Supporting Information.

Resolving ligand hyperfine couplings of type I and II Cu(II) in Ascorbate Oxidase by high field pulse EPR correlation spectroscopy

Alexey Potapov[+], Israel Pecht[#], Daniella Goldfarb[+*]
Departments of [+]Chemical Physics and [#]Immunology, Weizmann Institute of Science, Rehovot Israel 76100

ENDOR spectra.

The ENDOR spectra were simulated using the parameters given in the Figure caption to Figure S3.

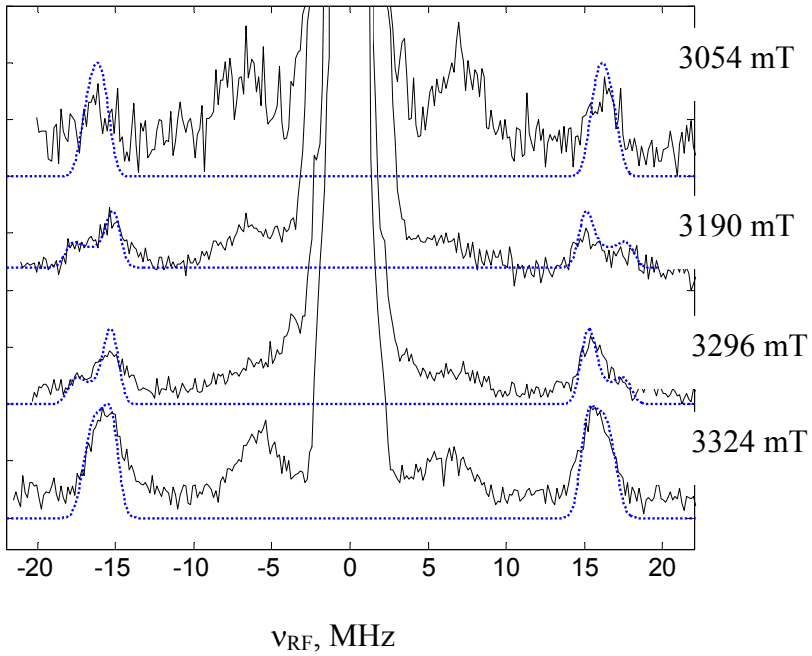

Figure S1 [1]H-ENDOR spectra recorded at different field positions(solid line) along with simulations (dotted lines). MW pulses t_{90}/t_{180}=100/200 ns, t_{inv}=200 ns t_{RF}=25 μs. Measurement temperature 10 K. Spectra were simulated with A_{xx},A_{yy},A_{zz}=[30 30 36] MHz and Euler angles $(\alpha,\beta,\gamma)=(0, 50, 0)^0$. The spectra are simulated using EasySpin[1]

HYSCORE spectra.

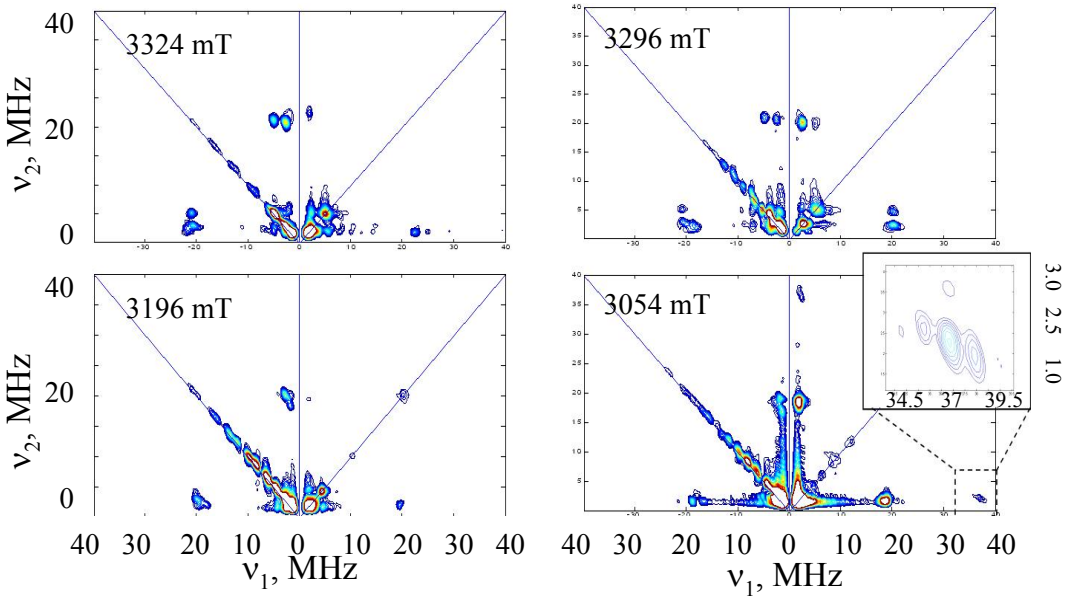

Figure S2 HYSCORE spectra recorded at the field positions marked with arrows in Fig. 3 of the main text. MW pulses t90/t180=12.5/25 ns, τ=137.5 ns, intervals t_1 and t_2 are incremented with dwell time of 12.5 ns in 90 steps; measurement temperature 10 K.

HYSCORE simulation.

In order to estimate the quadrupolar and hyperfine couplings we focus at the single quantum lines in the region ~(2, 20) MHz. The hyperfine coupling elongates the cross peaks perpendicular to diagonal of the (+,+) quadrant. The quadrupolar interaction in turn elongates the peaks along ω_α-axis and ω_β-axis. From the maximum spread of the frequencies parallel to the diagonal we can therefore estimate the largest quadrupole splitting to be ~4.5 MHz, yeilding Q_{zz} ~1.5 MHz and e^2qQ/h~3 MHz. This is in agreement with earlier W-band ESEEM measurements on a single crystals of azurin provide for e^2qQ/h 2.4 and 2.9 MHz.[2]

Taking e^2qQ/h~3 MHz together with estimates of the hyperfine coupling anisotropy from the ELDOR-detected NMR measurements we simulated the HYSCORE spectra. Due to the presence of two ^{14}N nuclei the total number of simulation parameters is rather large, specifically when the two different ^{14}N nuclei do not show distinct features that can be identified. Therefore, instead of attempting to carry out exact simulations we adapted a more qualitative approach where focused on line positions only, assuming that the two ^{14}N are equivalent. Accordingly, the simulations were

done using only one ^{14}N nucleus and these are shown in Fig. S3. We see that in general there is a reasonable agreement between the line positions and their trend with respect to the change in the magnetic field position, although at the lower field end the calculated frequencies of the high frequency manifold are somewhat higher than the experimental one.

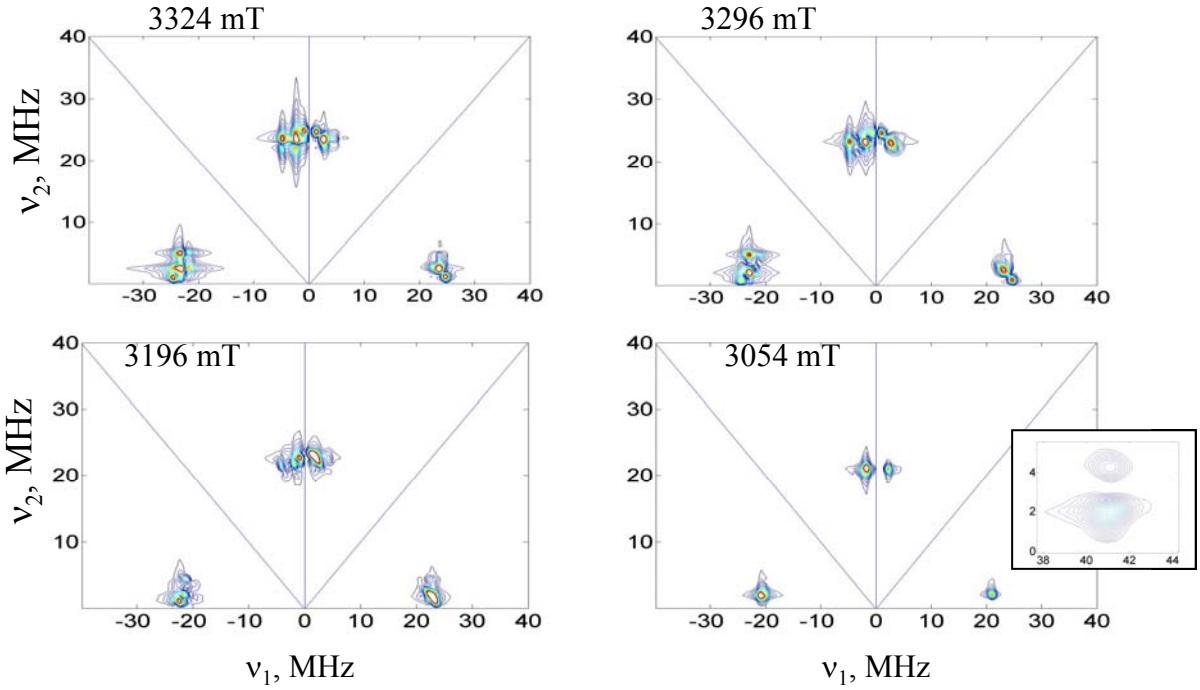

Figure S3 The simulation of nitrogen HYSCORE with parameters: Aiso=23 MHz, T=(-1,-1,2) MHz, hyperfine interaction Euler angles with respect to the g principal axes system (0,90,0)0. e2qQ/h=3 MHz, h=0.5 and quadrupole interaction Euler angles (0,60,0)0. The simulations were carried out using the SimBud program[3].

Table S1. The dihedral angles of the β-protons of AO determined by four different ways (see main text) and the sulfur spin density ρ_s derived from them for A_{iso}=32, 13 MHz.

Method	Angles φ, H1	Angles φ,H2	ρ_s
Pymol program	50.27	-71.2	0.59
MolDen program	44.23	-66.8	0.48
plastocyanin[6]	-44.68	66.92	0.72
cysteine[4]	-45.08	69.92	0.5

Table S2. The dihedral angles of the β-protons of several T1 determined by two different ways and the sulfur spin density derived from them.

	Hyperfine couplings, MHz		Angles as given by plastocyanin crystal structure		Angles as given by cysteine crystal structure[4]		ρ_S		
	A_{iso1}	**A_{iso2}**	**φ,H1**	**φ, H2**	**φ,H1**	**φ, H2**	**1st method**	**2nd method**	**refs**
Plastocyanin (poplar)	27	16	-50.41	66.18	-45.68	69.32	0.45	0.3	5,6
Azurin	25	18	-54.76	61.84	-50.16	64.84	0.45	0.22	2,7
ascorbate oxidase	32	13	-49.68	66.92	-45.08	69.92	0.72	0.5	this work,8
Stellacyanin (cucumber)	16	13	-57.8	58.8	-53.2	61.8	1.91	0.22	9,10
Plastocyanin (spinach)	23	17	-51.58	65.02	-46.98	68.02	0.29	0.18	9,11,

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