Supporting Information

for

A Conformational Switch to β-sheet Structure in Cytochrome c Leads to Heme Exposure; Implications for Cardiolipin Peroxidation and Apoptosis.

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S1. UVRR spectral analysis. Details of the UVRR spectral analysis have been described previously. [1] The 197 nm – excited spectrum (Fig 1(a)) contains strongly enhanced bands of phenylalanine (F) and tyrosine (Y), as well as backbone amide bands. To minimize the aromatic sidechain contributions, spectra of aqueous tyrosine and phenylalanine were subtracted, after scaling to the isolated Y1 and F12 bands in the cyt c spectrum. (Fig 1(b)). The subtraction is imperfect, because the intensity distributions are different in the protein and in aqueous solution, but the procedure serves to emphasize the amide III and amide S region (red) which is most sensitive to secondary structure. This is the region used to estimate the secondary structure contributions via least squares fitting of the standard spectra[1]. The quality of the modeling is shown in Fig. 1(c).

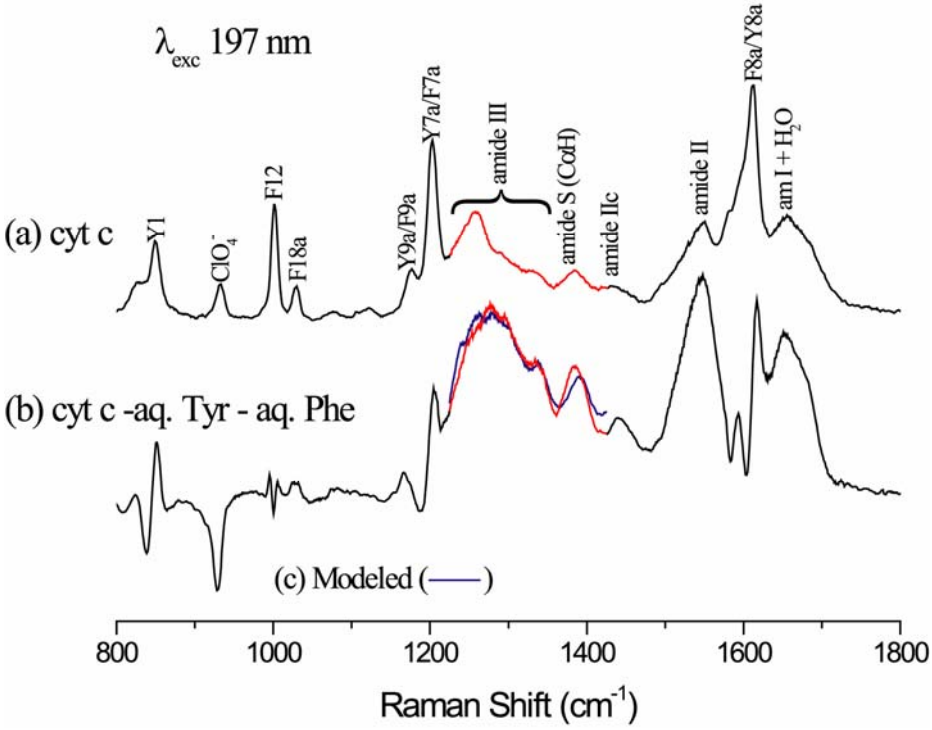

Fig 1. Illustration of UVRR spectral analysis for cyt c (50 μM) at pH 3 at 20 ^{o}C.

In order to test the robustness of our finding of β-sheet induction, we constrained the fitting to exclude β-sheet. Its place was taken by a combination of unordered and β-turn structure, and required an unphysical increase in β-turn population at elevated temperature. In addition, the goodness-of-fit parameter, R^2, steadily decreased from 0.95 at 20 ^{o}C to 0.81 at 80^{o}C, whereas inclusion of β-sheet lead to temperature independent R^2 value of 0.92 ± 0.01.

S2. CD spectroscopy of equine cyt c at pH 3. Far-UV circular dichroism spectra were measured with an AVIV 62DS spectropolarimeter equipped with a temperature controlled cuvette holder, using a 1 mm quartz cell. Inset: The CD spectra at the indicated temperatures were averages of three repeated scans with 1 nm/sec spacing from 320 to 190 nm. The temperature profile was recorded at 222 nm from 20 to 98 ^{o}C with 1 ^{o}C/sec steps and 1 min equilibration time(red). A reverse scan, from 98 to 20^{o}C (black), was carried out to verify reversibility. The cyt c concentration was 19.2 μM. The data were converted to mean residue ellipticity [θ] in deg.cm^2/dmol using the equation = (θ_{obs}/10lc)/r, where θ_{obs} is the ellipticity measured in millidegrees, l is the optical path length (cm), c is the concentration of the cyt c (M), and r is the number of residues.

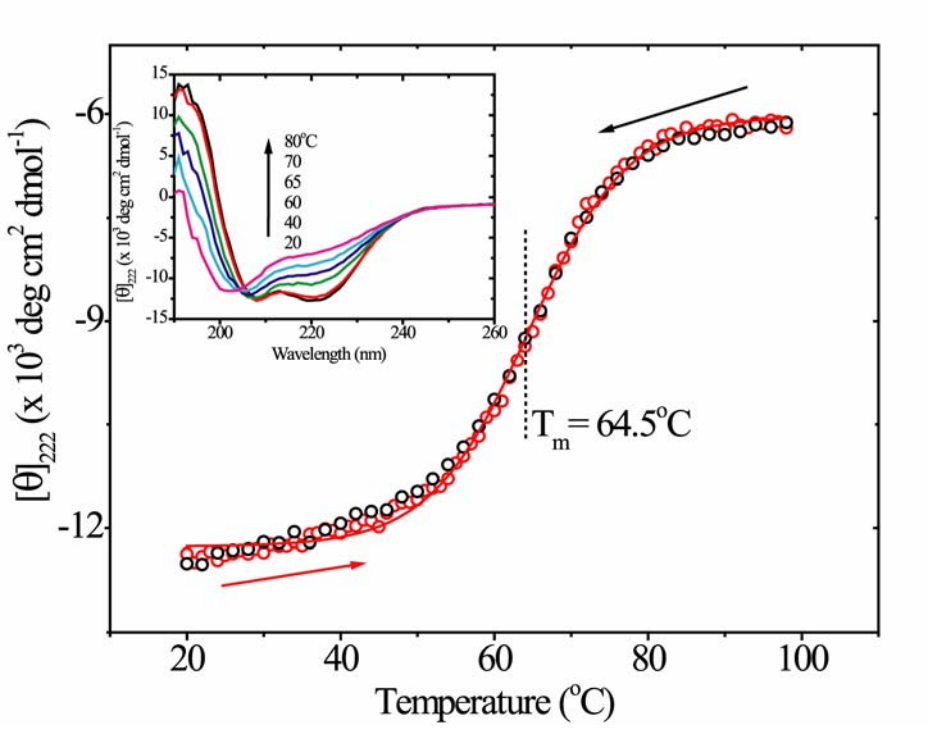

Fig 2. Thermal profile of the 222 nm ellipticity (θ_{222}) of cyt c at pH 3 (19.2 μM) associated with helical content. The continuous line is the sigmoidal fit. Inset: CD spectra at the indicated temperatures.

S3.Kinetics of β-sheet structure formation from T-jump/UVRR spectroscopy. The experimental setup and the spectral acquisition scheme have previously been described.[2] UV probe pulses (20 ns, 1 kHz) at 197 nm (~1 μJ/pulse) were obtained by frequency quadrupling the output of a Ti:sapphire laser, which was pumped (527 nm, ~10 mJ/pulse, 70 ns, 1 kHz) by an intracavity frequency-doubled Nd:YLF laser (GM30, Photonics International, Inc.). T-jump pulses were obtained from an intracavity diode laser-pumped Nd:YLF laser optical parametric oscillator (OPO ; Photonics International, Inc.), which can generate tunable near-infrared (NIR) at 1.6-2.0 μm (0.8-1.0 mJ/pulse, 20 ns, 1 kHz pulses). The final temperature of the sample after the T-jump (40 to 67 ^{o}C) was determined with a precision of 2 ^{o}C from the ~3400 cm^{-1} water Raman band intensity change. The wavelength of the T-jump pump pulse is within the near-IR water band centered at 1.9 μm. The UVRR spectra of cyt c at pH 3 at varying time delays were decomposed into the secondary structure contributions,[1] producing a relaxation curve for β-sheet formation that was well fit with a single exponential having a 2.2 μs time constant.

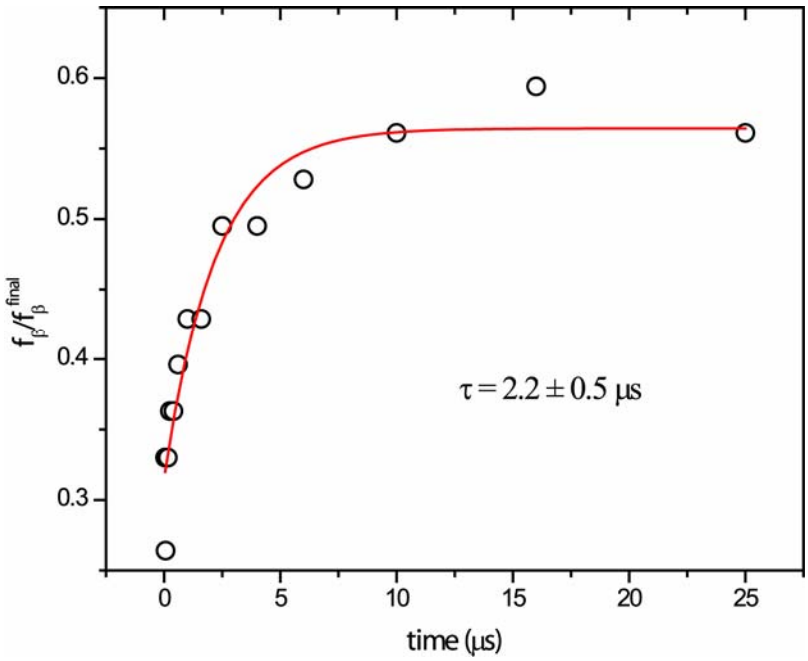

Fig 3. Time course of the β-sheet structure formation in cyt c at pH 3 after a T-jump from 40 to 67 ^{o}C. The continuous line is the fit to a single-exponential function.

S4. Peroxidase activity measurements[3,4]. Absorbance intensity of ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonate) radical, the product of ABTS oxidation, was monitored at 650 nm. Cyt c (0.5 μM) at pH 3 (50 mM phosphate buffer, 50 mM ClO_4^- and 0.1 mM DTPA(diethlenetriaminepentaacetic acid)) or at pH 7.2 (100 mM phosphate buffer with 0.1 mM DTPA) was equilibrated in the thermostatic cuvette holder block at the desired temperature for 10 min before adding ABTS (525 μM) and H_2O_2 (7.5 mM). Absorbance spectra of ABTS radical were collected for 3 min after rapidly mixing temperature-equilibrated H_2O_2 and ABTS with cyt c solution. A Cary Bio UV/Vis spectrophotometer with a thermostat was employed for the electronic spectra measurements in a 3.0 mL, 1 cm path length quartz cuvette. Due to the low concentration of cyt c in the reaction, the interference of its Q-band was negligible.

References:

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3. Childs, R. E.; Bardsley, W. G. *Biochem. J.* **1975,** *145*, 93.
4. Witting, P. K.; Mauk, A. G.; Lay, P. A. *Biochemistry* **2002,** *41*, 11495.