Self-Assembly of Peptide Porphyrin Complexes: Towards the Development of Smart Biomaterials

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Supporting Information

Peptide Synthesis and Purification. The peptide was synthesized by an Applied Biosystems Model 433A peptide synthesizer (Perseptive Biosystems) using standard Fmoc chemistry and PAL resin (Advanced ChemTech). The peptide was acetylated on the resin and cleaved using TFA. The peptide was purified by reversed-phase chromatography (Varian ProStar HPLC, Varian Dynamax C18 column) with H_2O (0.1% TFA) and acetonitrile (0.1% TFA) as the mobile phases. The identity of the purified peptide was verified by MALDI-TOF mass spectrometry (Synpep Corporation).

Solution preparation. Porphyrin and peptide stock solutions were prepared in MilliQ water. Peptide stock solution concentrations (1-2 mM) were determined by using a modified ninhydrin procedure from Rosen, H. *Arch. Biochem. Biophys.* **1957**, *67*, 10. Porphyrin stock solutions (< 1 mM) were prepared by dissolving $TPPS_4$ (Frontier Scientific Inc.) in 6 mM NaOH and measuring the concentration of this solution using $\varepsilon_{414} = 5.33\_10^5\ M^{-1}\ cm^{-1}$ from Fleischer, E. B.; Palmer, J. M.; Srivastava, T. S. Chatterjee, A. *J. Am. Chem. Soc.* **1971**, *93*, 3162. Small aliquots of the porphyrin and peptide stock solutions were added to 10 mM Tris-HCl at pH 7.6 to create solutions containing the complex for study.

UV-vis Spectroscopy. UV-vis measurements were made in 1 or 10 mm quartz cuvettes using either a Jasco V-570 UV-vis spectrophotometer or a Perkin-Elmer Lambda 25 spectrometer. The apparent isosbestic point in the UV-vis titration data were evaluated by determining the standard deviation about the mean for both the absorbance and wavelength values (0.474±0.025 A.U. at 406 nm; 406.88±0.84 nm). The UV-vis titration data collected at 413 nm were fit to a simple 1:1 binding equilibrium scheme to establish the dissociation constant. The data were also fit at a second wavelength (403 nm) but due to the much smaller change in absorbance at this wavelength, we were not able to independently establish a rigorous dissociation constant. However, we were able to fit the data using a fixed dissociation constant as determined from the 413 nm dataset with reasonable goodness of fit. A more complex, 2:2 scheme, assuming a coiled coil with two porphyrins bound, did not improve the fit. The appropriate equations for data analysis were encoded into MLAB (Knott, G. D. *Comput. Progr. Biomed.* **1979**, *10*, 271; Civilised Software, Inc., Bethesda, MD) for curve-fitting purposes as described previously (Grandori, R.; Khalifah, P.; Boice, J. A.; Fairman, R.; Giovanielli, K.; Carey, J. *J. Biol. Chem.* **1998**, *273*, 20960).

We tested the concentration dependence of the complex in a dilution experiment as measured by UV-vis spectroscopy (Figure S1). At high concentrations of the 1:1 complex, there are some changes in the normalized absorbance of the Soret bands, indicating a small amount of aggregation of the porphyrin. This aggregation was also evident in dilution experiments of the porphyrin alone; this problem is minimized if the porphyrin concentration is kept below 20 μM. At concentrations of 10 μM and below, we see a significant increase in the normalized signal, as expected for a dissociating

complex. Visual inspection suggests a dissociation constant from this experiment of about 4 μM, which is close to the value reported from curve-fitting analysis of the titration data as described above.

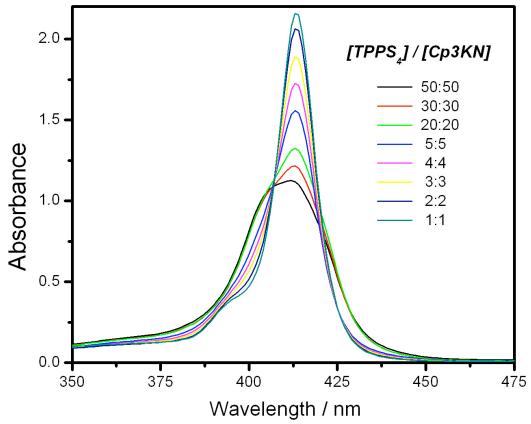

Figure S1. Dilution series of 1:1 $TPPS_4$:Cp3K-N complex from 50 μM to 1 μM as measured by UV-vis. Samples were prepared in 10 mM Tris-HCl, pH 7.6. The data have been scaled by normalizing the concentrations of the other samples relative to the 50 μM sample for graphing purposes (i.e., the absorbance for the 1:1 sample was multiplied by 50). This normalization highlights the crossover point at 403 nm, which approximates an isosbestic point particularly if the two highest concentrations are eliminated.

CD spectroscopy. CD spectra were collected using a 0.5 nm stepsize and averaged for 1-3 sec per data point, in 1 mm quartz cuvettes with an Aviv Model 202-01 circular dichroism spectrometer. To show that the interaction between the porphyrin and peptide requires the sulfonato groups of the $TPPS_4$, we compared CD spectra of solutions containing Cp3K-N with either $TPPS_4$ or TMPyP (meso-tetra(N-methyl-4-pyridyl) porphine tetra tosylate), a cationic porphyrin derivative. This experiment is shown in Figure S2.

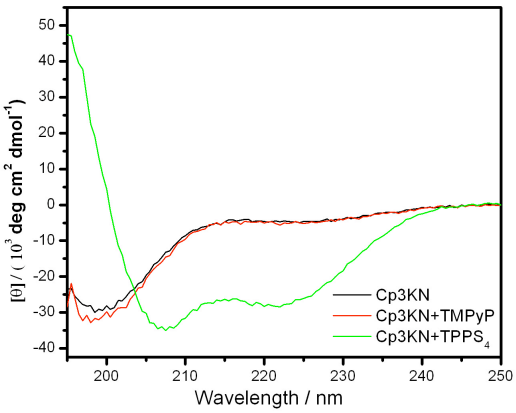

Figure S2. Comparison of binding of $TPPS_4$ *vs.* TMPyP to Cp3K-N as measured by CD. Sample conditions are: 50 μM Cp3K-N, 50 μM either $TPPS_4$ or TMPyP, 10 mM Tris-HCl, pH 7.6.

Analytical Ultracentrifugation. All samples were run in a Beckman model Optima XL-A analytical ultracentrifuge equipped with an An-Ti 60 rotor. Sedimentation equilibrium experiments were performed by using six-channel Epon charcoal-filled centerpieces, with 1.2 cm pathlengths. Absorbance scans were measured after a delay time of 8 hours at speeds of 30,000, 40,000, and 50,000 rpm at 25°C. Peptide Cp3K-N by itself was monitored at 232 nm, $TPPS_4$ at 441 nm, and Cp3K-N with $TPPS_4$ were monitored at both 232 nm and 441 nm respectively. Data were truncated by using WinReedit (v. 0.999, ©1998) and analyzed by using WinNonLin (v. 1.035, ©1997). More information on this procedure can be found in Johnson, M. L.; Correia, J. J.; Yphantis, D. A.; Halvorson, H. R. *Biophys. J.* **1981,** *36*, 575-588. For the analysis, the solution density and the peptide partial specific volume were calculated by using Sednterp as described in Laue, T. M.; Shah, B. D.; Ridgeway, T. M.; Pelletier, S. L. In *Analytical Ultracentrifugation in Biochemistry and Biopolymer Science;* Harding, S.; Rowe, A.; Horton, A. J., Eds.; Royal Society of Chemistry: Cambridge, 1992; pp. 90-125. The partial specific volume for

$TPPS_4$ was calculated by using a procedure described in Durchschlag, H.; Zipper, P. *Prog. Colloid Polym. Sci.* **1994,** *94*, 20. When AU data for each rotational speed are fit independently, the apparent molecular weight of the complex appears to decrease with increasing rotation rate as seen in Table S1. This is commonly used as evidence that several species are present in solution, prompting the use of multiple state models to fit the global dataset.

Table S1. Apparent molecular weight (MW) of porphyrin-peptide complex as a function of rotation rate from AU sedimentation equilibrium measurements.

Rate (rpm)	MW (Da)
30,000	7,829
40,000	6,813
50,000	6,704

Computer Modelling. The $TPPS_4$ structure was minimized *in vacuo* with B3LYP density functional theory and the 3-21G* basis set as implemented by Gaussian 2003 (Gaussian, Inc.). The Cp3K-N amino acid sequence was built with the Biopolymer module in InsightII 2000 (Biosym Technologies). The peptide side-chains were subjected to molecular mechanics minimizations with steepest descent and conjugate gradient methods implemented in the Discover module. The $TPPS_4$ molecule was then docked manually such that a subset of the sulfonate groups were in close contact with the three lysines in the peptide. Final adjustments were made to eliminate van der Waals clashes between $TPPS_4$ and Cp3K-N by using the Bump_Check function in InsightII.