Supporting Information

Expression and Purification of Non-His-tagged cyt c′. A non-His-tagged cyt *c′* expression system was developed, where the *cycP* open reading frame was amplified by PCR with the oligonucleotides c'8 and c'9. The resultant PCR product was cloned into the *Eco*RI and *Bam*HI sites of the vector pYSW35. The resultant plasmid was conjugated into *R. sphaeroides* 2.4.3 to yield a system for high expression of non-His-tagged cyt *c′*. Cells were grown as described above in the Materials Section. Cells were harvested for 10 min at 10,000 *g*, 4º C, washed in 500 mL 20 mM sodium phosphate, pH 7.0, 1 mM EDTA and resuspended in 30 mL of the same buffer. Resuspensions were then disrupted by passage through a French Pressure cell at 1280 psi. Cell extracts were obtained by an initial centrifugation for 30 min at 17,000 *g* and an additional centrifugation for 2 h at 255,629 *g*. Solid ammonium sulfate was added to the supernatants at 55% and then centrifuged for 10 min at 10,000 *g* at 4ºC to remove the precipitated proteins. The ammonium sulfate concentration was then raised to 85% and the precipitated proteins were pelleted by centrifugation at 10,000 g and resuspended in 20 mM sodium phosphate, pH 11.0 to neutralize the acidity of the salt solution (final pH was roughly 7.5). The His-tagged protein was generally easier to obtain in EPR-spectroscopically pure form than the non-His-tagged protein because without additional chromatography an unwanted paramagnetic protein, probably a bacterial ferredoxin with EPR features near g = 2, was present. In order to eliminate this unwanted paramagnetic protein, an anion exchange step using DEAE Fast Flow 1 mL prepackaged columns (Amersham Biosciences) was incorporated.[1] The non-His-tagged cyt *c′* was bound to the column at low ionic strength (0.03 M pH 7.2 Tris) and eluted at a slightly higher ionic strength (0.03 M pH 7.2 Tris, 0.07 M NaCl) while the unwanted protein was retained on the column.

Comparison of the sequences of cyt c' from *R. Sphaeroides* 2.4.3 and cyt c' from *R. Sphaeroides* 2.4.1 where the latter is indicated as being the same as *R. Sphaeroides* 2.4.1 from Ramirez et al.[2] The differences of 2.4.1 are denoted by bold face type.

2.4.3 ADAERVVEAR KGFFSLVALE FGPLAAMAKG EMPYDADAAK AHAADLVALT
R26 ADAE**H**VVEAR KG**Y**FSLVALE FGPLAAMAKG EMPYDA**A**AAK AHA**S**DLV**T**LT

2.4.3 KYNPSDLYAP GTSADDVKGT GAKAAIWQDM DAYQKKGMAF FEAVAALEPV
R26 KY**D**PSDLYAP GTSADDVKGT **A**AKAAIWQD**A** D**GF**Q**A**KGMAF FEAVAALEP**A**

2.4.3 AGNGQKELGA AVNKVGATCK SCHDDFRAKN
R26 AG**A**GQKEL**A**A AV**G**KVG**G**TCK SCHDDFRVK**R**

The sequence of cyt *c′* with His tag from *R. Sphaeroides* 2.4.3 has the His tagged C-terminal sequence attached after the final N, HHHHHH.

For comparison to the relevant His-tagged cyt *c′* EPR spectrum of Figure 2, proton ENDOR of Figures 3c and 4c, and nitrogen ENDOR of Figure 5c, we provide the corresponding spectra of the non-His-tagged protein respectively in Figure 1S (EPR), 2S and 3S (proton ENDOR), and 4S (nitrogen ENDOR).

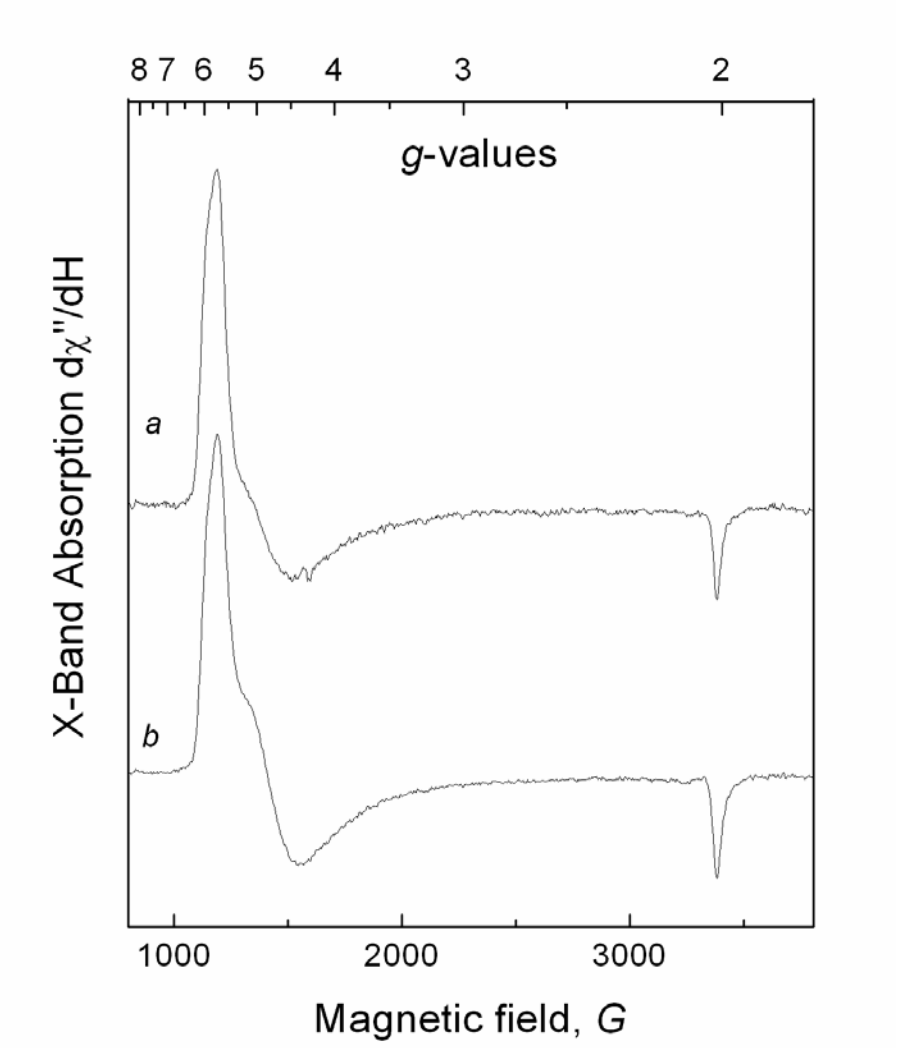

Figure 1S. This figure provides a comparison of the absorption (dχ”/dH) X-band EPR spectra of His-tagged cyt *c*' (**a**) and non-His-tagged (**b**) cyt *c′* together with an overlay of the g-values. The conditions used were the conditions used for Figure 2 in the body of the paper.

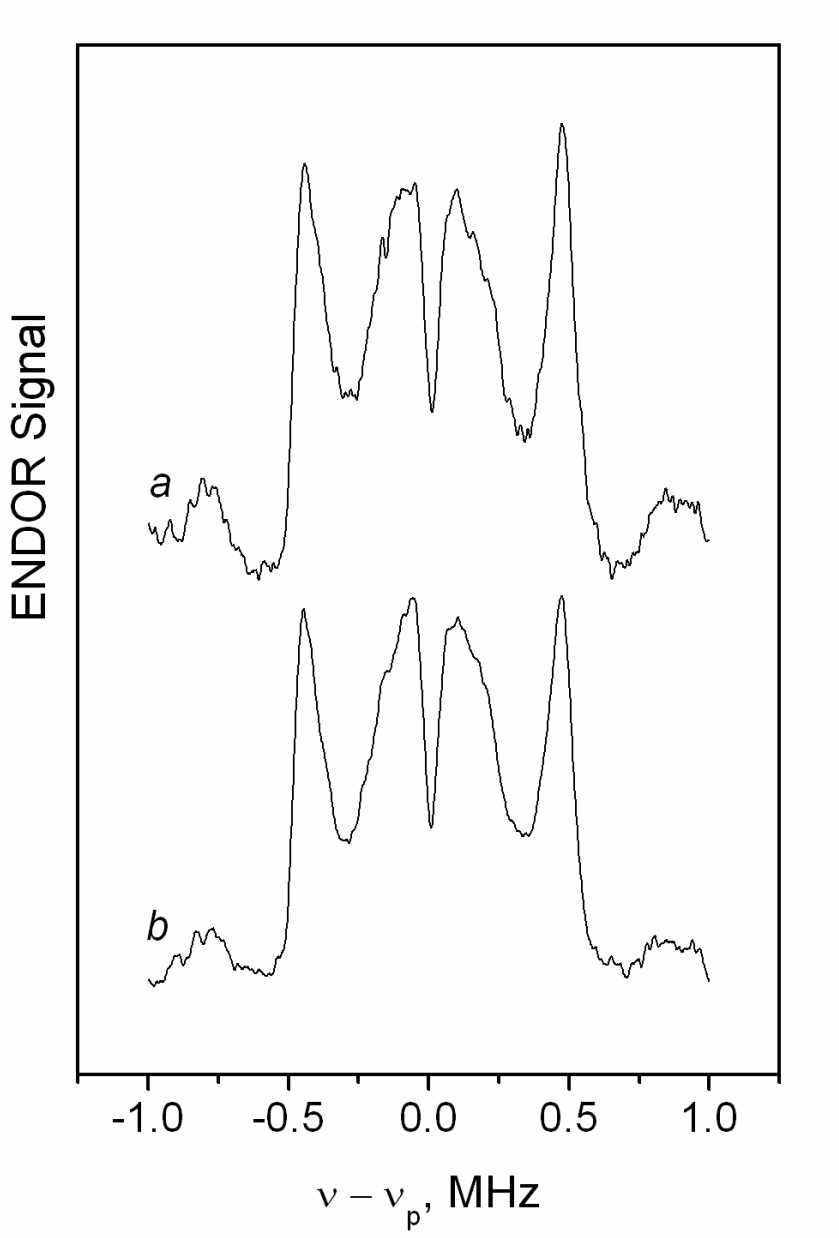

Figure 2S. This figure provides a comparison of weakly coupled proton features from His-tagged cyt *c*' (**a**) and non-His-tagged (**b**) cyt *c*′ as they occur within $\pm$ 1 MHz of ${}^{P}\nu_{NMR}$. The conditions used were the conditions used for Figure 3 in the body of the paper.

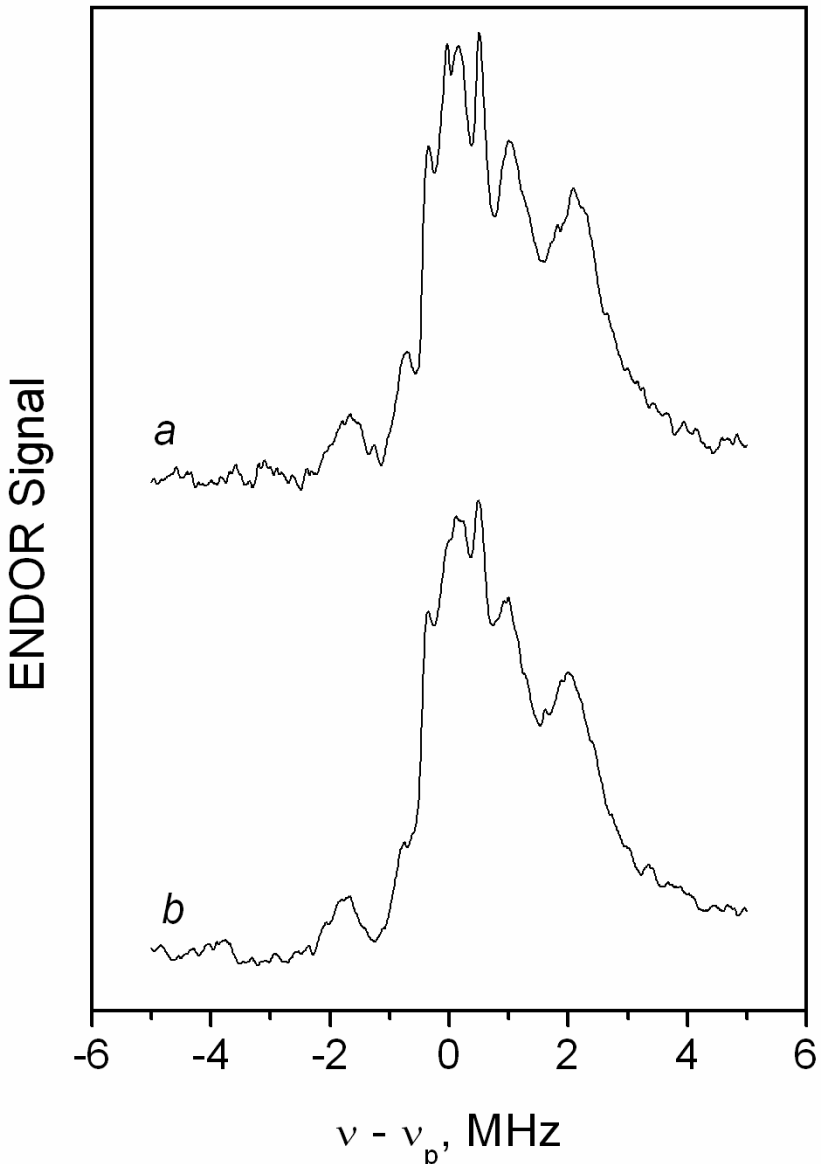

Figure 3S. This figure provides a comparison of strongly coupled proton features from His-tagged cyt *c*' (**a**) and non-His-tagged (**b**) cyt *c'*. The conditions used were the conditions used for Figure 4 in the body of the paper.

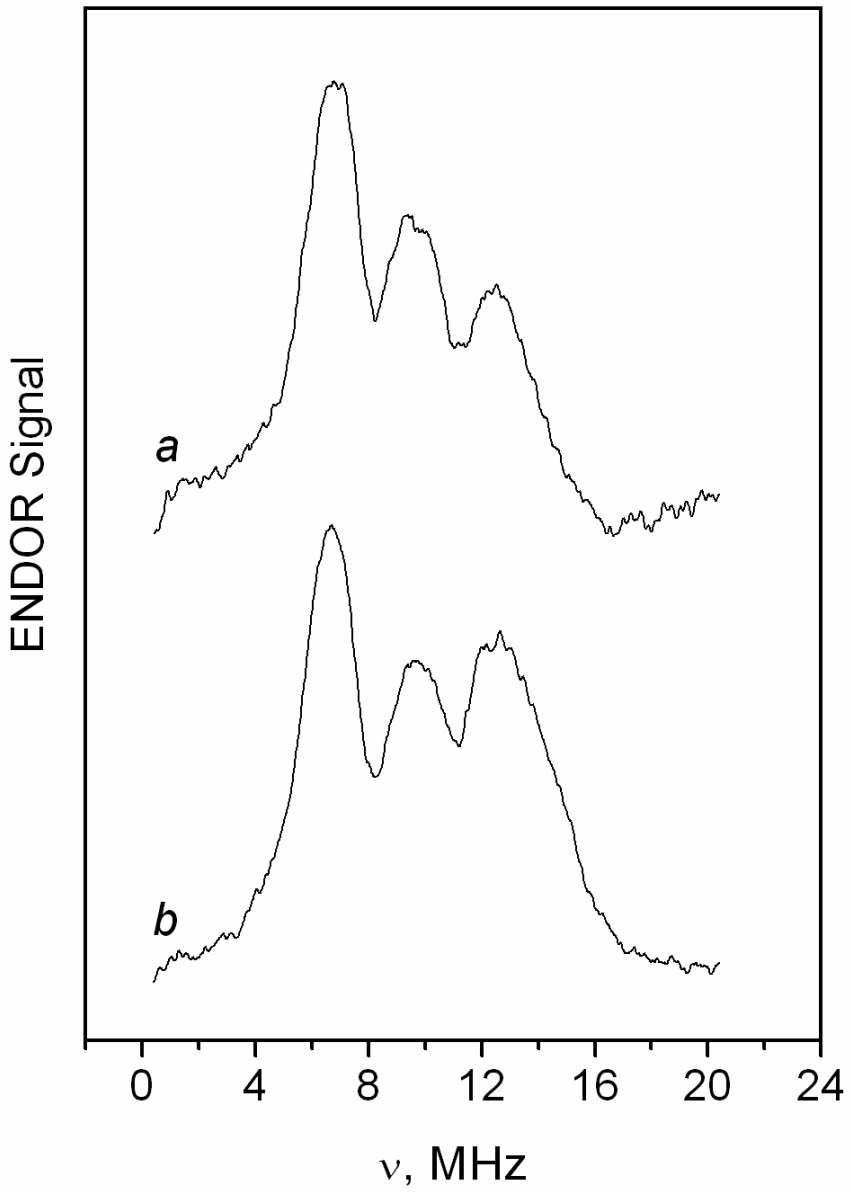

Figure 4S. This figure provides a comparison of nitrogen ENDOR from His-tagged cyt *c*' (**a**) and non-His-tagged (**b**) cyt *c'*. The conditions used were the conditions used for Figure 5 in the body of the paper.

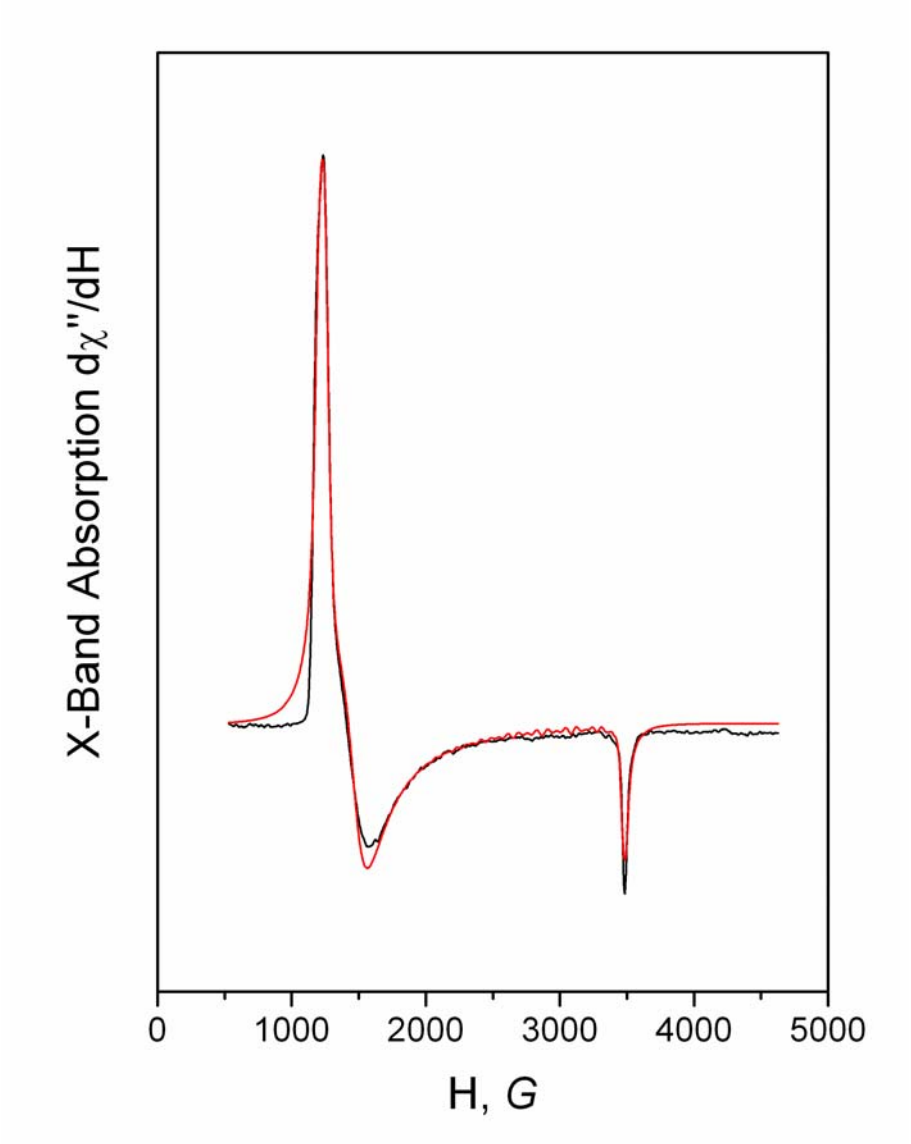

Figure 5S. This figure compares the X-band EPR spectrum of non-his-tagged cyt c' with a simulated EPR spectrum, where the purpose of the simulation was to provide estimates of the g-values of the low field region. The program SIMPIPM[3] was used to calculate the spectrum and to obtain parameters which internally optimize the fit of experimental and fitted data. A rhombic g-tensor was used and the fitted g-values were g_x, g_y, g_z = 5.55, 4.70, 2.00; corresponding fitted linewidths were W_x, W_y, W_z = 240, 860, and 60 Gauss. This main rhombic component accounted for 95 % of the integrated simulated spectrum. In the fitting procedure a small axial component with $g_\perp$ = 5.72 and $g_\parallel$ = 2.05 accounted for the other 5 %.

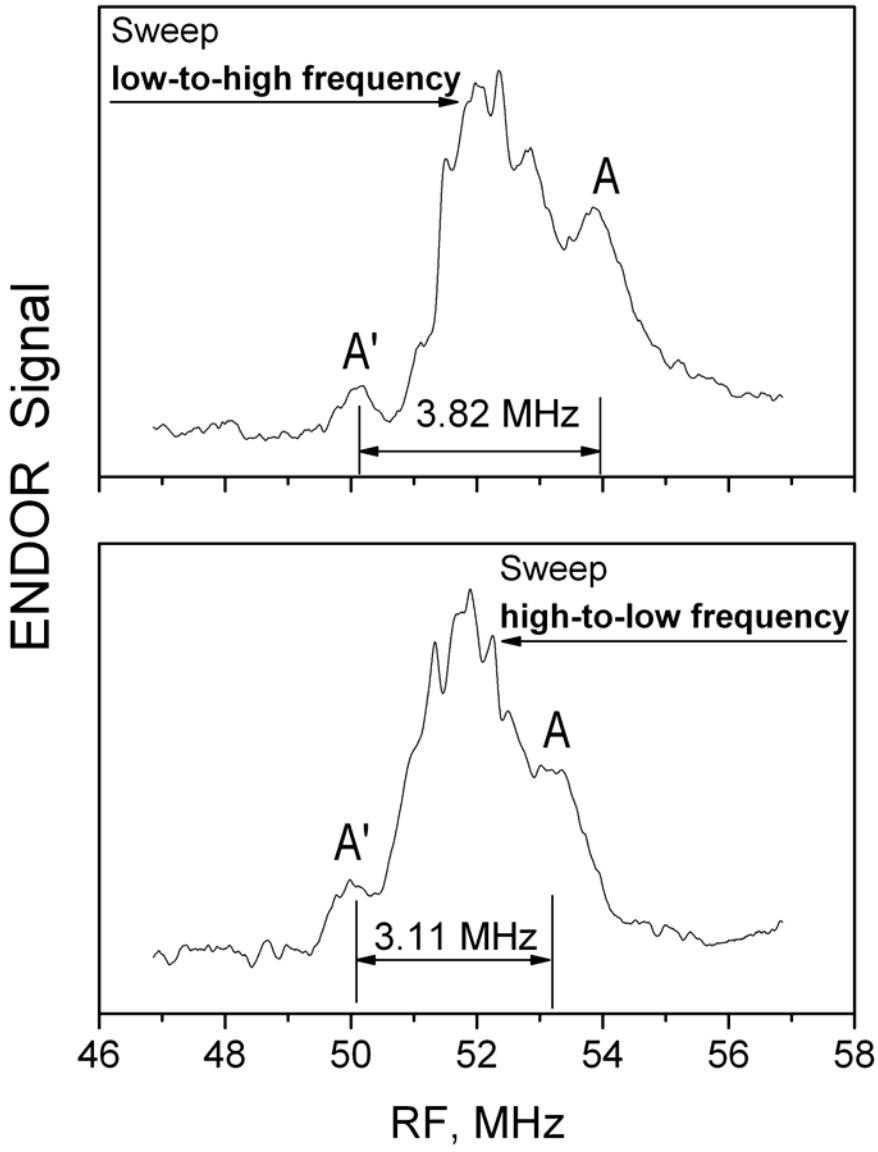

Figure 6S. Figure 6S shows the strongly coupled proton ENDOR spectra of cyt *c*' in protonated solvent from both upward and downward frequency sweeps within $\pm$ 5 MHz of the free proton frequency over the range shown in Figure 4c. The purpose was to obtain estimates of the splitting of features A,A' which are broad features whose passage ENDOR signals are distorted in the direction of the sweep. The average splitting of these features is 3.47 ± 0.35 MHz.

References

(1) Meyer, T. E.; Cusanovich, M. A. *Biochimica et Biophysica Acta* **1985**, *807*, 308-319.

(2) Ramirez, L. M.; Axelrod, H. L.; Herron, S. R.; Rupp, B.; Allen, J. P.; Kantardjieff, K. A. *Journal of Chemical Crystallography* **2003**, *33*, 413-424.

(3) SIMPIPM IS AN EXTENSION OF PIP WHICH IS AN EXTENSION OF QPOW. Nilges, M. J., Belford, R. L., and Francesconi, L. C. "Simulation of strain in EPR spectra using the method of gradients," 40th Rocky Mountain Conference on Anal. Chem., Denver, Colorado, July 1998. For QPOW, see Nilges, M. J., Thesis, University of Illinois, Urbana, 1979.