

SUPPORTING INFORMATION: Figures summarizing biophysical characterization of proteins designed to bind iron porphyrin cofactors.

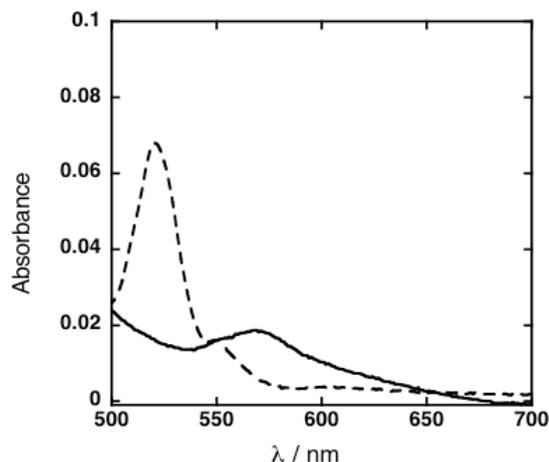


Figure S1. Oxidized (solid line) and reduced (dashed line) UV-vis spectra of a SEC fraction containing the 4PA and iron porphyrin cofactor. An aliquot of the SEC fraction was oxidized with potassium ferricyanide and reduced with sodium dithionite. Spectra were used to determine the concentration of DPP-Fe^{III} present in the SEC fraction.

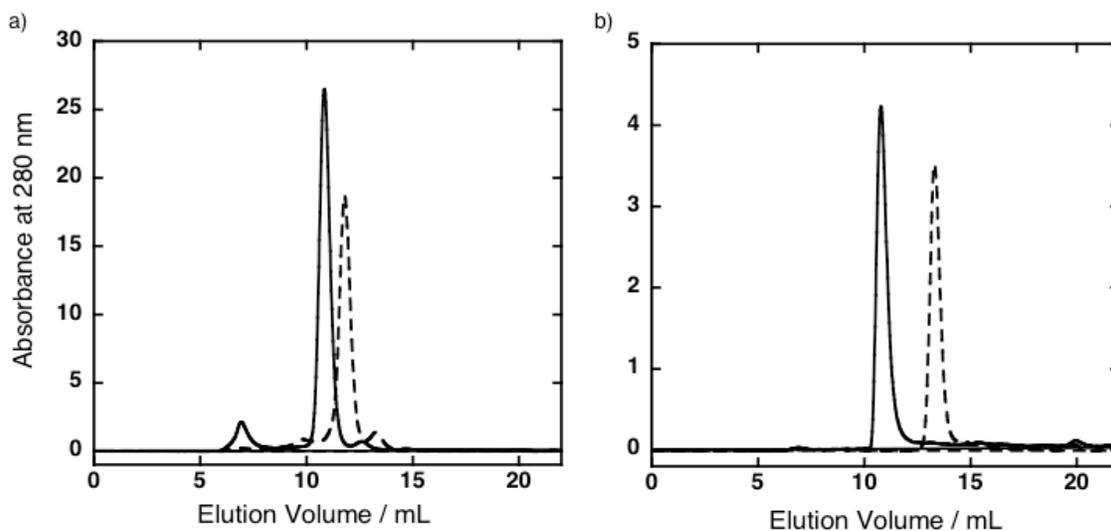


Figure S2. Size exclusion chromatograms of 50 μ M solutions of the four-porphyrin binding peptide in the (a) presence and (b) absence of 50 μ M DPP-Fe^{III} porphyrin. Solid line represents the 4PA peptide. Dashed line represents the 4PA_{without cap}. Mobile phase and sample buffer: 20 mM sodium phosphate, 100 mM NaCl, pH 7.5. Flow rate: 0.5 mL/min.

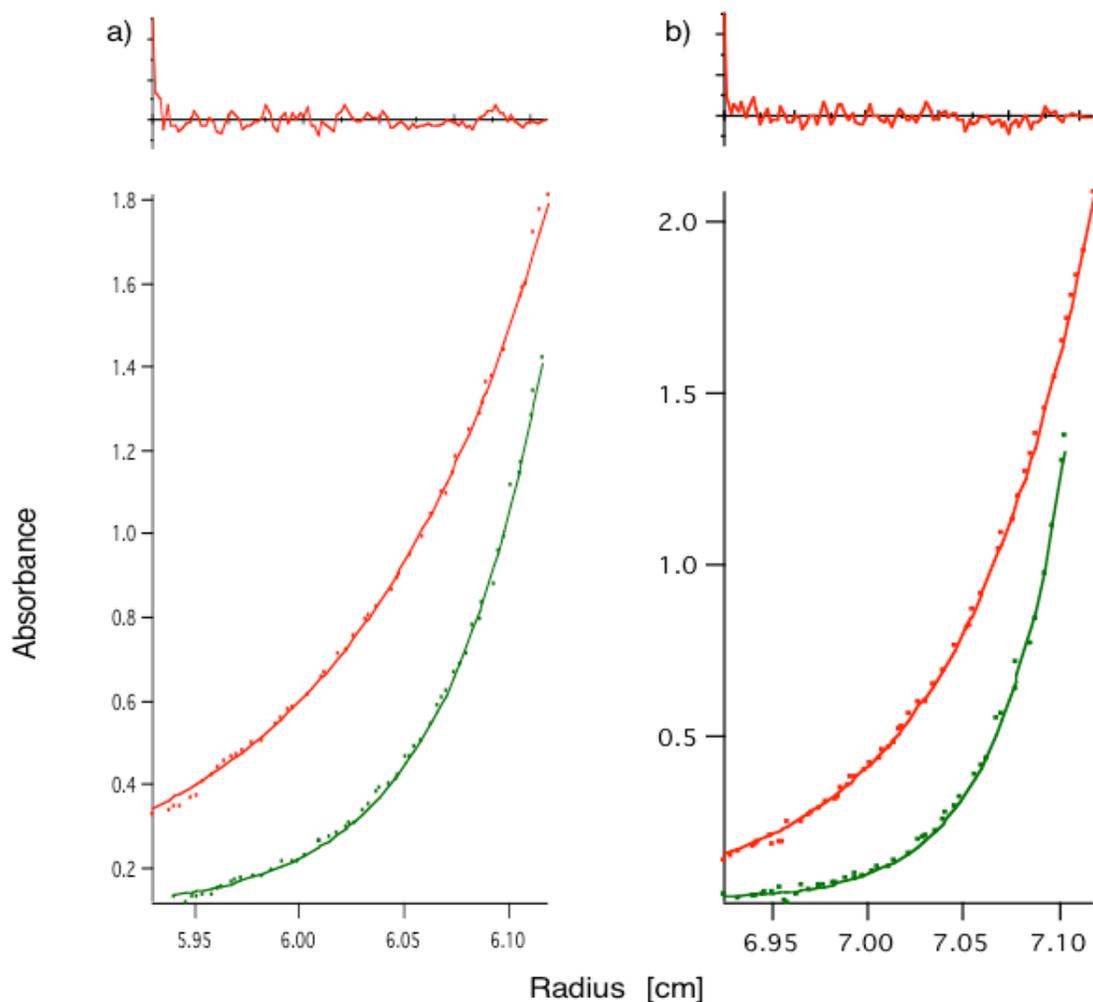


Figure S3. Sedimentation equilibrium profiles of (a) 50 μM 4PA peptide (b) and 10 μM 4PA peptide with 10 μM DPP-Fe^{III} in 20 mM sodium phosphate, 100 mM NaCl, pH 7.5 measured at (a) 230 nm and (b) 409 nm and acquired at centrifugation speeds of 25,000 rpm (red line) and 35,000 rpm (green line) at 25 °C. (a) Determined molecular weight = 23511 ± 156 D. Theoretical molecular weight of tetramer = 25,328 D. (b) Determined molecular weight = $27,914 \pm 129$ D. Theoretical molecular weight of tetramer = 27,988 D. The data were analyzed using a modified global fitting routine in Igor Pro (Wavemetric, Inc.).¹ The molecular weights were determined by holding the partial specific volume and buffer density fixed at 0.732 mL/g and 1.012 g/mL, respectively. Top traces in each panel show normalized auto-correlation function of concatenated residuals.

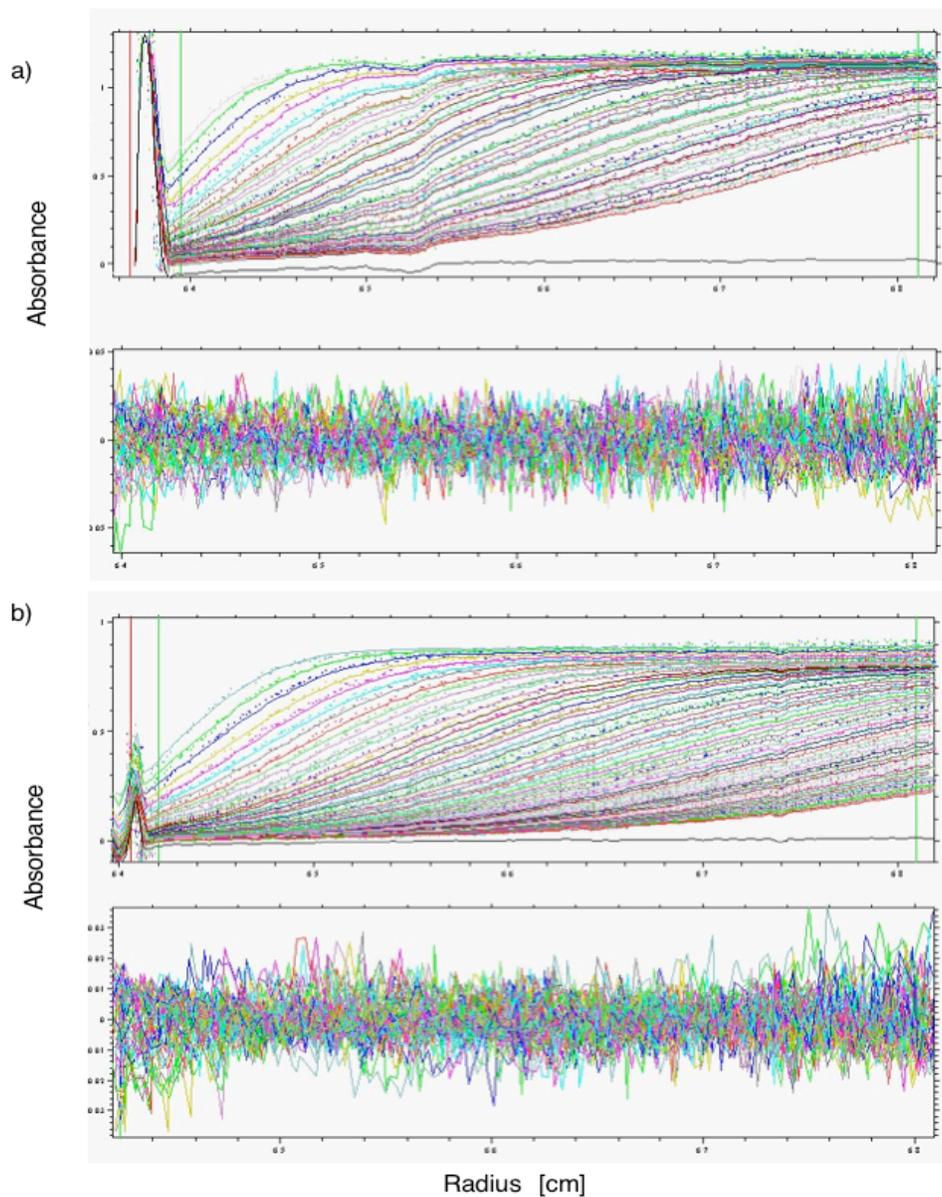


Figure S4. Sedimentation velocity profiles of (a) 50 μM 4PA peptide (b) and 10 μM 4PA peptide with 10 μM DPP- Fe^{III} in 20 mM sodium phosphate, 100 mM NaCl, pH 7.5 measured at (a) 230 nm and (b) 409 nm and acquired at 25 $^{\circ}\text{C}$. Bottom images in (a) and (b) represent the residuals.

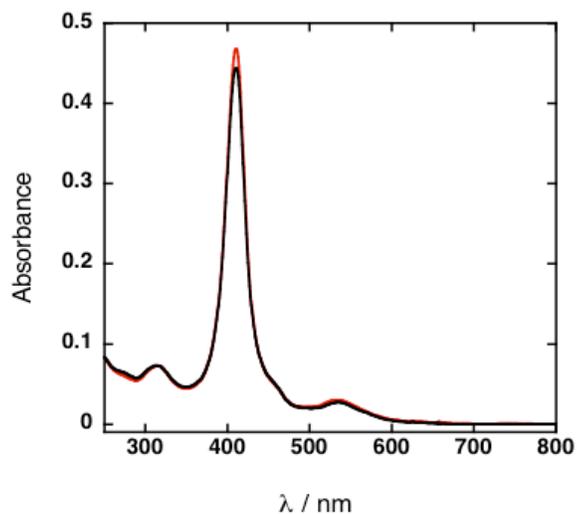


Figure S5. UV-vis spectra of proteins in 20 mM sodium phosphate, pH 7.5 acquired with a 0.1 cm path length quartz cuvette. Red line represents 50 μM 4PA and 50 μM DPP-Fe^{III}. Black line represents 50 μM 4PA_{without cap} peptide and 50 μM DPP-Fe^{III}.

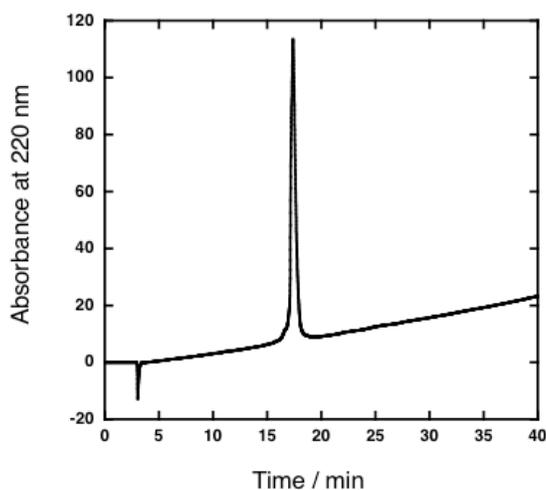


Figure S6. HPLC chromatogram of purified 4PA peptide. 9 μg of 4PA peptide was injected onto a 4.6 mm x 250 mm Vydac C₄ column. A linear gradient of acetonitrile in water containing 0.1% TFA flowed through the column at 1 mL/min starting with 18% acetonitrile and increasing at a rate of 0.9% acetonitrile/min.

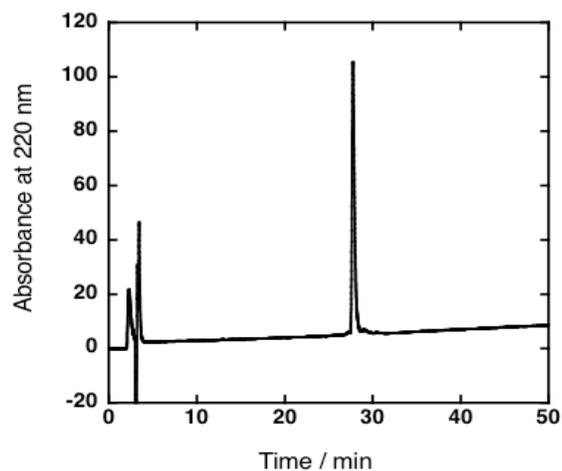


Figure S7. HPLC chromatogram of purified 4PA_{without cap} peptide. Approximately 10 μg of the 4PA_{without cap} peptide was injected onto a 4.6 mm x 250 mm Vydac C₄ column. A linear gradient of acetonitrile in water containing 0.1% TFA flowed through the column at 1 mL/min starting with 22.5% acetonitrile and increasing at a rate of 0.27% acetonitrile/min.

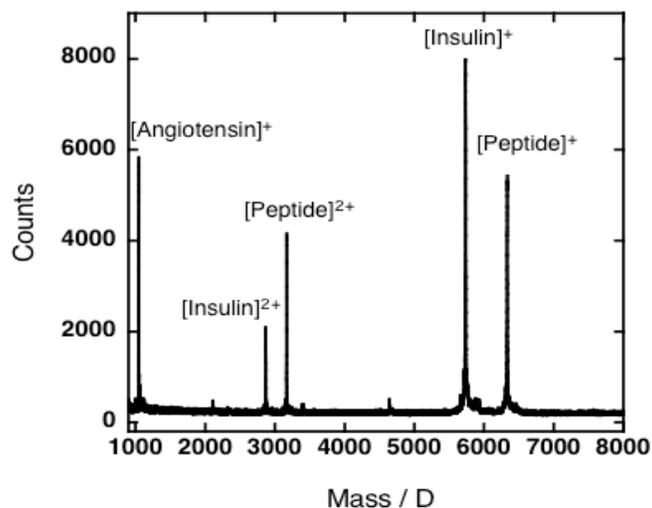


Figure S8. MALDI-TOF MS spectrum of purified 4PA peptide. α -cyano-4-hydroxy-cinnamic acid was used as the matrix. Insulin and angiotensin I were used as internal standards. Calculated mass is 6332.91 D. Measured mass is 6332.71 D.

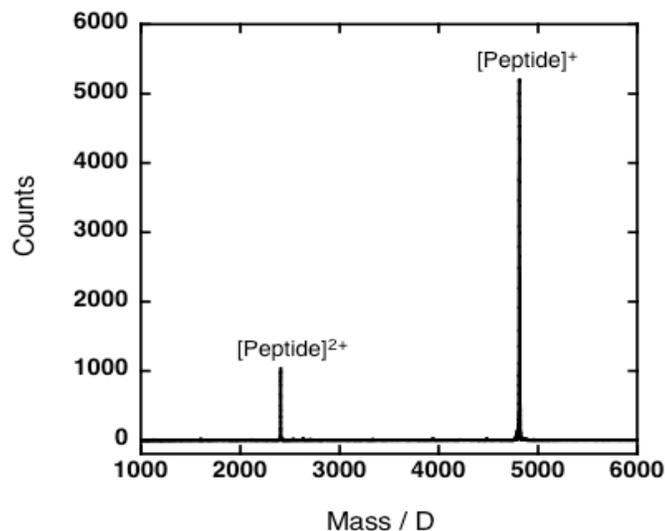


Figure S9. MALDI-TOF MS spectrum of purified 4PA_{without cap} peptide. α -cyano-4-hydroxycinnamic acid was used as the matrix. Angiotensin I was used as an external standard. Calculated mass is 4812.17 D. Measured mass is 4812.9 D.

References for Supporting Information

- (1) Brooks, I.S.; Sonesson, K.K.; Hensley, P. *Biophys. J.*, **1993**, *64*, A244.

Complete reference (6).

- (6) MacKerrell, A.D.; Bashford, D.; Bellott, M.; Dunbrack, R.L.; Evanseck, J.D.; Field, M.J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, J.D.; Kuchnir, L.; Kuczera, K.; Lau, F.T.K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D.T.; Prodhom, B.; Reiher, W.E.; Roux, B.; Schlenkrich, M.; Smith, J.C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. *J. Phys. Chem. B.* **1998**, *102*, 3586-3616.