

Supplementary material to

‘A Multi-Frequency Pulse EPR and ENDOR Approach to Study Strongly Coupled Nuclei in Frozen Solutions of High-Spin Ferric Heme Proteins’

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Figure captions

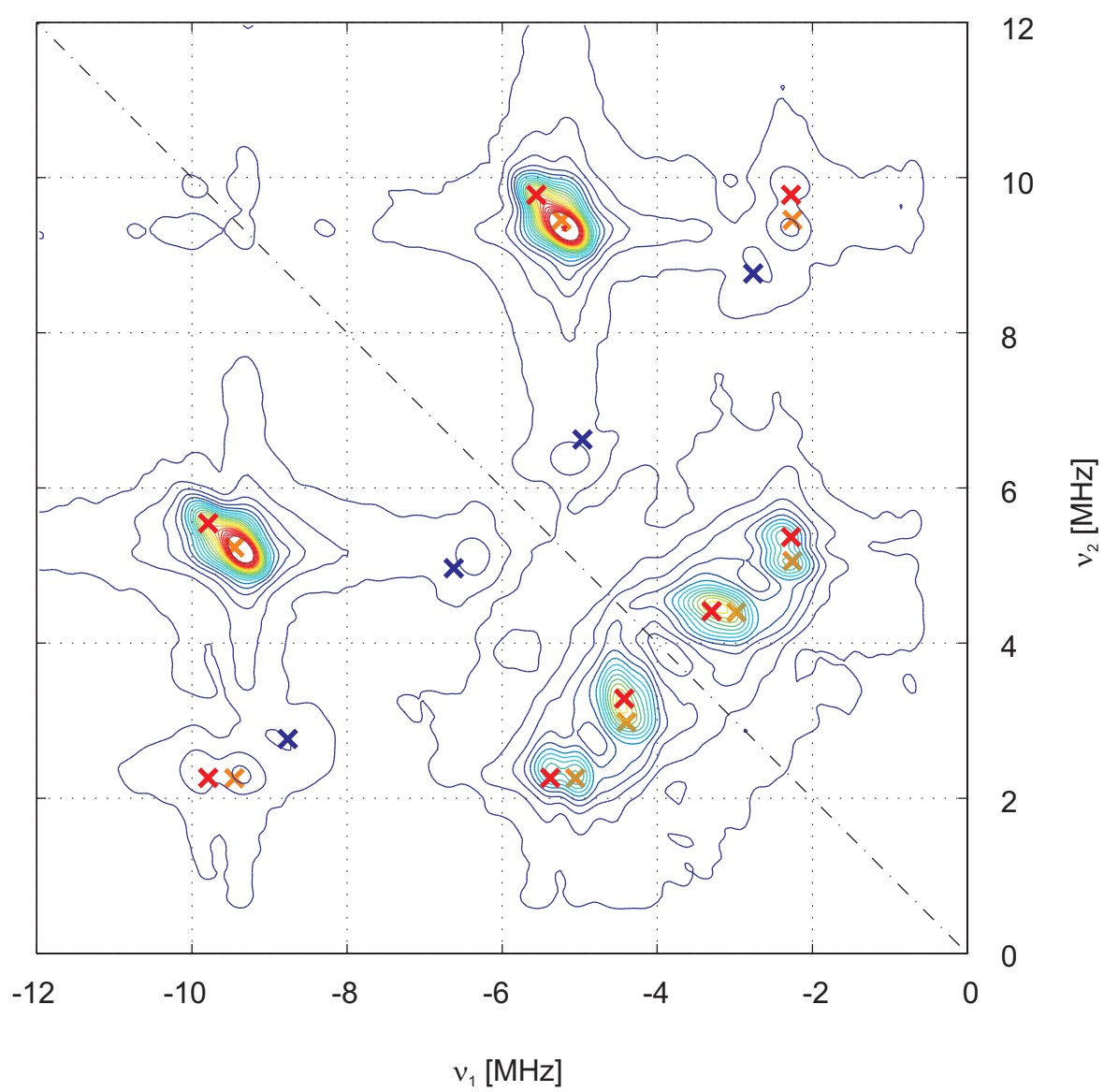
Figure S1. X-band HYSCORE spectrum of a frozen solution of metMb taken at 3.8 K and $B_0 = 349$ mT corresponding with $g = g_z^{eff}$. $\pi/2$ and π pulse lengths of 16 ns were taken and $\tau = 80$ ns. Simulations of the correlation ridges of the two porphyrin (red and orange) and histidine nitrogens (blue) using the parameters collected in Table 2 are shown.

Figure S2. X-band SMART HYSCORE of metMb in $^2\text{H}_2\text{O}$. The high turning angle (HTA) pulses lengths were tuned to optimize the modulation depth of the coupled nitrogens. (A) Spectrum acquired at $B_0 = 117$ mT $g \approx g_y^{eff}$, HTA pulse length was 52 ns and $\tau = 132$ ns. (B) $B_0 = 240$ mT, HTA pulse length was 24 ns and $\tau = 96$ ns. Simulations of the correlation ridges of the porphyrin (red) and histidine nitrogens (purple) using the parameters collected in Table 2 are shown.

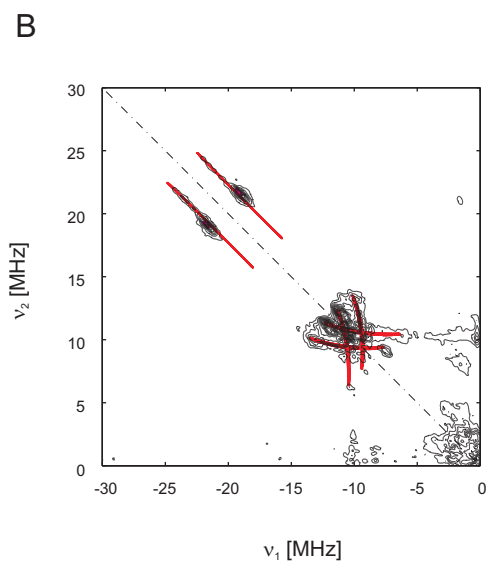
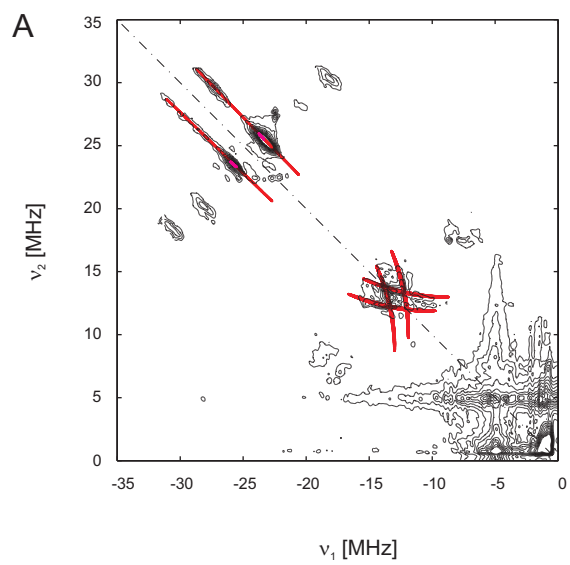
Figure S3. Simulations of the N(His) X-band HYSCORE spectrum at $g \approx g_y^{eff}$ ($B_0 = 117.2$ mT) performed using the GAMMA HYSCORE program.

Figure S4. (A) Q-band standard HYSCORE spectrum of a frozen solution of metMb taken close to $g \approx g_y^{eff}$, $B_0 = 462$ mT, $T = 5.5$ K. The $\pi/2$ pulse lengths were 24 ns, the length of the inversion π pulse was 16 ns. $\tau = 80$ ns. (B) W-band standard HYSCORE spectrum of a frozen solution of metMb at $g = g_y^{eff}$, $B_0 = 1.146$ mT, $T = 3.8$ K, The $\pi/2$ pulse lengths and the length of the inversion π pulse were 16 ns. The spectrum is the sum of three spectra with $\tau = 80, 128$ and 192 ns. Simulations of the correlation ridges calculated of the two porphyrin nitrogens (red and orange) using the parameters collected in Table 2 are shown. (C) Closer view of (A).

Figure S5: (B) X-band standard HYSCORE spectrum of metMb labelled with ^{17}O -labeled water at $B_0 = 320$ mT measured at 3.8 K. $\pi/2$ and π pulse lengths of 16 ns and $\tau = 128$ ns were used. (C) The simulation of correlation ridges of the ^{17}O (green), of the porphyrin (red) and histidine (magenta) nitrogens using the parameters given in Table 2 are shown.

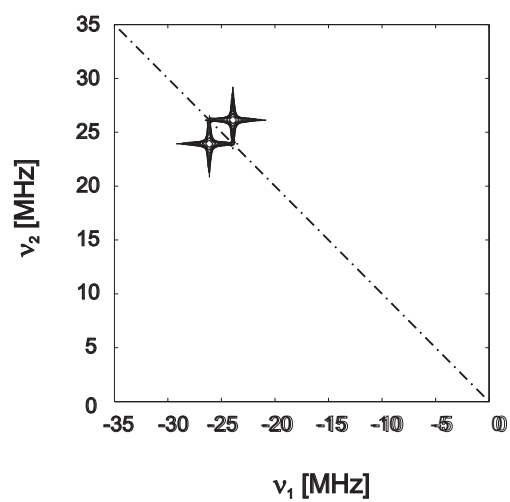


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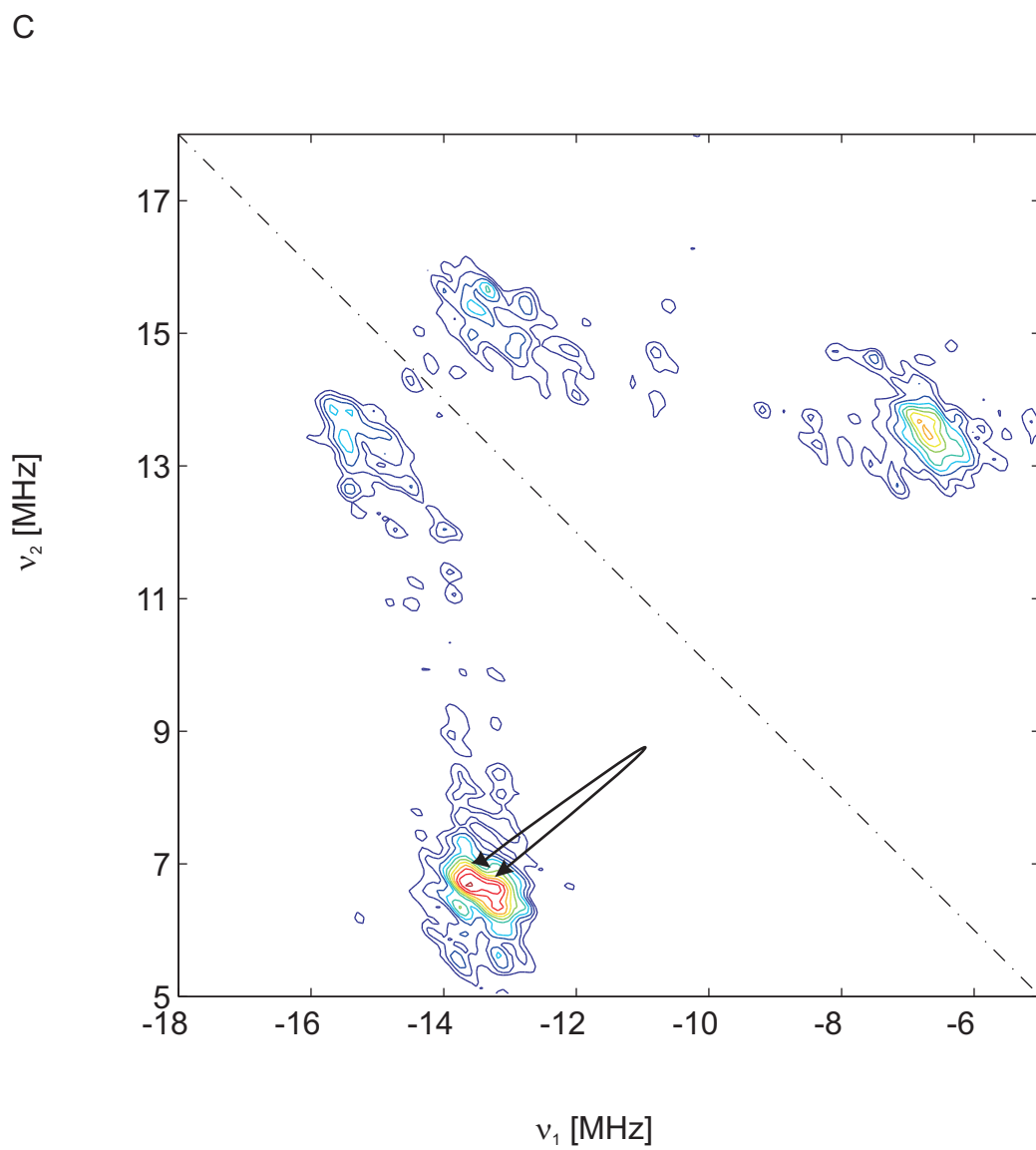
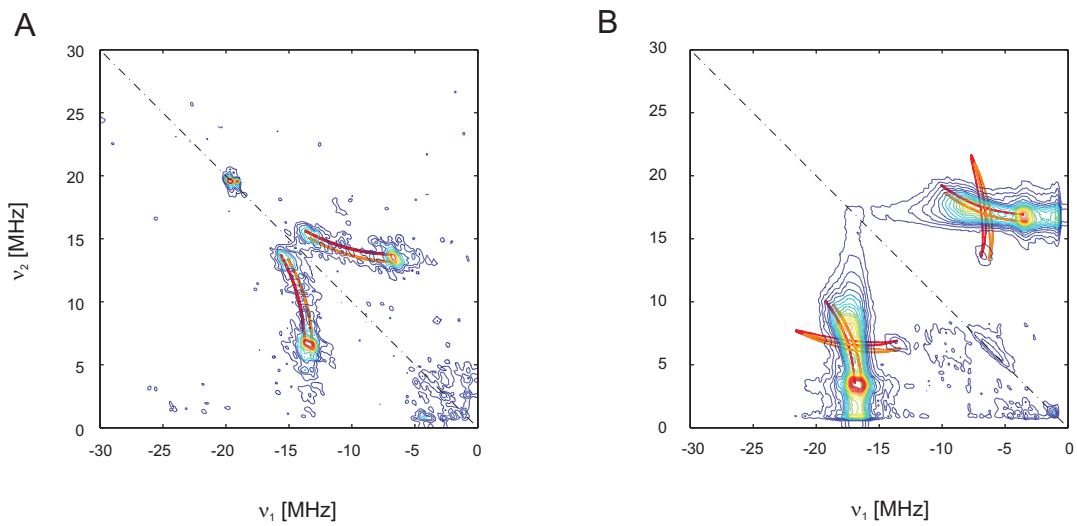


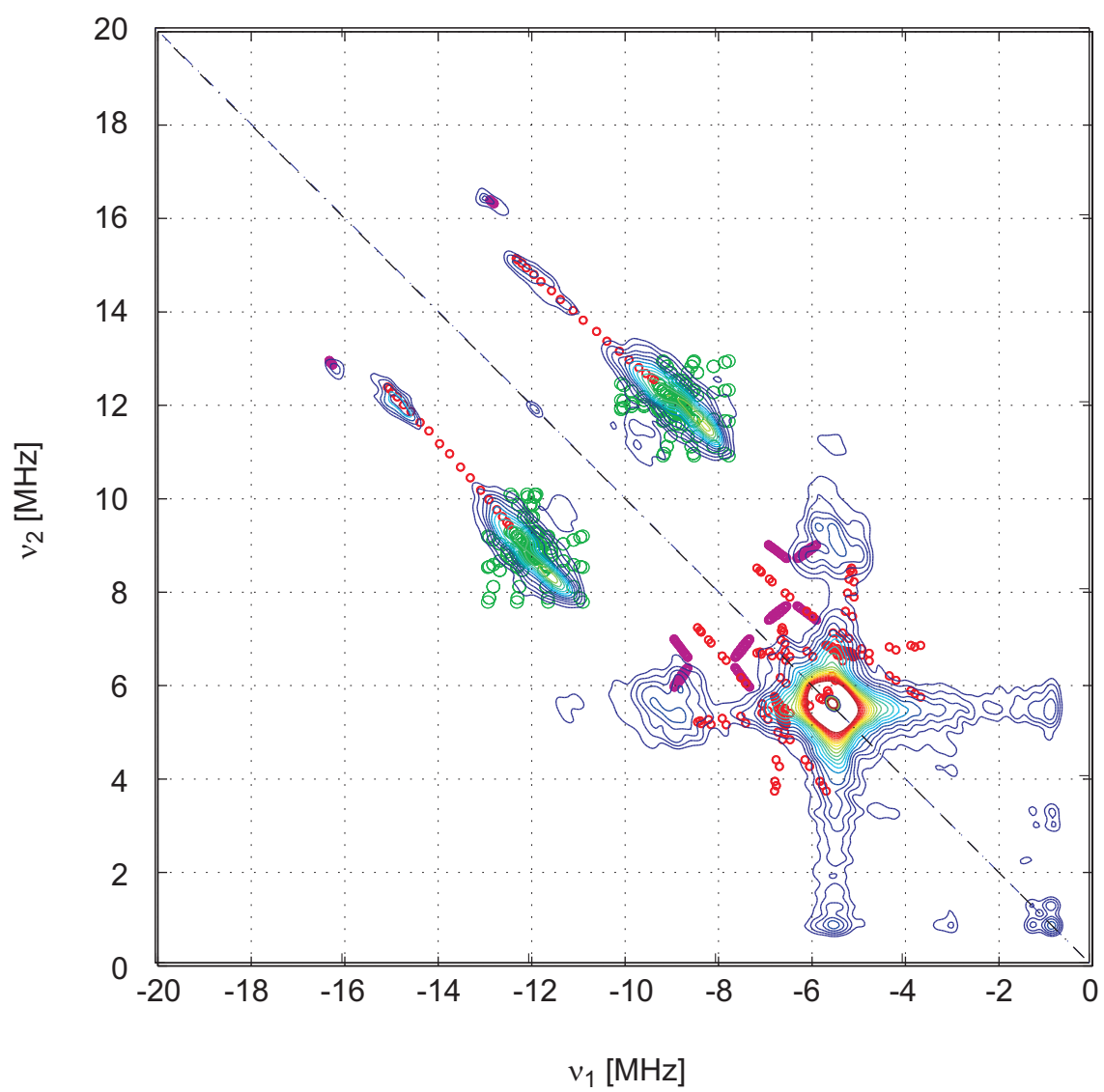
S2

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S3





S5