SUPPLEMENTARY MATERIAL OF

Determining large hyperfine interactions of a model flavoprotein in the semiquinone state by pulse-EPR techniques

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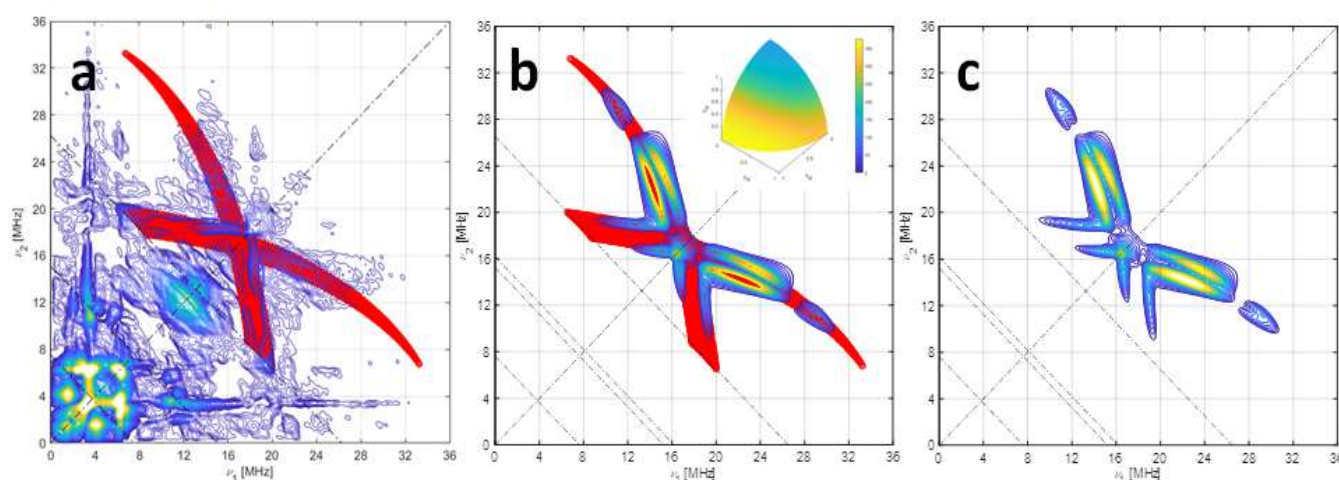


Figure S.1: HYSCORE of ^{13}C labelled Fld variants. a) [^{13}C(2,4a)-FMN]-Fld spectrum with the calculated HYSCORE pattern for a ^{13}C nucleus with the parameters specified in the text superimposed in red, b) Simulated spectrum of ^{13}C(4a) using the function saffron from EasySpin. Superimposed in red, the calculated HYSCORE pattern. c) Simulated spectrum of ^{13}C(4a) using the function saffron from EasySpin. The inset in the central spectrum shows the orientation selection of the experimental spectra in a sphere octave according to the 20   colors of the accompanying scale.

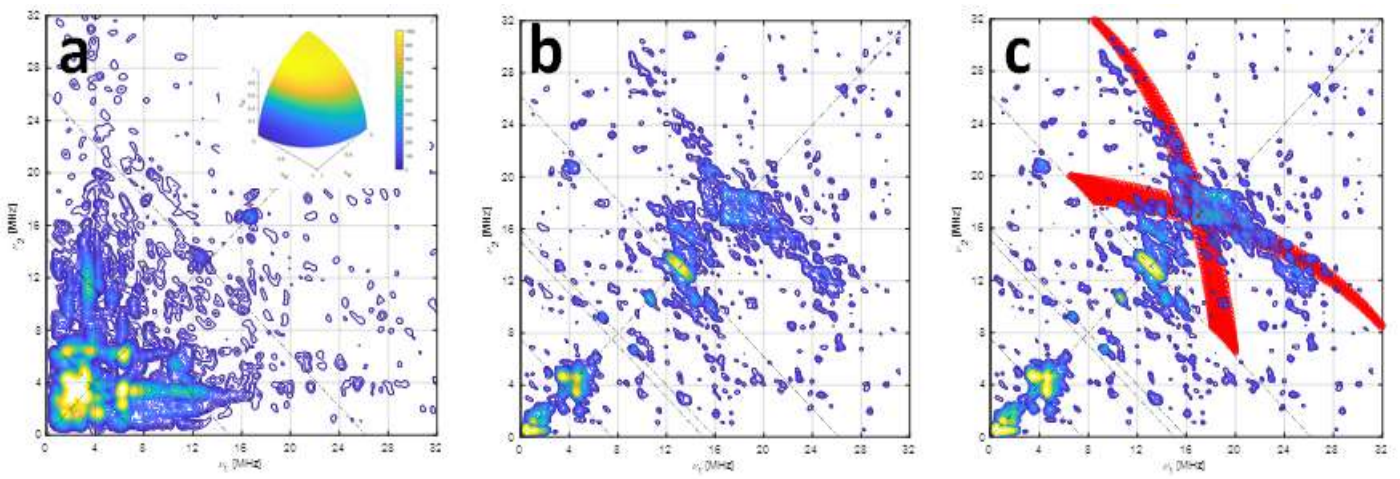
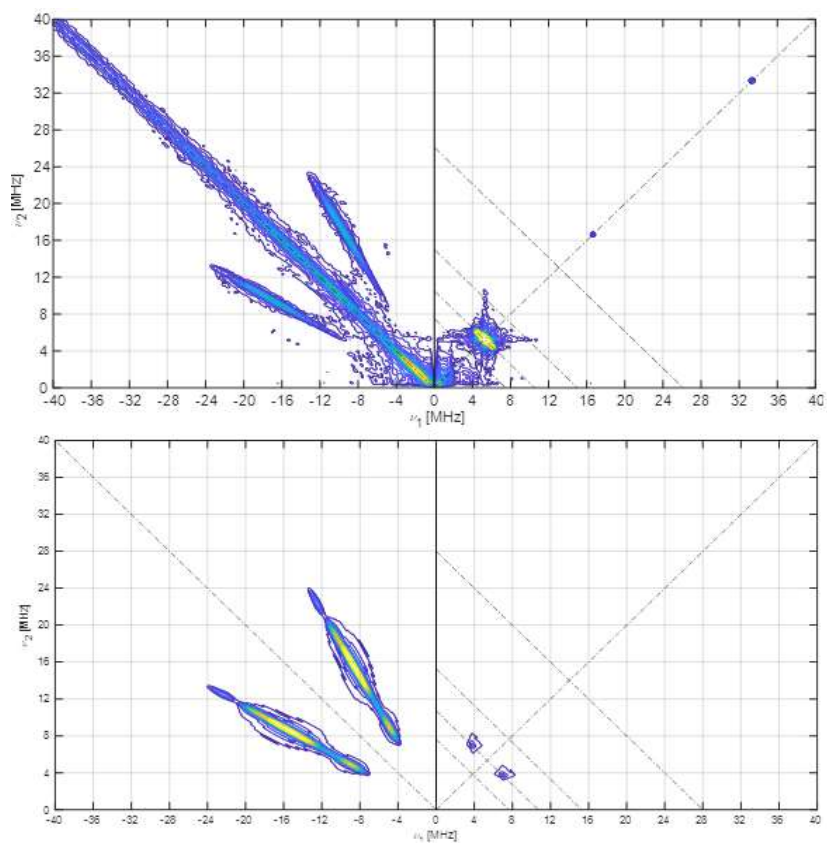



Figure S.2: HYSCORE of ^{13}C labelled Fld variants at the low-field tail of the CW-EPR spectrum. a) [^{13}C(2)-FMN]-Fld spectrum, b) [^{13}C(2,4a)-FMN]-Fld spectrum, c) [^{13}C(2,4a)-FMN]-Fld spectrum with the calculated HYSCORE pattern for a ^{13}C nucleus with the parameters specified in the text superimposed in red. Both experimental spectra were taken at the low-field tail of the CW-EPR spectrum corresponding to the loose paralel orientation selection shown in the inset of spectrum a. B = 1221.4 mT, τ = 124 ns and T = 50 K. Antidiagonal lines cross the diagonal at the Larmor frequencies ν_{14N}, $2 \cdot \nu_{14N}$ and ν_{13C}.

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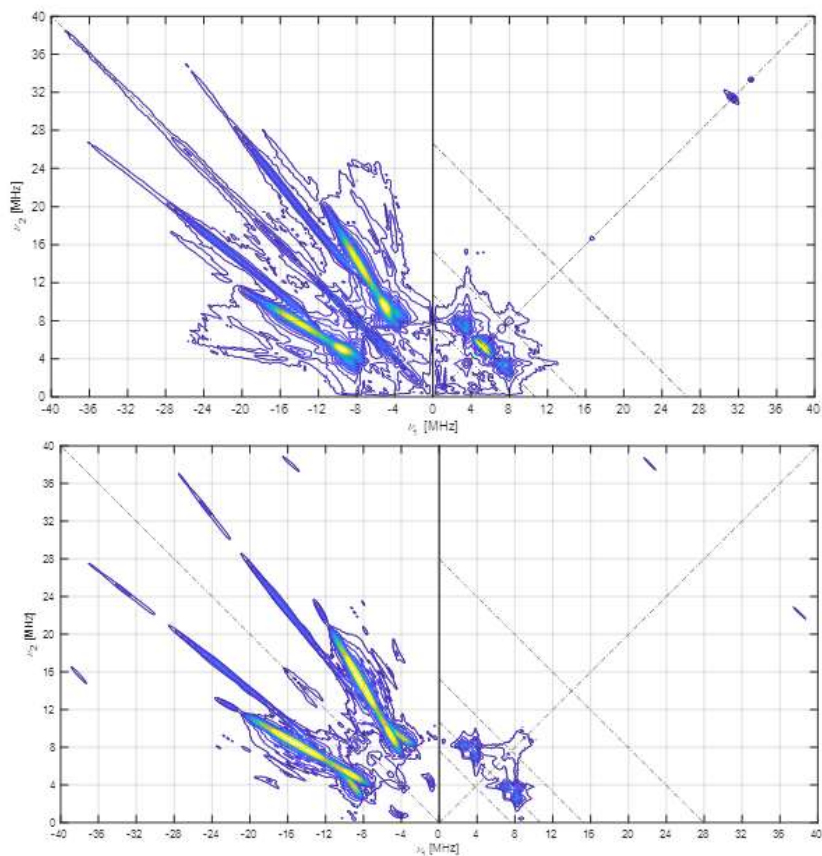
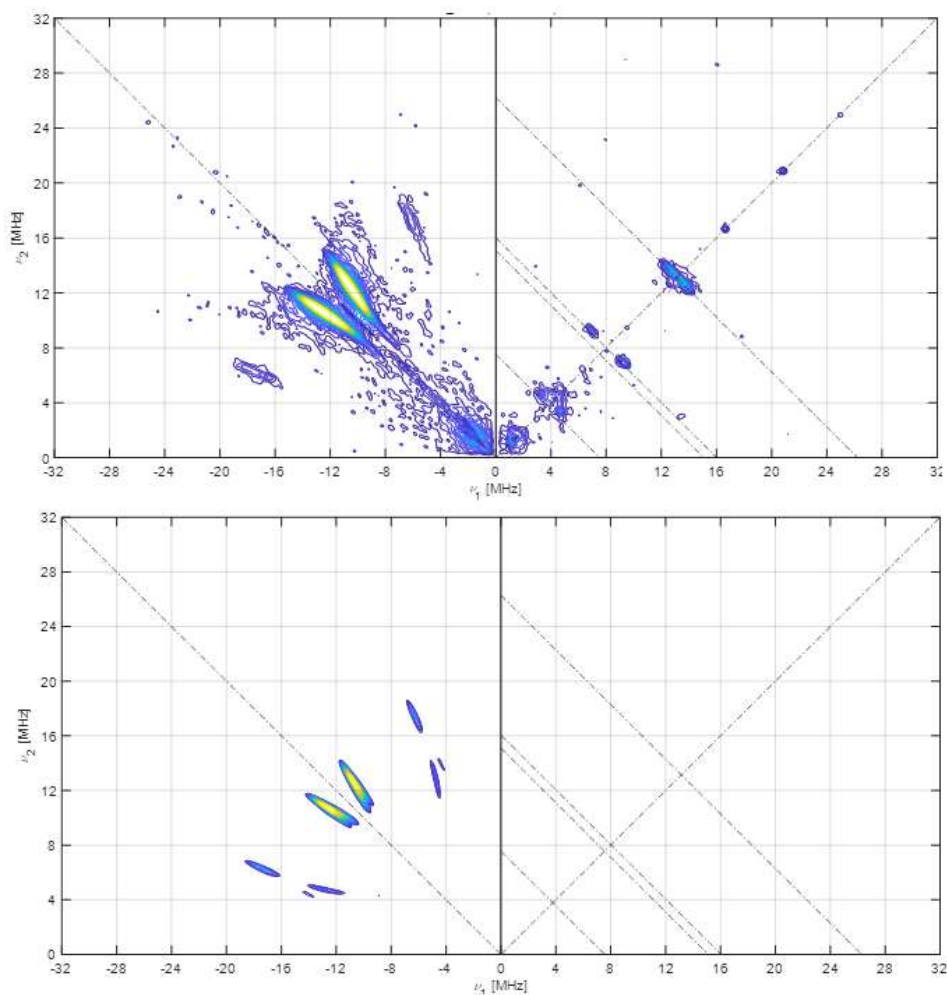
30   **Figure S.3: HYSCORE simulation of [^{15}N-FMN]-Fld variant at the high-field edge of the EPR spectrum.** a) Experimental spectrum, B = 1219.7 mT, sum of τ values of 96, 124, 144 and 168 ns. It is the same shown in Fig. 5.a of the main text but is depicted also here for comparative purposes. b) Simulation for the same experimental conditions using the parameters specified in the text and in Table 1. The antidiagonal line at the Larmor frequency ν_{15N} has been included for reference.

40 **Figure S.4: HYSCORE simulation of [^{15}N-FMN]-Fld variant at the high-field edge of the EPR spectrum.** a) Experimental spectrum, B = 1217.2 mT, sum of τ values of 96, 144 and 168 ns. It is the same shown in Fig. 5.b of the main text but is depicted also here for comparative purposes. b) Simulation for the same experimental conditions using the parameters specified in the text and in Table 1. The antidiagonal line at the Larmor frequency ν_{15N} has been included for reference.

45 **Figure S.5: HYSCORE simulation of ^{14}N in [^{13}C(2)-FMN]-Fld at the low-field edge of the EPR spectrum.** a) Experimental spectrum, B = 1225.0 mT, sum of τ values of 96, 112, 128, 144 and 176 ns. It is the same shown in Fig. 6.a of the main text but is depicted also here for comparative purposes. b) Simulation for the same experimental conditions using the parameters specified in the text and in Table 1. The antidiagonal line at the Larmor frequencies ν_{14N}, $2·\nu_{14N}$, ν_{2H} and ν_{13C} have been included for reference.

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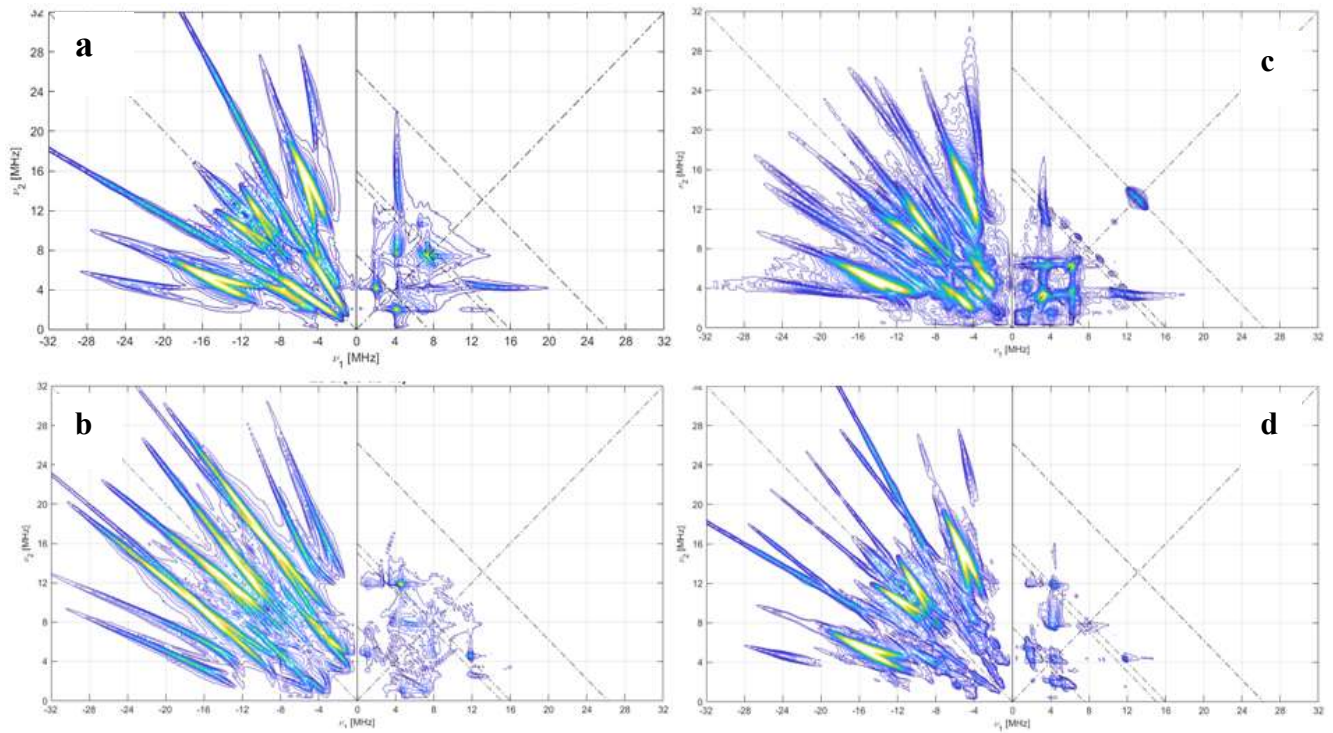
Figure S.6: HYSCORE simulations of ^{14}N in [^{13}C(2)-FMN]-Fld at the center of the EPR spectrum. a) Simulation of features associated to N(10), b) Simulation of features associated to N(5), c) Simulation of N(10) and N(5), d) Spectrum obtained from the sum of experimental spectra taken at τ values of 96, 128 and 208 ns, B = 1221.0 mT and T = 50 K. The simulations have been performed using the same τ values together with the coupling parameters specified in the text and in Table 1. The antidiagonal lines crossing the (+,+) diagonal at the Larmor frequencies ν_{14N}, $2 \cdot \nu_{14N}$, ν_{2H} and ν_{13C} have been included for reference.

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