Conformations of a Model Protein Revealed by an Aggregating Cu^{II} Porphyrin: Sensing the Difference

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Electronic Supporting Information

Experimental Section

Chemicals. 5,15-bis(N-methylpyridinium-4-yl)-10,15-bis-diphenylporphine (t-H_2P) was purchased from Mid-Century Chemicals as the chloride salt and used as received. Its copper(II) derivative (t-CuP) was prepared via previously reported procedures.[1] Porphyrins stock solutions were prepared from the solids in Millipore purified water and stored in the dark. Solution concentrations were determined from known molar extinction coefficient at the Soret maximum (*t*-CuP: 2.34×10^5 $M^{-1}cm^{-1}$).[2]

Sodium salts of poly-L-glutamic acid (batches of different lengths) were obtained from Aldrich Chemicals Co. Stock solution of the polypeptides were prepared by dissolving the solids in Millipore purified water, and dialyzing exhaustively against buffer at the appropriate pH and ionic strength (IS = 2 mM). Concentrations of the peptides solutions were determined spectrophotometrically, using $\varepsilon_{205} = 2240$ M^{-1} cm^{-1}.[3]

All other reagents were supplied by Fisher Chemical and used without further purification.

Methods. Mixing of the reactants was carried out in methacrylate cuvettes at room temperature, by using a protocol in which a small volume of porphyrin stock was added to poly-L-Glu in a 2 mM IS buffer (acetate or phosphate) at the proper pH. In the case of experiments at high ionic strength (0.15 M) conditions, NaCl was added last. This protocol led to highly reproducible results.

Extinction measurements were conducted on a JASCO V550 spectrophotometer, and resonance light scattering (RLS) measurements were performed on a SPEX Fluorolog spectrofluorimeter using a synchronous scan mode.[4] Whenever possible, plastic cuvettes were used to reduce porphyrin staining of the cuvette surface. Quartz cuvettes were employed only in the case of measurements in the UV region, or for circular dichroism experiments carried out on an Aviv 62DS spectropolarimeter.

References.

1. O. Herrmann, S. H. Mehdi and A. Corsini, *Can. J. Chem.*, 1978, **56**, 1084; R. F. Pasternack, L. Francesconi, D. Raff and E. Spiro, *Inorg. Chem.*, 1973, **12**, 2606.
2. R. F. Pasternack, C. Bustamante, P. J. Collings, A. Giannetto and E. J. Gibbs, *J. Am. Chem. Soc.*, 1993, **115**, 5393.
3. K. Rosenheck and P. Doty, *Proc. Natl. Acad. Sci. U. S. A.*, 1961, **47**, 1775.
4. R. F. Pasternack and P. J. Collings, *Science*, 1995, **269**, 935.

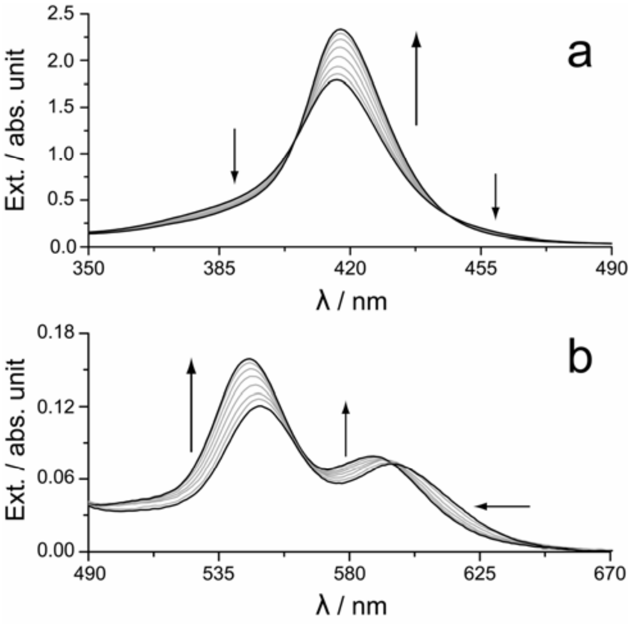

Figure S1. UV/vis extinction spectra of t-CuP in MilliQ water (8 μM; a: Soret band, b: Q-bands), as a function of increasing temperature (from 10°C to 70°C, marked by arrows). The presence of several isosbestic points suggests there are only two chromophores present under these conditions.

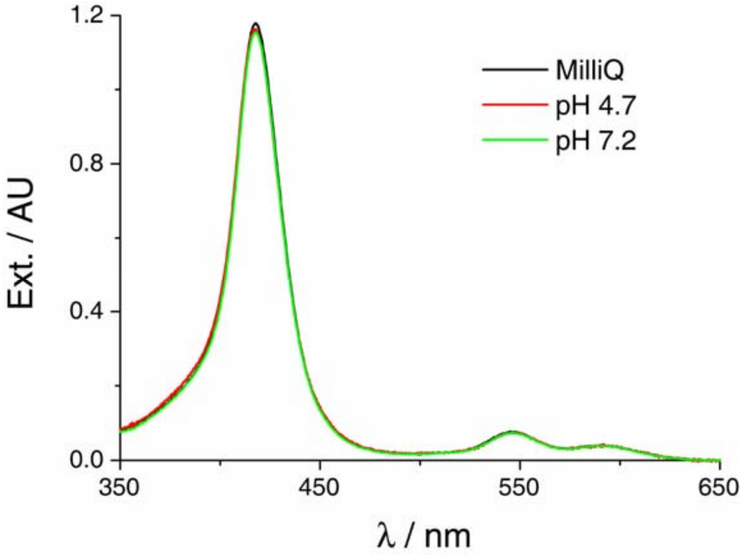

Figure S2. UV/vis extinction spectra of 5 μM t-CuP in MilliQ water (black), pH 4.7 acetate buffer (red), and phosphate buffer (green), showing that under the ionic strength conditions (IS = 2 mM) used in the various experiment the presence of the buffers has a negligible effect on the porphyrin extinction features.

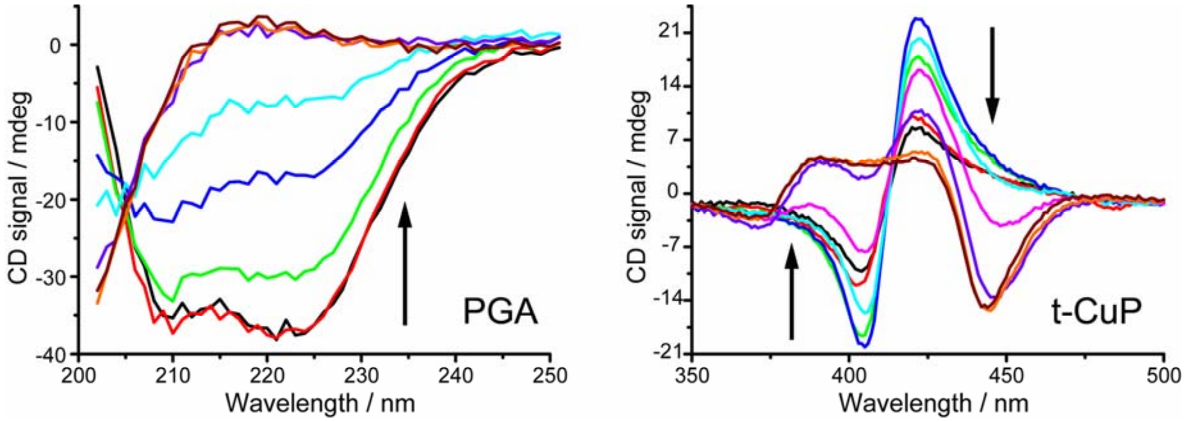

Figure S3. Changes observed in the CD spectral features of a batch of t-CuP / PGA solutions with pH that ranges between 4.53 and 7.96. The UV region (left) highlights the helix-coil transition of the polypeptide, while in the visible region (right) the CD signals induced in the Soret region of t-CuP parallel the protein conformational changes. The arrows mark the increase in pH. [t-CuP] = 5 μM; [Glu] = 100 μM; buffer IS = 2-3 mM.

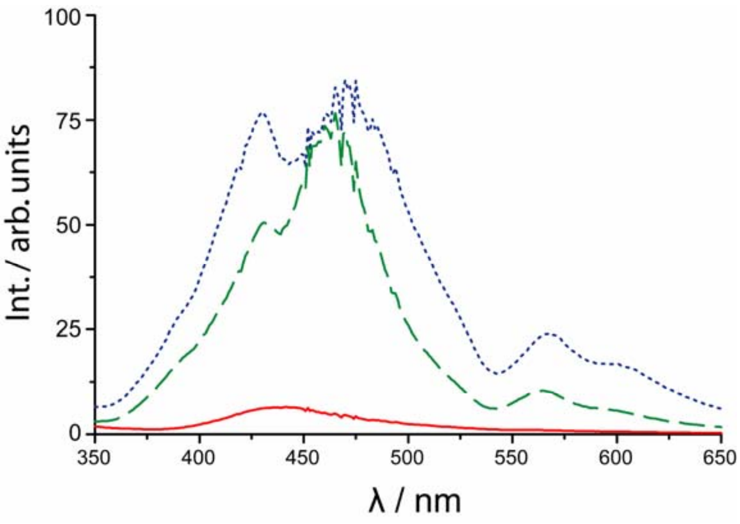

Figure S4. RLS spectra of t-CuP in the presence of PLG (pH = 4.58, solid; pH = 5.5, dotted; pH = 6.86, dashed), at high ionic strength. [t-CuP] = 5 μM; [Glu] = 500 μM, FW ~ 17 kD; [buffer] = 2 mM; [NaCl] = 0.15 M.