Supplementary Information for:

Multifrequency EPR Studies on the Mn(II) Centers of Oxalate Decarboxylase

by

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Supplementary Information for Materials & Methods:

Several different batches of OxDC enzyme preparations were used. They were prepared according to the procedures listed in the Materials & Methods section of this paper. Final concentrations ranged from 7.7 to 12.3 mg/mL. Samples in HMTA pH6.0 were used without further modifications. Samples in 50mM acetate buffer (AB) pH5.2 were prepared from stock by addition of a concentrated acetate buffer solution (500 mM, pH5.2). Typically, 90 μL of sample were mixed with 10 μL of concentrated AB buffer. The volumes were scaled in the same ratio for experiments requiring smaller (W-band) or larger quantities (sub-mm bands). The same procedure was used for the addition of formate to the HMTA buffered samples. 10 μL of 500 mM formate solution was added to 90 μL of HMTA pH6.0 buffered sample to arrive at a final concentration of 50 mM formate.

To convince ourselves that there was no nonspecifically bound Mn(II) in the enzyme samples used for data collection, two methods were used to prepare samples and blanks for determination of metal content by ICPMS (University of Wisconsin Soil and Plant Analysis Lab). In the first method, approximately 0.2 mM enzyme sample (200 μL of ~10 mg/mL) was prepared in 1 mM ethylenediaminetetracetic acid (EDTA) and incubated on ice for 15 minutes. The sample was then desalted on a G-25 pasteur pipet column equilibrated with dH2O with the desalting column having been previously treated with EDTA. 200 μL of enzyme storage buffer was put through an identical procedure for use as a blank. In the second method, divalent cations were removed from the final enzyme storage buffer by passing through a 1.5 x 16 cm column containing Chelex 100 (Bio-Rad) in the Na^+ form. Purified protein samples were exchanged into the resulting buffer by washing 2.5 mg sample three times with ten-fold volumes of the "scrubbed" buffer in Centricon or Centriprep 30 (Amicon) concentrators. The final filtrates were recovered and used as blanks and did not possess significant metal content. This second method is identical to the method used to prepare samples for EPR data collection. ICP-MS results for the two methods were nearly identical, indicating that our samples do not have nonspecifically bound Mn(II). EPR measurements were performed on the metal-free storage buffer and the flowed-through solution of the Centricon 30 concentrators as controls and revealed no significant presence of Mn(II).

EPR Spectroscopy:

For X-band the OxDC samples were placed in 3x4 mm^2 (IDxOD) homemade CFQ (clear fused quartz) tubes and frozen in liquid nitrogen before insertion into the Oxford ESR900 cryostat which had been pre-cooled to ~10 K.

For W-band the samples were placed into 0.7x0.79 mm^2 (IDxOD) CFQ capillaries. The samples were then frozen in liquid nitrogen prior to insertion into the precooled Bruker ER4118CF-W cryostat.

For V-band and sub-mm bands the samples were placed into 7.2x8.2 mm^2 (IDxOD) home-made Teflon cups. The cups have a depth of 9.5 mm and were supplied with a Teflon stopper. Typically, 200 μL of sample was inserted into the cup which was then closed with the stopper to protect the sample from contamination. The sample was pre-frozen in liquid nitrogen, the field standard (P-doped Si sample) was then placed on top of the stopper before it was inserted into the sample holder. The sample holder was also pre-cooled to liquid nitrogen temperatures before it was inserted into the pre-cooled Oxford Spectrostat CF DY LT cryostat. Field calibration was performed using a piece of P-doped silicon as described before by Petrenko *et al*. in *Biochemistry* **2004**, *43*, 1781. This standard has a g-value of 1.99850 and a hyperfine coupling constant of 117.53 MHz (41.986 G) as given by Feher in *Physical Review* **1959**, *114*, 1219, and its EPR lines show up in many of the high-field EPR spectra as two weak but sharp lines.

The physical meaning of the fine structure parameters *D* and *E* can be found in the deviation of the ligand field from spherical and axial symmetry, respectively. *D*- and *E*-strain denotes the inhomogeneity of these values which depend critically on the metal-ligand distances and bond angles. Fundamentally, the fine structure parameters describe the splitting of the ^6S ground state of the Mn^{2+} ion due to spin-spin and spin-orbit interactions which are anisotropic because the ligand field is anisotropic. In first order these interactions lead to a splitting of the various EPR transitions between the electron multiplet states. In higher order they lead to line broadening and possibly splitting effects which are mainly apparent on the narrow central sextet that arises from the $|+½\rangle \leftrightarrow |-½\rangle$ transitions. With increasing external magnetic field these contributions to the lineshape of the EPR signals become less pronounced because the Zeeman interaction becomes stronger and the higher order spin-spin and spin-orbit interactions become less important in comparison. For an excellent review see ref. (19) of the main paper.

The spin-lattice relaxation rates of the Mn(II) ions detected was fairly substantial. Therefore, the temperature for the EPR measurements was adjustd to achieve maximum signal intensities while minimizing spectral distortions. Typically, X-band experiments were performed at 5 K while the experiments at V-band and higher frequencies were carried out at 20-50 K.

The EPR instrumental parameters for each data set shown in figures 1-3 of the manuscript are given in the captions of the figures S1-S15 in this supplement together with the spectral simulations.

Supplemental Material for Spectral Simulations of EPR spectra at different field/frequency combinations of OxDC in storage buffer (SB) pH6.0:

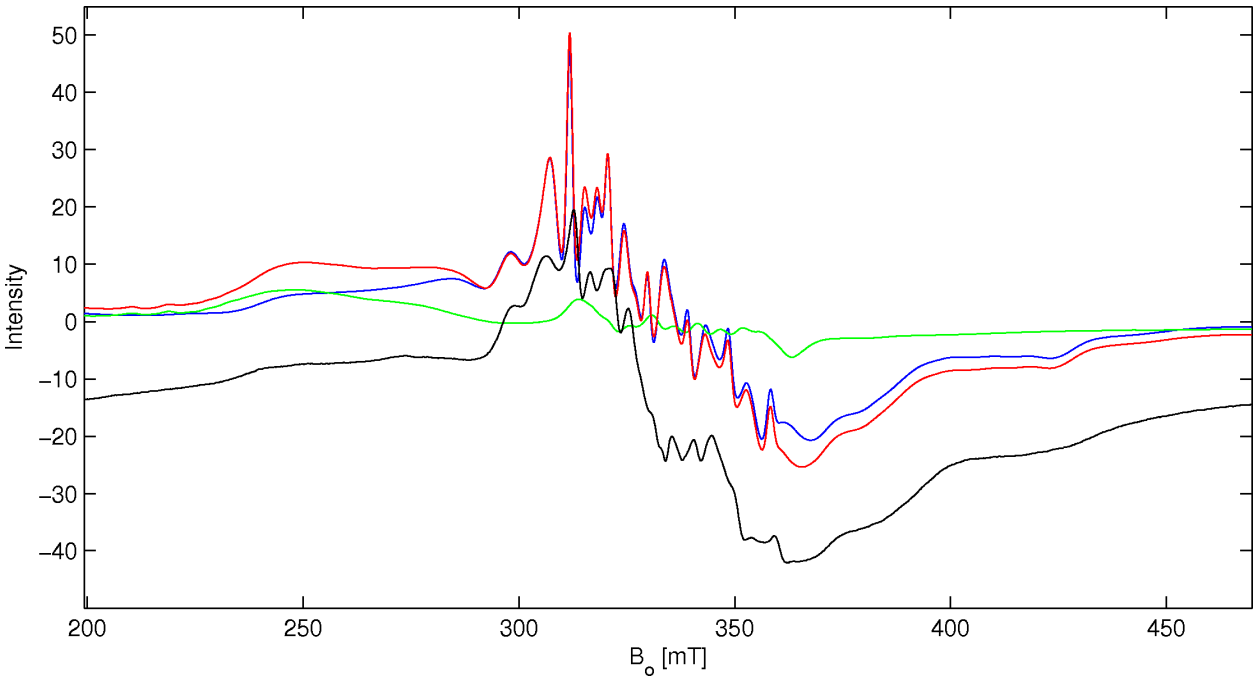

Figure S1: X-band spectrum of OxDC in SB pH6.0 at T = 10 K. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation of the two sites is shown in red.
Experimental parameters: Microwave frequency 9.48731 GHz, microwave power 0.64 mW, modulation frequency 100 kHz, modulation amplitude 10 G, receiver gain 60 dB, time constant 41 ms, conversion time 41 ms, 1 sweep, 1.465 G/data point.
Simulation parameters for site I: g_{iso} = 2.000865, A_{iso} = 254 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.24×D, E-Strain = 0.24×E, linewidth$_{iso}$ = 33 MHz.
Simulation parameters for site II: g_{iso} = 2.00094, A_{iso} = 248 MHz, D = 2750 MHz, E = 660 MHz, D-Strain = 0.20×D, E-Strain = 0.20×E, linewidth$_{iso}$ = 33 MHz.

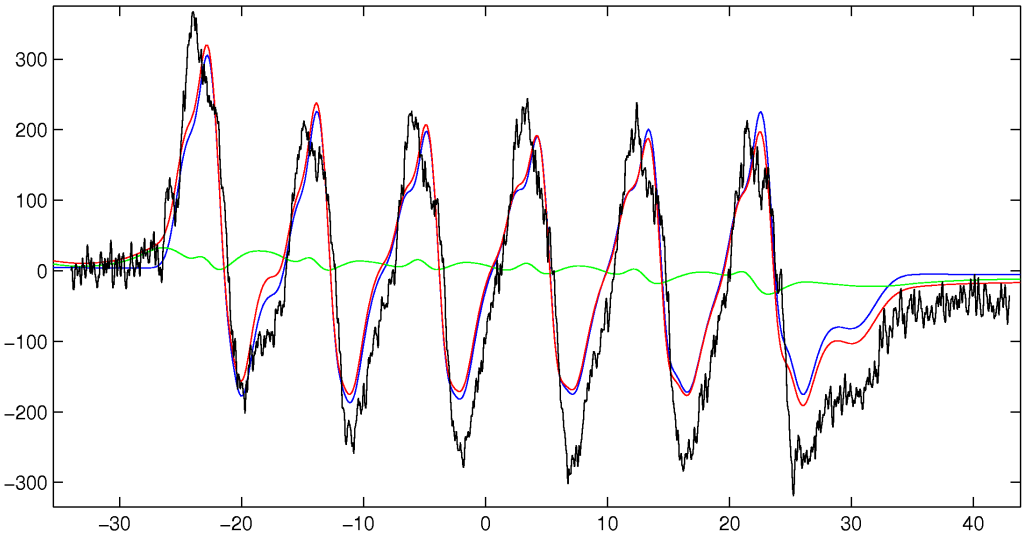

Fig. S2: V-band spectrum of OxDC in SB pH6.0 at T = 20 K. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation of the two sites is shown in red. The zero point of the x-axis (magnetic field) is at 1.7530 T. Tic marks are given in mT.
Experimental parameters: Microwave frequency 49.200 GHz, microwave power corresponding to a dector signal of 500 mV, modulation frequency 41.68 kHz, modulation amplitude 4 G, lock-in sensitivity 200 μV, time constant 300 ms, sweep speed 1 G/s, 1 sweep, 0.250 G/data point, center field 1.753 T.
Simulation parameters for site I: g_{iso} = 2.000865, A_{iso} = 254 MHz, D = 1200 MHz, E = 276 MHz, D-Strain = 0.24×D, E-Strain = 0.30×E, linewidth$_{iso}$ = 33 MHz.
Simulation parameters for site II: g_{iso} = 2.00094, A_{iso} = 248 MHz, D = 2700 MHz, E = 675 MHz, D-Strain = 0.25×D, E-Strain = 0.20×E, linewidth$_{iso}$ = 33 MHz.

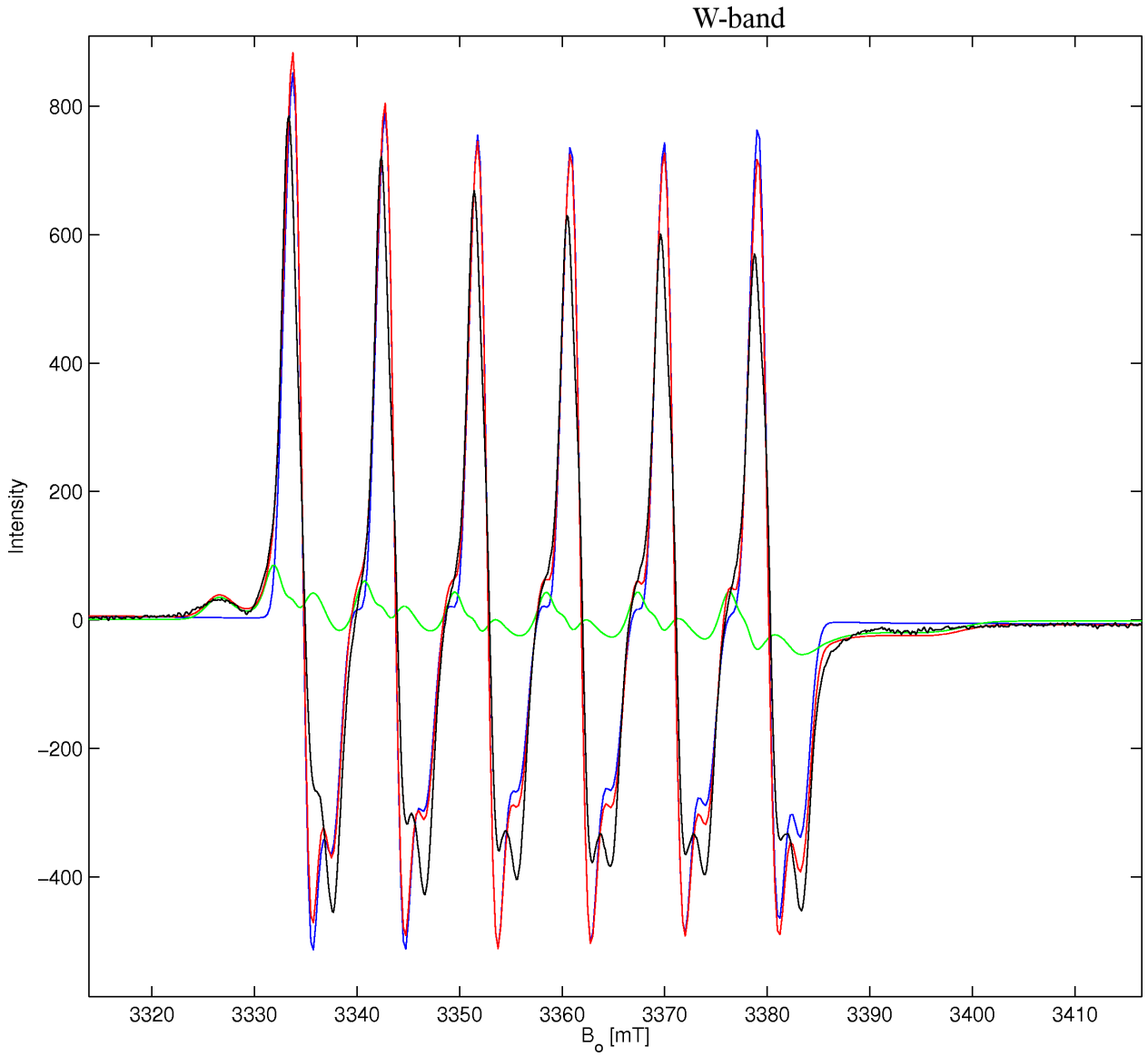

Figure S3: W-band EPR spectrum of OxDC in SB pH6.0 at T = 50 K. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation traces of the two sites is given in red.
Experimental parameters: Microwave frequency 94.02141 GHz, microwave power 0.6 µW modulation frequency 100 kHz, modulation amplitude 2 G, receiver gain 40 dB, time constant 164 ms, conversion time 164 ms, 1 sweep, 1.172 G/data point.
Simulation parameters for site I: g_{iso} = 2.000865, A_{iso} = 254 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.24×D, E-Strain = 0.24×E, linewidth$_{iso}$ = 33 MHz.
Simulation parameters for site II: g_{iso} = 2.00094, A_{iso} = 248 MHz, D = 2700 MHz, E = 648 MHz, D-Strain = 0.20×D, E-Strain = 0.20×E, linewidth$_{iso}$ = 33 MHz.

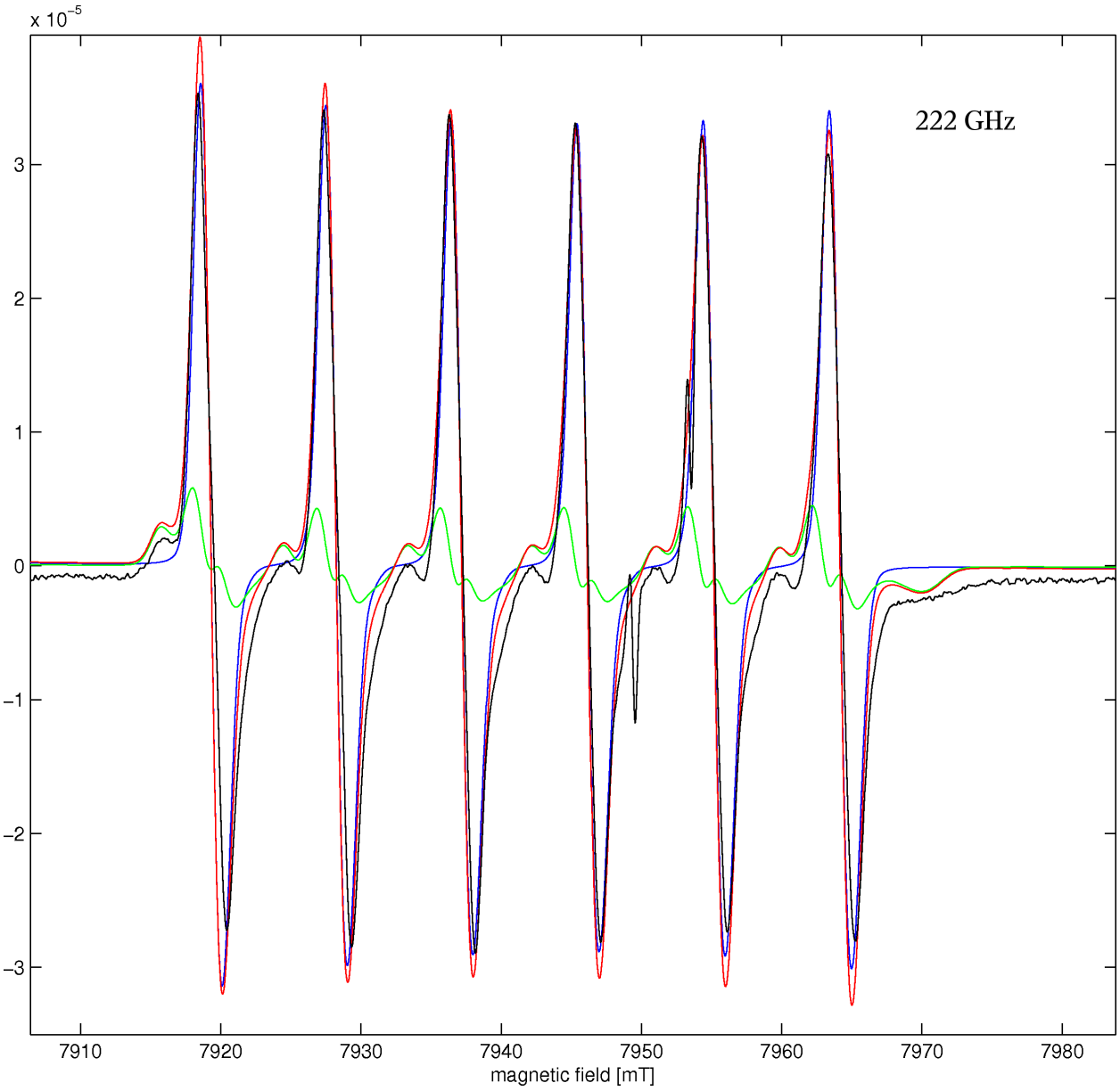

Figure S4: sub-mm EPR spectrum of OxDC in SB pH6.0 at T = 20 K and 222 GHz. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation of the two sites is shown in red.
Experimental parameters: Microwave frequency 222.400 GHz, modulation frequency 41.8 kHz, modulation amplitude 0.5 G, lock-in sensitivity 500 µV, time constant 100 ms, sweep speed 5.01 G/s, 1 sweep, 0.368 G/data point.
Simulation parameters for site I: g_{iso} = 2.000865, A_{iso} = 251 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.24×D, E-Strain = 0.24×E, linewidth$_{iso}$ = 33 MHz.
Simulation parameters for site II: g_{iso} = 2.00094, A_{iso} = 247 MHz, D = 2700 MHz, E = 675 MHz, D-Strain = 0.20×D, E-Strain = 0.20×E, linewidth$_{iso}$ = 33 MHz.

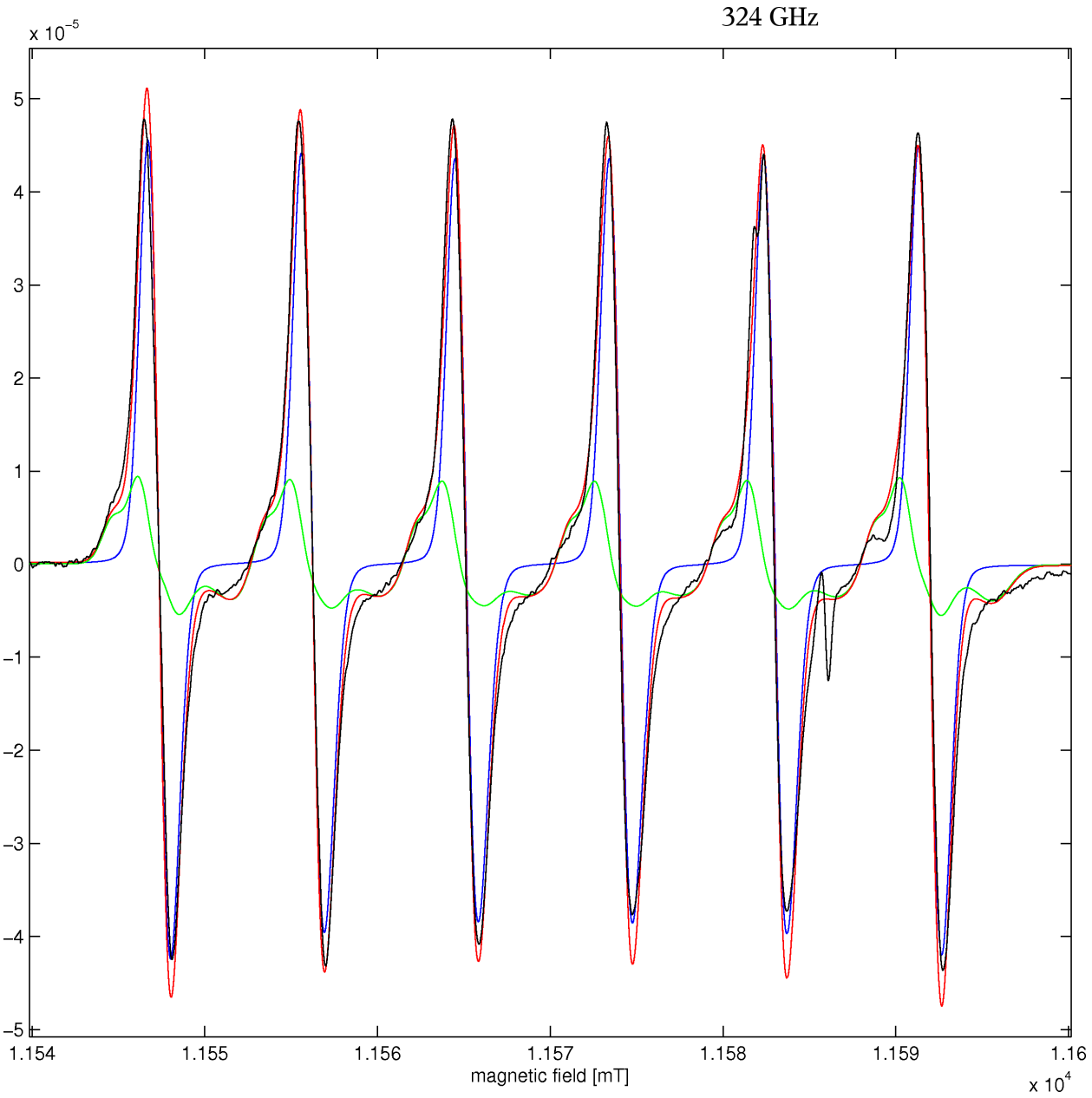

Figure S5: sub-mm EPR spectrum of OxDC in SB pH6.0 at T = 20 K and 324 GHz. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation traces of the two sites is given in red. Experimental parameters: Microwave frequency 324.00 GHz, modulation frequency 41.8 kHz, modulation amplitude 0.5 G, lock-in sensitivity 50 µV, time constant 100 ms, sweep speed 5.01 G/s, 1 sweep, 0.367 G/data point.
Simulation parameters for site I: g_{iso} = 2.000865, A_{iso} = 250 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.24×D, E-Strain = 0.24×E, linewidth$_{iso}$ = 33 MHz.
Simulation parameters for site II: g_{iso} = 2.00094, A_{iso} = 247 MHz, D = 2700 MHz, E = 675 MHz, D-Strain = 0.20×D, E-Strain = 0.20×E, linewidth$_{iso}$ = 33 MHz.

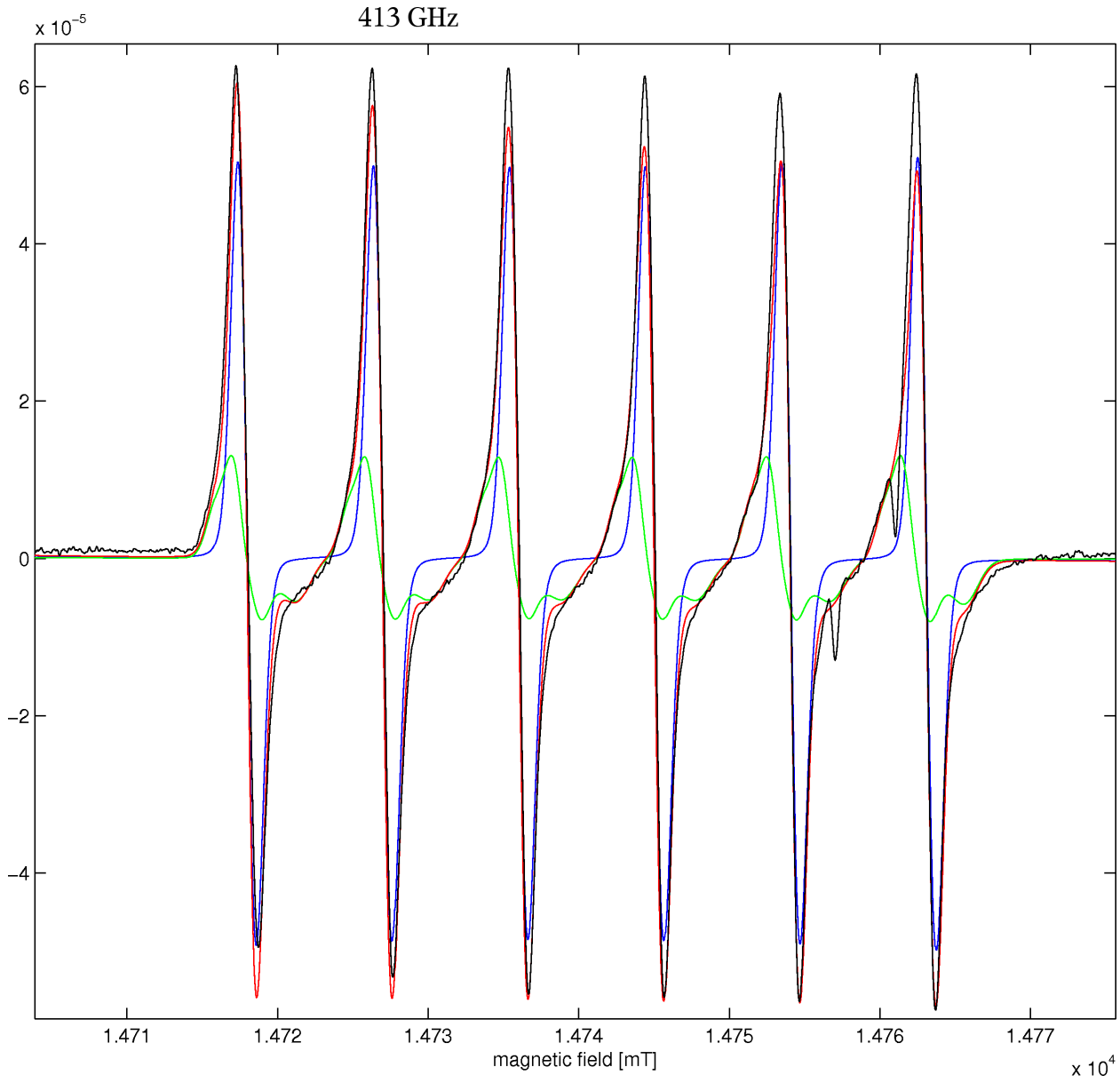

Figure S6: sub-mm EPR spectrum of OxDC in SB pH6.0 at T = 20 K and 412.8 GHz. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation traces of the two sites is given in red. Experimental parameters: Microwave frequency 412.800 GHz, modulation frequency 41.8 kHz, modulation amplitude 4 G, lock-in sensitivity 500 µV, time constant 100 ms, sweep speed 5.01 G/s, 1 sweep, 0.369 G/data point.
Simulation parameters for site I: g_{iso} = 2.000865, A_{iso} = 253 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.24×D, E-Strain = 0.24×E, linewidth$_{iso}$ = 33 MHz.
Simulation parameters for site II: g_{iso} = 2.00093, A_{iso} = 249 MHz, D = 2700 MHz, E = 675 MHz, D-Strain = 0.25×D, E-Strain = 0.25×E, linewidth$_{iso}$ = 33 MHz.

Spectral Simulations for EPR spectra at different field/frequency combinations of OxDC in acetate buffer (AB) pH5.2:

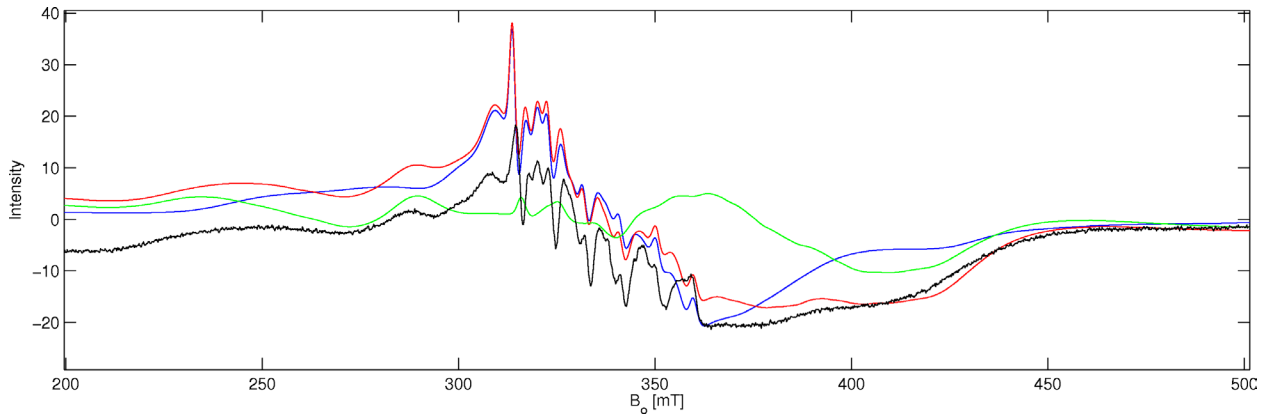

Figure S7: X-band spectrum of OxDC in AB pH5.2 at T = 10 K. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation of the two sites is shown in red.
Experimental parameters: Microwave frequency 9.48531 GHz, microwave power 0.20 mW, modulation frequency 100 kHz, modulation amplitude 8 G, receiver gain 60 dB, time constant 20 ms, conversion time 41 ms, 1 sweep, 1.465 G/data point.
Simulation parameters for site I: g_{iso} = 2.00086, A_{iso} = 252 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.40×D, E-Strain = 0.40×E, linewidth$_{x,y,z}$ = 50, 30, 20 MHz.
Simulation parameters for site II: g_{iso} = 2.00086, A_{iso} = 250 MHz, D = 2150 MHz, E = 108 MHz, D-Strain = 0.33×D, E-Strain = 0.60×E, linewidth$_{iso}$ = 35 MHz.

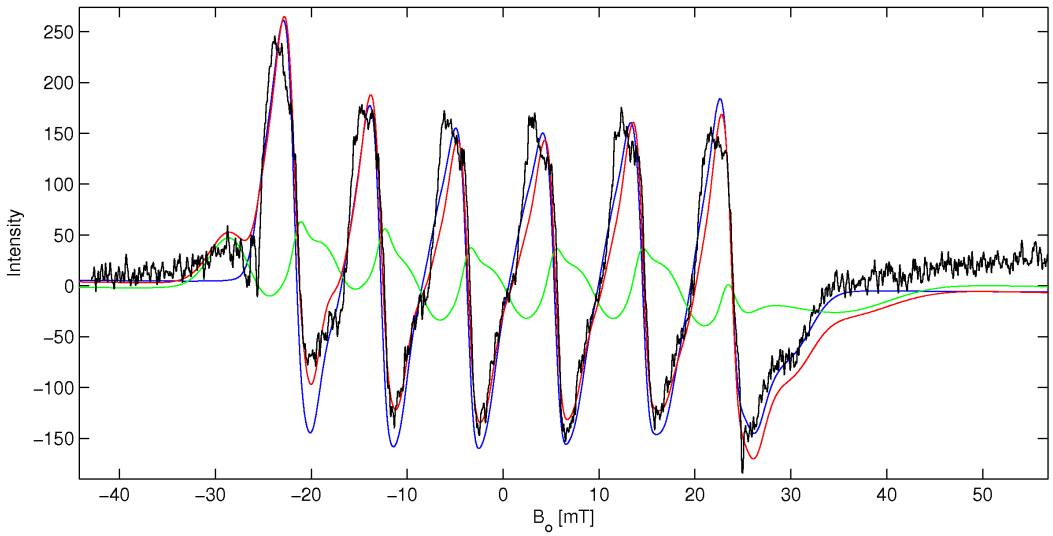

Figure S8: V-band spectrum of OxDC in AB pH5.2 at T = 20 K. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation of the two sites is shown in red.

Experimental parameters: Microwave frequency 49.200 GHz, microwave power corresponding to a 1000 mV receiver signal, modulation frequency 41.68 kHz, modulation amplitude 0.4 G, lock-in sensitivity 200 μV, time constant 300 ms, sweep speed 1 G/s, 1 sweep, 0.250 G/data point, center field 1.757 T. Tic marks are given in mT.
Simulation parameters for site I: g_{iso} = 2.00086, A_{iso} = 255 MHz, D = 1200 MHz, E = 288 MHz, D-Strain = 0.40×D, E-Strain = 0.40×E, linewidth$_{iso}$ = 33 MHz.
Simulation parameters for site II: g_{iso} = 2.00086, A_{iso} = 250 MHz, D = 2150 MHz, E = 108 MHz, D-Strain = 0.40×D, E-Strain = 0.60×E, linewidth$_{iso}$ = 35 MHz.

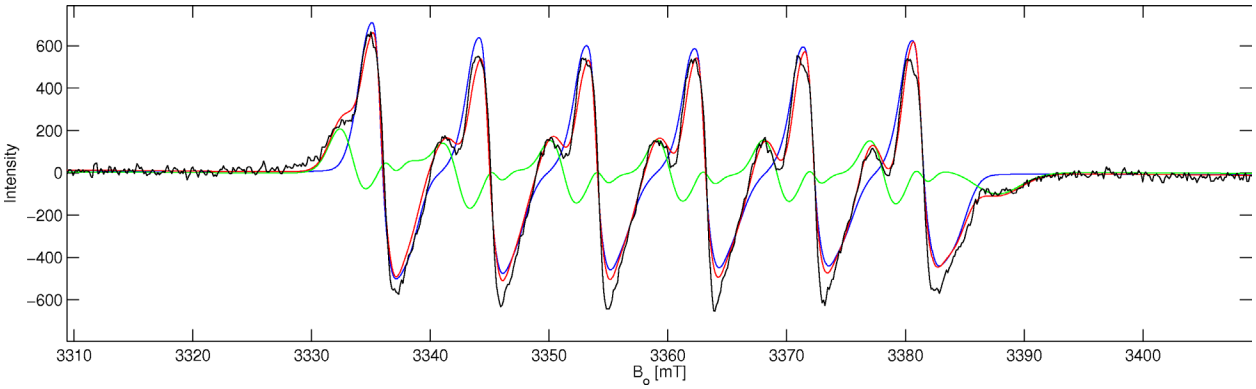

Figure S9: W-band spectrum of OxDC in AB pH5.2 at T = 50 K. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation of the two sites is shown in red.
Experimental parameters: Microwave frequency 94.02073 GHz, microwave power 60 nW, modulation frequency 100 kHz, modulation amplitude 2 G, receiver gain 40 dB, time constant 164 ms, conversion time 164 ms, 1 sweep, 1.172 G/data point.
Simulation parameters for site I: g_{iso} = 2.00086, A_{iso} = 255 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.40×D, E-Strain = 0.40×E, linewidth$_{x,y,z}$ = 50, 30, 20 MHz.
Simulation parameters for site II: g_{iso} = 2.00086, A_{iso} = 250 MHz, D = 2150 MHz, E = 108 MHz, D-Strain = 0.33×D, E-Strain = 0.60×E, linewidth$_{iso}$ = 35 MHz.

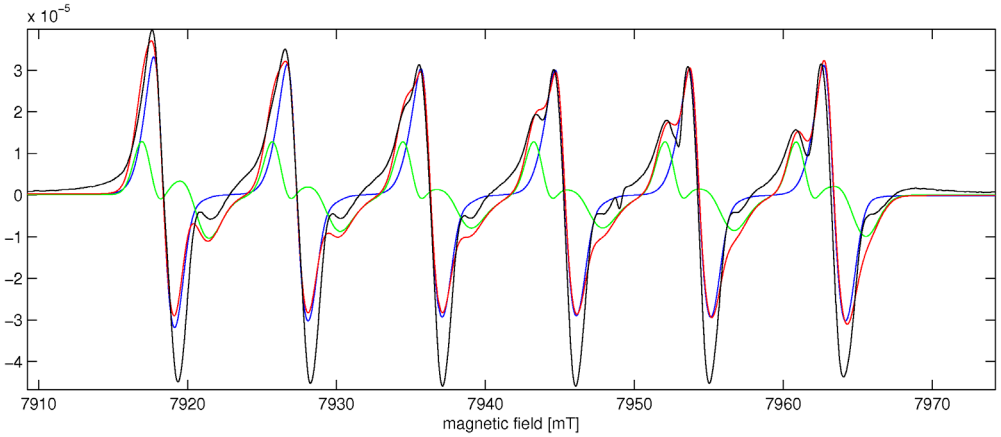

Figure S10: sub-mm EPR spectrum of OxDC in AB pH5.2 at T = 20 K at 222 GHz. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation of the two sites is shown in red.
Experimental parameters: Microwave frequency 222.400 GHz, modulation frequency 41.8 kHz, modulation amplitude 0.5 G, lock-in sensitivity 500 µV, time constant 100 ms, sweep speed 5.01 G/s, 1 sweep, 0.350 G/data point.
Simulation parameters for site I: g_{iso} = 2.00086, A_{iso} = 252 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.40×D, E-Strain = 0.40×E, linewidth$_{x,y,z}$ = 50. 30, 20 MHz.
Simulation parameters for site II: g_{iso} = 2.00086, A_{iso} = 246 MHz, D = 2150 MHz, E = 108 MHz, D-Strain = 0.30×D, E-Strain = 0.60×E, linewidth$_{iso}$ = 35 MHz.

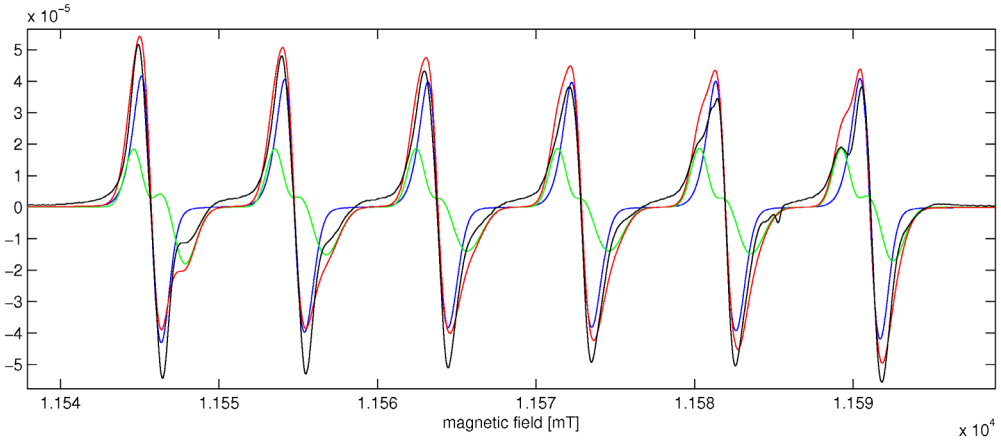

Figure S11: sub-mm EPR spectrum of OxDC in AB pH5.2 at T = 20 K at 324 GHz. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation traces of the two sites is given in red.
Experimental parameters: Microwave frequency 324.00 GHz, modulation frequency 41.8 kHz, modulation amplitude 0.5 G Lock-In sensitivity 500 µV, time constant 100 ms, sweep speed 5.01 G/s, 1 sweep, 0.361 G/data point.
Simulation parameters for site I: g_{iso} = 2.00086, A_{iso} = 254 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.40×D, E-Strain = 0.40×E, linewidth$_{x,y,z}$ = 50, 30, 20 MHz.
Simulation parameters for site II: g_{iso} = 2.00086, A_{iso} = 250 MHz, D = 2150 MHz, E = 108 MHz, D-Strain = 0.30×D, E-Strain = 0.60×E, linewidth$_{iso}$ = 35 MHz.

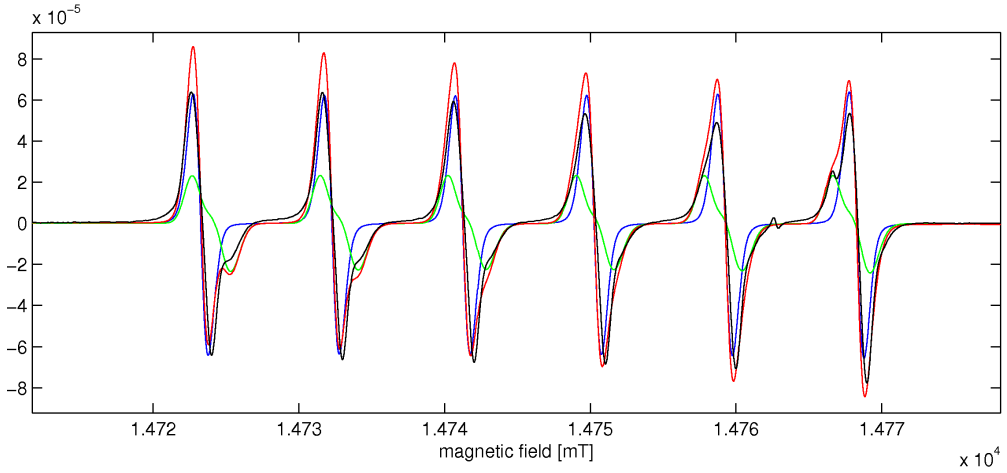

Figure S12: sub-mm EPR spectrum of OxDC in AB pH5.2 at T = 10 K at 412.8 GHz. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation traces of the two sites is given in red. Experimental parameters: Microwave frequency 412.800 GHz, modulation frequency 41.8 kHz, modulation amplitude 0.5 G, lock-in sensitivity 500 µV, time constant 100 ms, sweep speed 5.01 G/s, 1 sweep, 0.359 G/data point.
Simulation parameters for site I: g_{iso} = 2.00086, A_{iso} = 252 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.40×D, E-Strain = 0.40×E, linewidth$_{x,y,z}$ = 30, 30, 20 MHz.
Simulation parameters for site II: g_{iso} = 2.00086, A_{iso} = 246 MHz, D = 2150 MHz, E = 108 MHz, D-Strain = 0.33×D, E-Strain = 0.60×E, linewidth$_{iso}$ = 35 MHz.

Spectral Simulations for EPR spectra at different field/frequency combinations of OxDC in storage buffer (SB) pH6.0 with 25 mM formate present:

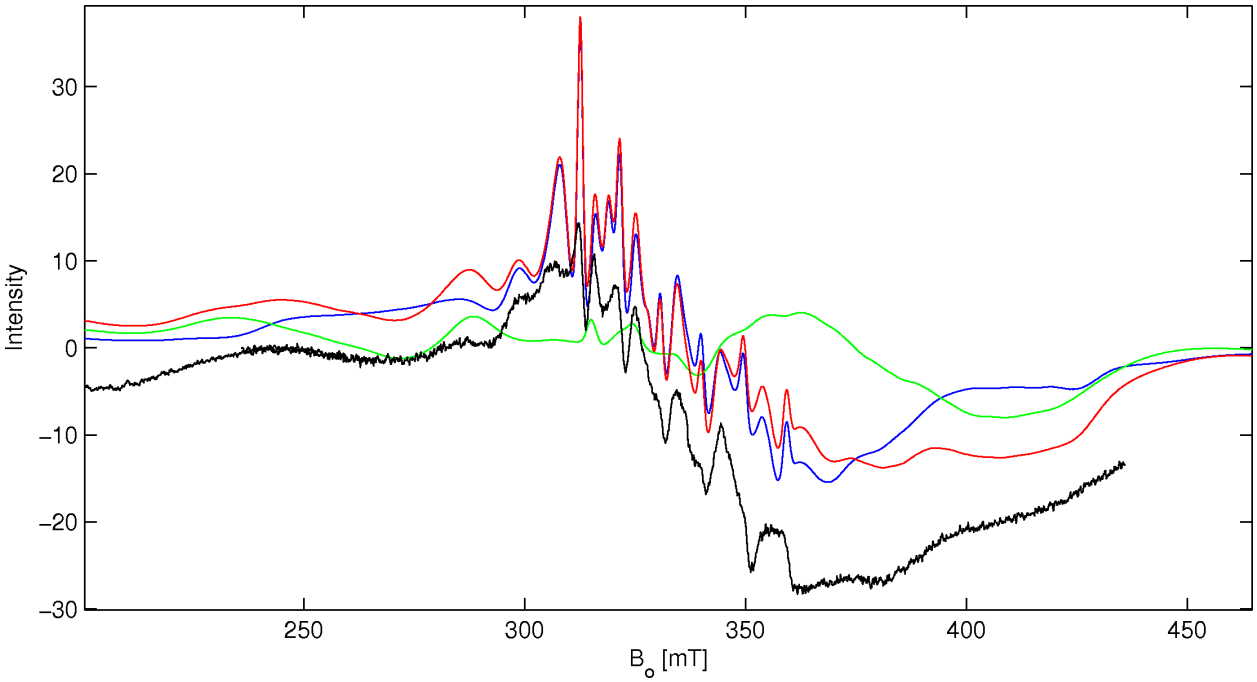

Figure S13: X-band spectrum of OxDC in SB pH6.0 with 50 mM formate at T = 10 K. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation of the two sites is shown in red.
Experimental parameters: Microwave frequency 9.46289 GHz, microwave power 2.0 mW, modulation frequency 100 kHz, modulation amplitude 7 G, receiver gain 60 dB, time constant 164 ms, conversion time 164 ms, 1 sweep, 0.977 G/data point.
Simulation parameters for site I: g_{iso} = 2.00078 A_{iso} = 255 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.24×D, E-Strain = 0.24×E, linewidth$_{x,y,z}$ = 50, 40, 30 MHz.
Simulation parameters for site II: g_{iso} = 2.00086, A_{iso} = 250 MHz, D = 2150 MHz, E = 108 MHz, D-Strain = 0.30×D, E-Strain = 0.60×E, linewidth$_{iso}$ = 35 MHz.

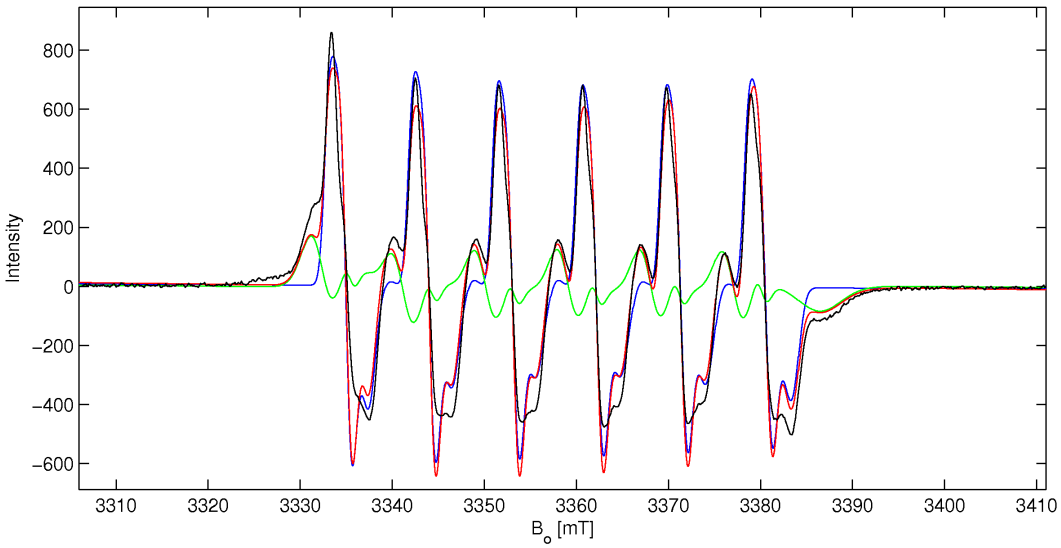

Figure S14: W-band spectrum of OxDC in SB pH6.0 with 50 mM formate at T = 10 K. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation of the two sites is shown in red.
Experimental parameters: Microwave frequency 94.02059 GHz, microwave power 600 nW, modulation frequency 100 kHz, modulation amplitude 2 G, receiver gain 40 dB, time constant 164 ms, conversion time 164 ms, 1 sweep, 1.172 G/data point.
Simulation parameters for site I: g_{iso} = 2.00078 A_{iso} = 255 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.20×D, E-Strain = 0.20×E, linewidth$_{x,y,z}$ = 30, 40, 30 MHz.
Simulation parameters for site II: g_{iso} = 2.00086, A_{iso} = 250 MHz, D = 2150 MHz, E = 108 MHz, D-Strain = 0.30×D, E-Strain = 0.60×E, linewidth$_{iso}$ = 35 MHz.

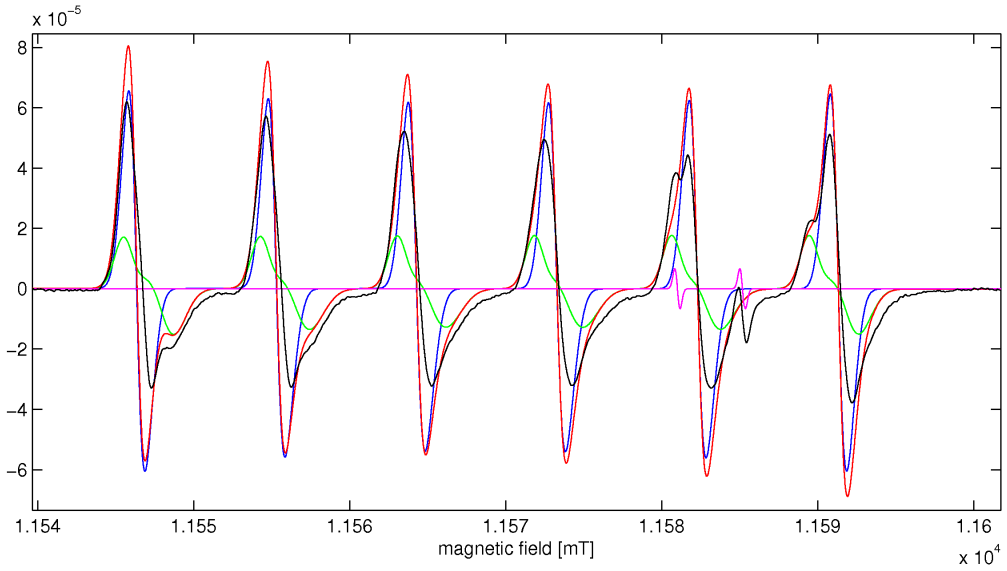

Figure S15: sub-mm EPR spectrum of OxDC in SB pH6.0 with 50 mM formate at T = 20 K at 324 GHz. Experimental spectrum is shown in black. Simulations for sites I and II are shown in blue and green, respectively. The sum of the simulation traces of the two sites is given in red. Experimental parameters: Microwave frequency 324.00 GHz, modulation frequency 41.8 kHz, modulation amplitude 0.5 G, lock-in sensitivity 500 µV, time constant 100 ms, sweep speed 5.01 G/s, 1 sweep, 0.364 G/data point.
Simulation parameters for site I: g_{iso} = 2.00086, A_{iso} = 252 MHz, D = 1200 MHz, E = 252 MHz, D-Strain = 0.20×D, E-Strain = 0.20×E, linewidth$_{x,y,z}$ = 30, 40, 20 MHz.
Simulation parameters for site II: g_{iso} = 2.00086, A_{iso} = 246 MHz, D = 2150 MHz, E = 108 MHz, D-Strain = 0.30×D, E-Strain = 0.60×E, linewidth$_{iso}$ = 35 MHz.