Design of a Five-coordinate Heme Protein Maquette: A Spectroscopic Model of deoxyMyoglobin

Supplementary Material

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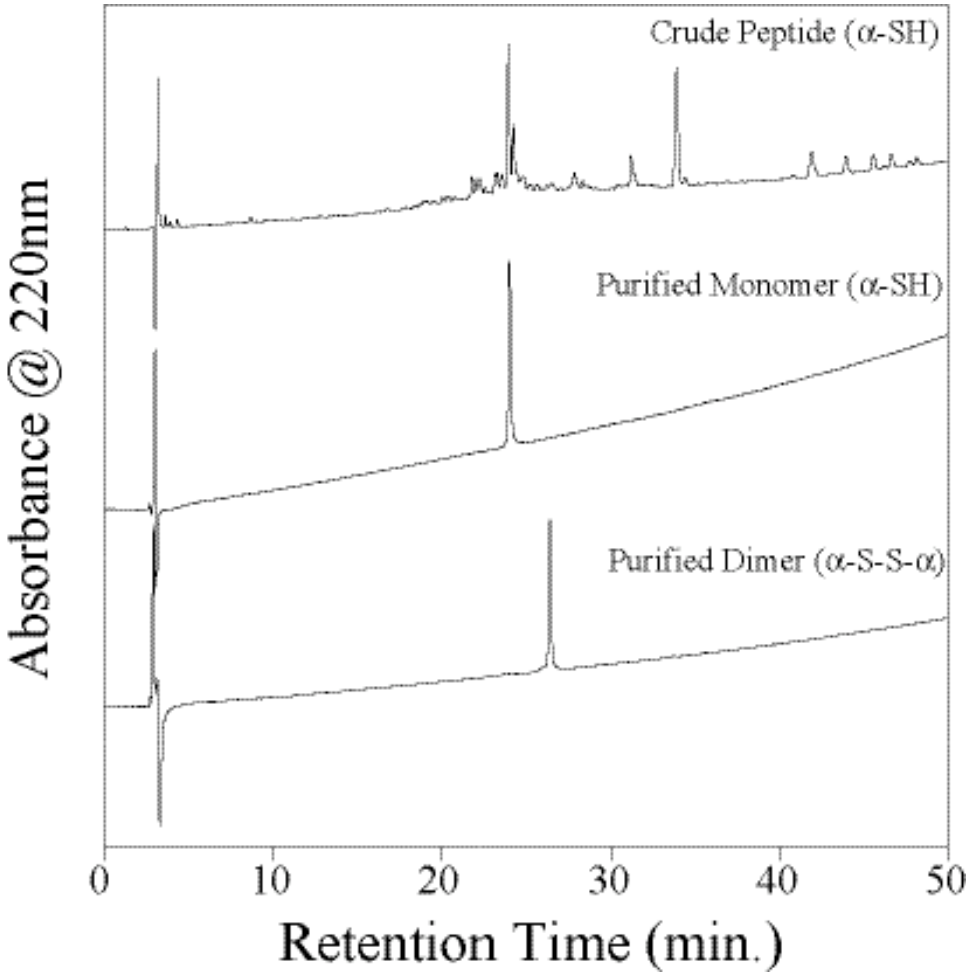

Figure S1. HPLC purification of the peptide ligand □7-H1m$_{10}$I$_{14}$I$_{21}$. HPLC chromatograms of the crude peptide product (top), the HPLC purified peptide (middle) and the disulfide bridged peptide dimer, □7-H1m$_{10}$I$_{14}$I$_{21}$,(bottom) are shown. All chromatograms were recorded on a Beckman-Coulter System Gold HPLC equipped with a Vydac C$_{18}$ reverse phase column using a 20-70% acetonitrile in water (0.1% v/v trifluoroacetic acid) gradients over 50 minutes.

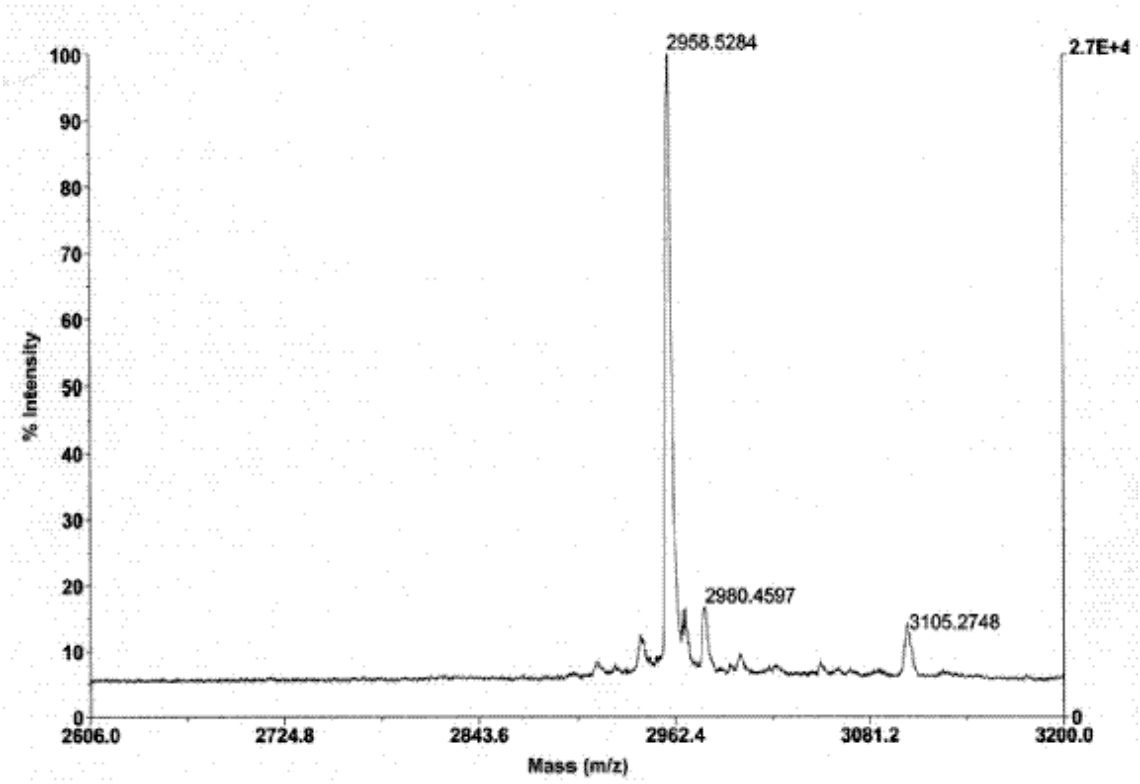

Figure S2. MALDI mass spectrum of the peptide ligand □7-H1m$_{10}$I$_{14}$I$_{21}$. Observed mass: 2958.5 amu; expected mass 2958.6 amu.

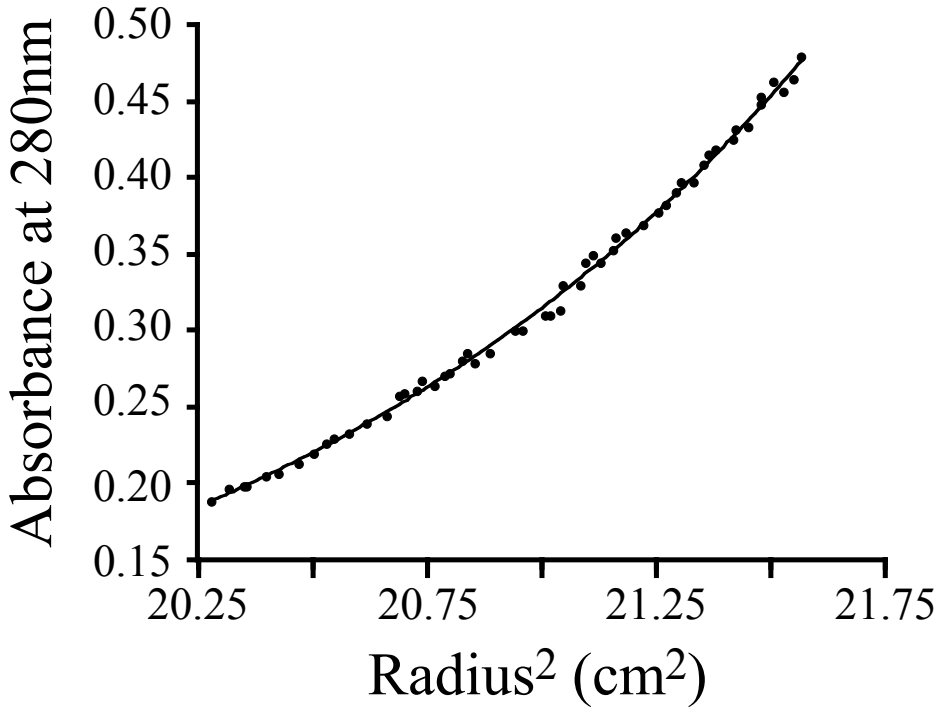

Figure S3. Sedimentation equilibrium analytical ultracentrifugation analysis of the peptide oligomerization state for $[\square 7\text{-}H1m_{10}I_{14}I_{21}]_2$. The sample was equilibrated for 24 hrs at 25,000 RPM in a Beckman XL-I ultracentrifuge at 20°C. The initial loading concentration was 16.5 μM in 20mM potassium phosphate, 100 mM KCl, pH 8. Data reduction and analysis was performed using WinNonlin, WinReedit, WinMatch and Sednterp. The radial distribution absorbance scan data at 280 nm (tryptophan) was fit to a single exponential using WinNonlin. The partial specific volumes ($\overline{\square}$) for the peptide was calculated to be 0.7595 using the method of Cohn and Edsall. $[\square 7\text{-}1Mh_{10}I_{14}I_{21}]_2$ sediments as single homogenous species with molecular weights of 11500 ± 750 amu, a value close to the expected mass of a dimer, 11828 amu.

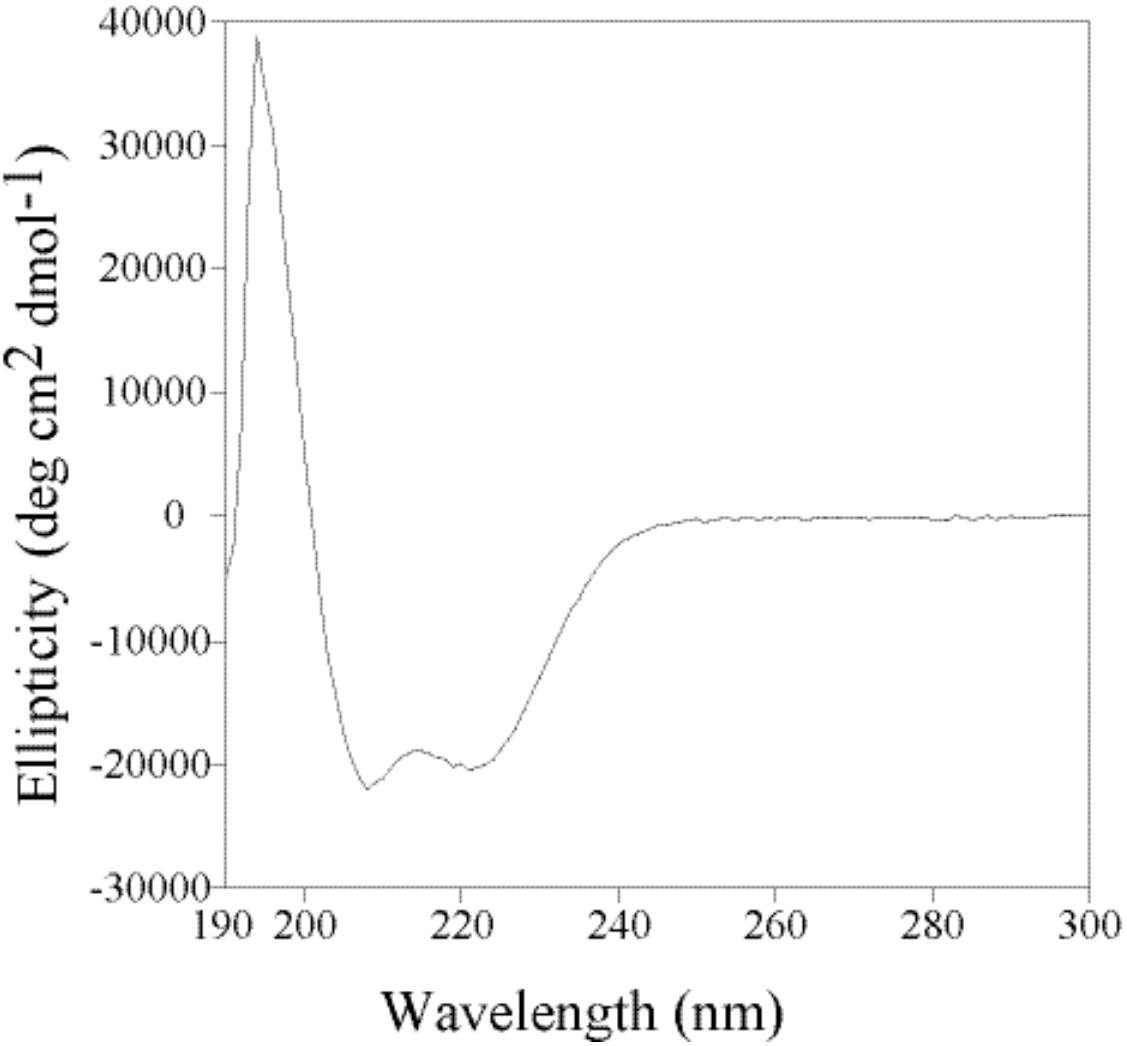

Figure S4. Circular Dichroism spectropolarimetry analysis of peptide secondary structure. A sample of 7.5μM [□7-H1$m_{10}I_{14}I_{21}$]$_2$ in 20 mM potassium phosphate, 100 mM KCl, pH 8.0 at 25°C was recorded in a 2 mm path length cell on an Aviv Model 215 CD spectrophotometer.

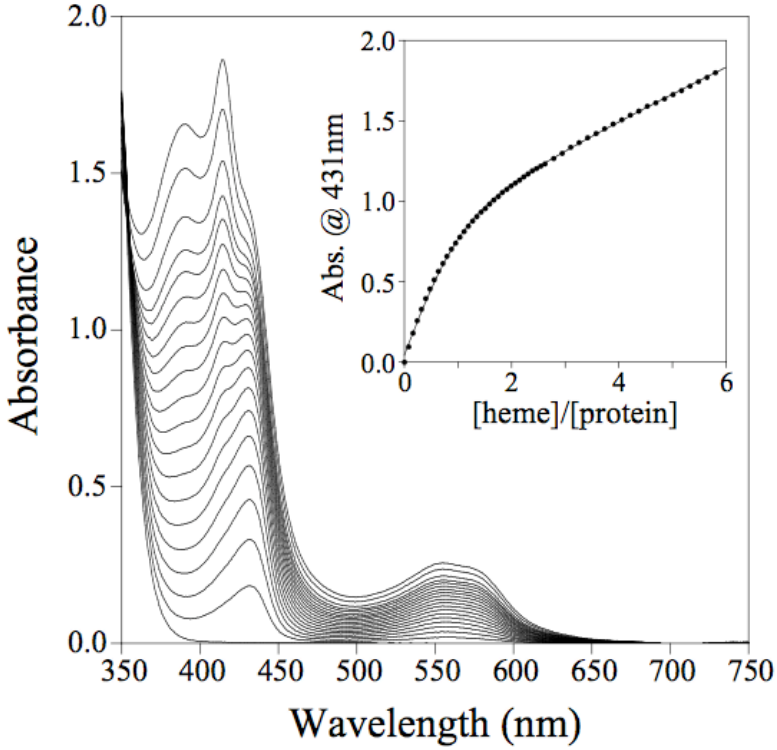

Figure S5. Spectroscopic determination of the ferrous heme dissociation constant at 29μM four helix bundle concentration. Spectra shown contain 0.0, 0.15, 0.29, 0.44, 0.59, 0.74, 0.89, 1.04, 1.18, 1.33, 1.48, 1.63, 1.78, 1.93, 2.07, 2.22, 2.37, 2.59, 2.89 and 3.19 equivalents of heme per bundle. In the inset, the absorbance at 431 nm is fit to a 1:1 heme:four helix bundle binding model with a dissociation constant, K_{d1}, value of 5μM. The experiment was performed in 20 mM potassium phosphate, 100 mM KCl, pH 8.0.

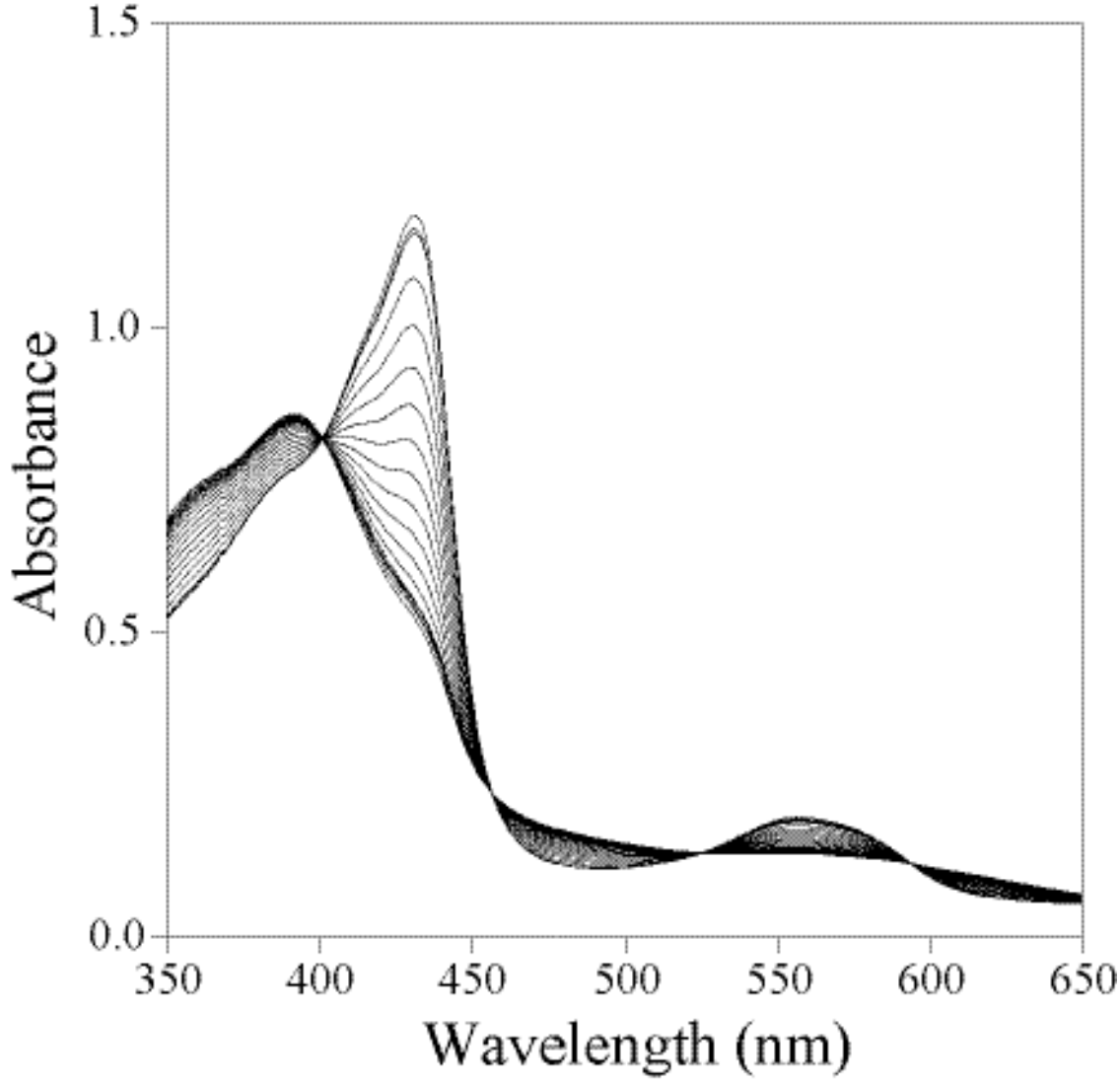

Figure S6. UV-visible evidence for the reactivity of [□7-H1m$_{10}$I$_{14}$I$_{21}$]$_2$ with oxygen. Ferrous monoheme [□7-H1m$_{10}$I$_{14}$I$_{21}$]$_2$ at 14.7μM concentration in 20 mM potassium phosphate, 100 mM KCl, pH 8.0 was exposed toambient dioxygen. The time interval between scans is 3 minutes.

Resonance Raman Data Collection and Analysis

Raman spectra of peptide samples at 114μM in 1 mm diameter capillary tubes were obtained at room temperature using a SPEX 1404 0.85 m double spectrometer with a liquid N_2 cooled, 13.6 mm back-thinned SPEX Spectrum-1 CCD chip detector. Raman scattering was excited by the 441.6 nm line (~30 mW at the sample) of a Kimmon IK series HeCd laser. Raman shifts were calibrated against the laser emission at 441.6 nm. Three separate data sets consisting of 25, 25, and 10 spectra with 120 s integration time were averaged (weighted by number of spectra) after a linear baseline correction was applied to each of the three data sets.

Magnetic Circular Dichroism Data Collection and Analysis.

Ferrous monoheme-[□7-H1m$_{10}$I$_{14}$I$_{21}$]$_2$ was generated under N_2 in a rubber septum sealed cuvette (1 cm) by adding a few grains of solid sodium dithionite to the ferric sample. Reduction was monitored spectrophotometrically using a Varian Cary 400 spectrophotometer. The MCD spectra were obtained at 4 ^{0}C using a Jasco J600 spectopolarimeter equipped with a Jasco MCD-1B electromagnet operated at a magnetic field of 1.41 T.