Supporting Information for

## His26 Protonation in Cytochrome c Triggers Microsecond βsheet Formation and Heme Exposure: Implications for Apoptosis

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Figure S1. Temperature-dependent, 197-nm excited UVRR spectra of hh cyt c at pH 3.0, showing loss of intensity in the signals from backbone amide, Tyr and Phe bands. Assignments<sup>1,2</sup> are indicated in the top spectrum.



Figure S2. 197-nm excited UVRR spectrum of hh cyt c (pH 3.0). For secondary structure analysis the interfering bands from Tyr and Phe are suppressed by subtracting aqueous Tyr and Phe spectra at the appropriate molar concentration. Subtraction is inexact because of the altered cross-sections in the protein, but is sufficient to reliably expose backbone features, particularly in the central 1200-1500 cm<sup>-1</sup> region.



Figure S3. Temperature-dependent, 229 nm-excited UVRR spectra of *hh* cyt c at pH 3.0, showing loss of intensity in the signals from Tyr and Trp. Assignments are indicated in the top spectrum.<sup>1,2</sup>



Figure S4. 220 nm-excited UVRR spectrum of hh cyt c in D<sub>2</sub>O, showing the 1408 cm<sup>-1</sup> HisD<sub>2</sub><sup>+</sup> band.

## REFERENCES

- (1) Harada, I.; Takeuchi, H. In *Spectroscopy of biologicalsystems*; Clark, R. J. H., Hester, R. E., Eds.; John Wiley: New York, 1986, p 113-175.
- (2) Austin, J. C.; Rodgers, K. R.; Spiro, T. G. *Metallobiochemistry, Part C* **1993**, *226*, 374-396.