibrated for 10 minutes, and the absorbances of the oxidized and reduced spectra at 520 nm were recorded (Cary 300 Bio UV-visible spectrophotometer).

The second aliquot of the SEC complex was used to determine the amount of protein present. The FeDPP(III) cofactor was removed from the complex by adding 2 µL trifluoroacetic acid to 100 µL of sample, and filtering through a 0.22 µm PVDF membrane centrifuge filter (Millipore) so that only protein remained. The protein concentration was measured by reverse-phase HPLC at 220 nm using an Agilent 1100 Series Liquid Chromatograph with a Vydac C_4 4.6 mm x 250 mm column. The protein eluted from a linear gradient of acetonitrile in water with 0.1% TFA. A standard curve was generated from samples containing 10 to 500 µM protein

(a)



(b)

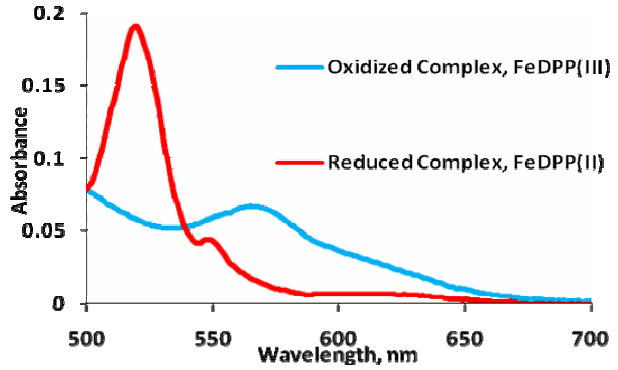
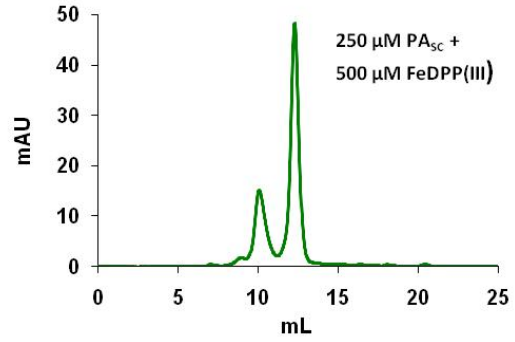


Figure S3. **Size Exclusion Chromatography and Stoichiometry**.

(a) **Size exclusion profile** for 250 µM PA_{SC} with 500 µM DPP-Fe(III). 100 µL of the sample was injected onto a Superdex 75 10/300GL column, flow rate 0.5mL/min, with a mobile phase of 50 mM phosphate buffer with 100 mM NaCl, pH 7.5 (GE Healthcare Akta FPLC System). The monomeric species eluted at 12.7 mL, yielding MW_{app} of 18,500 D. MW_{app} was calculated from a standard curve done with mass standards blue dextran, V_o, aprotinin, 6,500 D, cytochrome C, 12,400 D, carbonic anhydrase, 29,000 D, and albumin, 66,000 D.

(b) **Binding Stoichiometry.** Binding stoichiometry was determined to be 2:1, as there was no significant increase in absorbance at 410 nm with excess cofactor. Binding stoichiometry was determined using the monomeric species isolated through SEC. By holding the protein concentration fixed, various cofactor concentrations were evaluated using UV-visible spectroscopy to monitor the formation of the Soret band at 410 nm.

(a)



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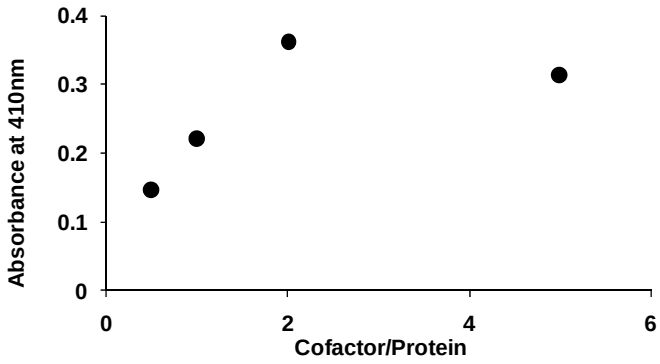
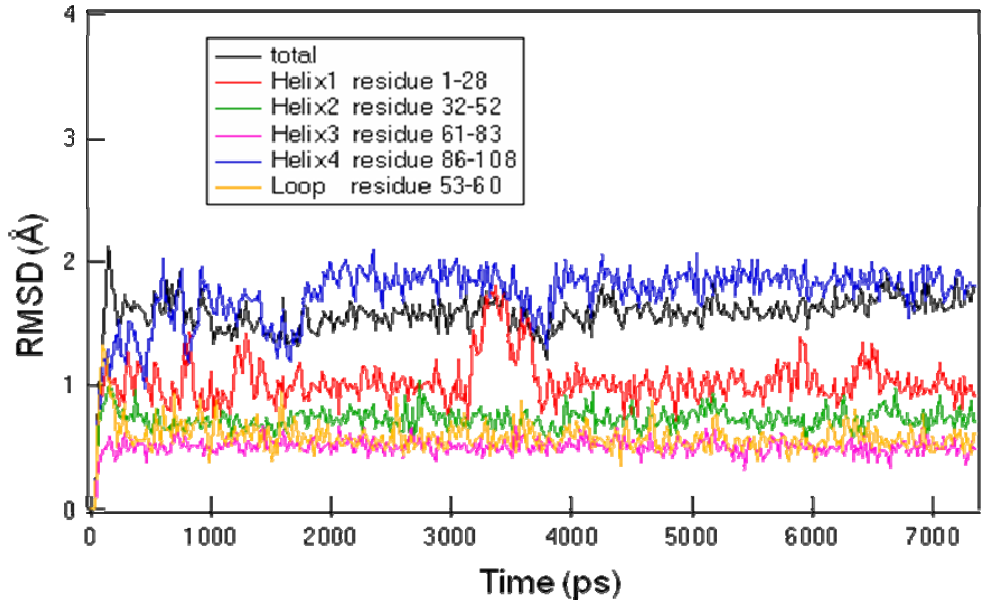


Figure S4. MD Simulations. The simulations were performed on two different models: wild type PA_{SC} with (a) FeDPP(III) and (b) PA_{SC}-ALA with FeDPP(III). The individual helix RMSD versus time is represented here for each model.

(a)



(b)

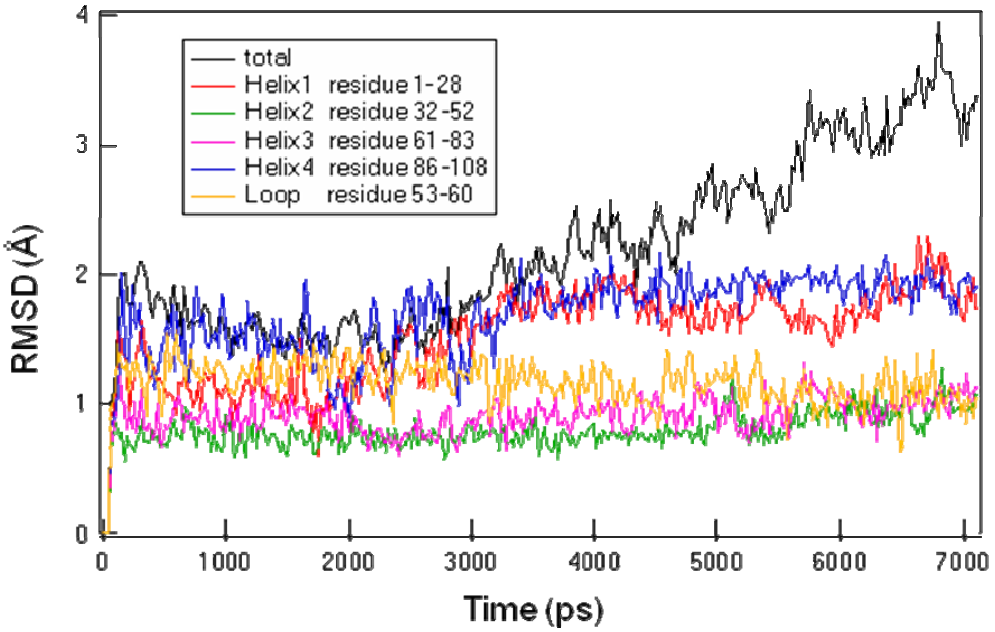


Table S1. Stability of Inter-helical Turns and Loops. The mean and standard deviation of the φ/ψ angles for PA_{SC}/FeDPP(III) loops and turns after the simulation. The values for PA_{SC}/FeDPP(III) were calculated after it reached equilibrium (averaged over the last 4 ns of the 7 ns trajectory). The right hand columns indicate the φ/ψ angles for the starting model (before equilibrium), PA_{SC}/FeDPP(III). The loop includes residues 53-60, and the two turns include residues 29-31 and residues 82-84, respectively.

Residue	Φ-equilibrium	ψ-equilibrium	Φ-model	ψ-model
K29	-70.7 ± 11.5	-48.4 ± 16.2	-69.9	-3.6
G30	131.5 ± 50.9	6.5 ± 40.2	56.6	22.7
D31	-94.7 ± 20.0	93.9 ± 21.2	-102.2	89.7
M53	-65.6 ± 10.8	158.4 ± 10.6	-68.9	166.3
N54	-105.1.6 ± 11.8	117.5 ± 9.1	-115.5	80.1
P55	-77.2 ± 8.8	-8.3 ± 10.8	-51.9	-32.7
Q56	-86.9 ± 11.2	-32.7 ± 12.8	-69.4	-20.3
A57	-71.7 ± 12.6	-35.4 ± 15.6	-98.2	-6.4
I58	-76.7 ± 13.6	151.9 ± 14.0	-87.7	133.0
S59	-92.7 ± 15.5	177.3 ± 8.9	-94.6	170.6
E60	-63.3 ± 10.5	-40.1 ± 10.3	-53.2	-37.7
K82	-72.2 ± 10.9	-46.1 ± 17.5	-69.9	-3.7
G83	105.1 ± 19.1	51.5 ± 19.4	56.7	22.6

D84	-111.2 ± 21.4	116.2 ± 19.9	-102.1	89.7

References

(1) Berry, E.A.; Trumpower, B.L.: Simultaneous Determination of Hemes *a, b* and *c* from Pyridine Hemochrome Spectra. Analytical Biochemistry 161 (1987) 1 – 15.

(2) Laue, T.M., Shah, B.D., Ridgeway, S.L., Pelletier, S.L.: Computer-aided interpretation of analytical sedimentation data for proteins in *Analytical Ultracentrifugation in Biochemistry and Polymer Science*. (Eds: S.E. Harding, A.J. Rowe, J.C. Horton), Cambridge Royal Society of Chemistry (1992) 90 – 125.