Supporting Information

Advanced electron paramagnetic resonance on the catalytic iron sulphur cluster bound to the CCG domain of heterodisulfide reductase and succinate: quinone reductase

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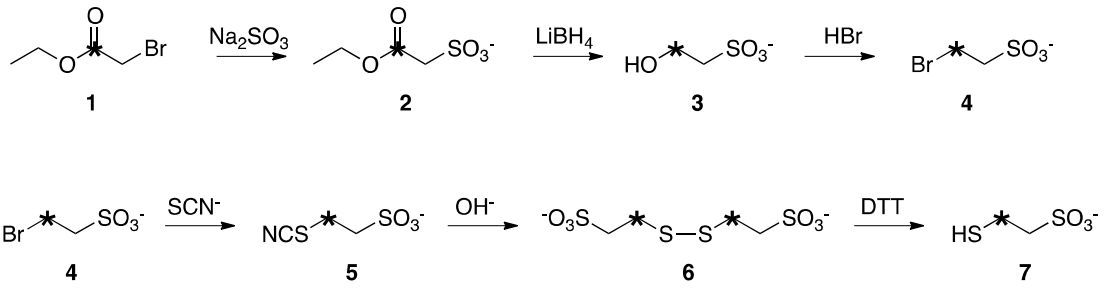
Synthesis of [2-^{13}C]-coenzyme M

Coenzyme M labeled with ^{13}C at position 2 ([2-^{13}C]-mercaptoethane sulfonate (**7**)) was synthesized from [1-^{13}C]-ethyl bromoacetate (**1**), according to the following reaction sequence:



[1-^{13}C]-Ethyl 2-sulfoacetate sodium salt (2)

[1-^{13}C]-Ethyl bromoacetate (**1**) (980 mg, 5.83 mmol, Aldrich, 99% ^{13}C) was added under nitrogen to a suspension of sodium sulfite (1.00 g, 7.93 mmol) in ethanol (1.8 ml) and water (1.5 ml). The suspension was stirred at 50 °C for 15 h. The solvents were evaporated at 80 °C under reduced pressure and the residue re-suspended in ethanol (6 ml) and dried under high vacuum. The residue was twice suspended in ethanol (200 ml), filtrated and evaporated under high vacuum in order to remove excess sodium sulfite. This crude product was used for the next step.

[2-^{13}C]-2-Hydroxyethane sulfonic acid (3)

[1-^{13}C]-Ethyl 2-sulfoacetate sodium salt (**2**) (crude product, ca. 5.8 mmol) and dry lithium bromide (605 mg, 7.0 mmol) was suspended under nitrogen in diethylene glycol dimethyl ether (10 ml, freshly distilled from CaH_2) and heated to 80 °C. Sodium borohydride (895 mg, 23.6 mmol) in dry diethylene glycol dimethyl ether (15 ml) was slowly added under vigorous stirring. The suspension was stirred at 100 °C for 17 h and cooled to room temperature. HCl (aq., 10%, ca. 10 ml) and water (6 ml) was slowly added. Ultrasonication yielded a clear

solution with pH = 1 that was neutralized with Na_2CO_3 to pH = 7 and evaporated under high vacuum. The residue was twice dissolved in water and dried under high vacuum in order to remove most of the diethylene glycol dimethyl ether (< 10% present according to NMR). Amberlite IR 120 (strongly acidic ion exchanger resin, 80 ml suspension in water) was added and equilibrated for 3 h. The solution was filtered and the resin washed 10 times with water (15 ml) until pH = 4 of the washing. The combined aqueous parts were lyophilized. The lyophilisate was dissolved in methanol (150 ml) and distilled at 110 °C bath temperature in order to remove boron compounds as $B(OMe)_3$. This procedure was repeated once. The crude product was dried under high vacuum for 4 days to yield 1.185 g of crude product that was used for the next step.

[2-^{13}C]-2-Bromoethane sulfonate ammonium salt (4)

[2-^{13}C]-2-Hydroxyethane sulfonic acid (**3**) (crude product, 5-6 mmol) was added to HBr (62%, 20 ml, ca. 250 mmol) and refluxed at 150 °C for 18 h (90% conversion to the bromide). Air was blown over the reaction mixture at 150 °C until all liquid was evaporated, resulting in 93% conversion to the bromide. Amberlite IR 120 (strongly acidic, 15 ml suspension in water) was added and equilibrated for 1 h. The solution was filtered and the resin washed 8 times with water (8 ml) until pH = 5.5 of the washing. The combined aqueous parts were concentrated to ca. 10 ml and centrifuged in order to remove dispersed insoluble particles. The supernatant was dried under high vacuum. Kugelrohr distillation at 0.007 mbar and 120 °C yielded 265 mg distillate and 754 mg residue. The residue was treated with Amberlite IR 120 as before and redistilled (0.007 mbar and 140 °C) to yield additional 580 mg distillate and 111 mg residue. The distillates were each diluted with water, NH_3 (aq., conc., ca. 3 ml) added, evaporated and checked for purity. The two distilled fractions (pure) were dissolved in methanol, combined and dried for 24 h at high vacuum to yield [2-^{13}C]-ammonium 2-

bromoethane sulfonate (**4**) as a colorless powder (884 mg, 4.27 mmol, 73% yield over all 3 steps, ≥ 98% purity according to NMR).

^{1}H-NMR (400 MHz, D_2O, ref. external DSS): 3.60 dxm, 2H, $^{1}J_{CH}$ = 174.1 Hz; 3.42 m, 2H. $^{13}C\{^{1}H\}$-NMR (100 MHz, D_2O): 53.6 d, $^{1}J_{CC}$ = 36.3 Hz, rel. integral 1.06; 24.5 (rel. integral 100).

[2-^{13}C]-2-Thiocyanatoethane sulfonate ammonium salt (5)

[2-^{13}C]- 2-Bromoethane sulfonate ammonium salt (**4**) (207.1 mg, 1.00 mmol) was added under nitrogen to ammonium thiocyanate (91 mg, 1.20 mmol) in fresh DMF (1.0 ml). The solution was heated to 100 °C for 20 h leading to full conversion. The DMF was removed under high vacuum and twice dissolved in water and dried under high vacuum. This product was directly used for the next step.

[2,2'-$^{13}C_2$]-2,2'-Dithioethane sulfonate diammonium salt (6)

[2-^{13}C]-2-Thiocyanatoethane sulfonate ammonium salt (**5**) (crude product, 1 mmol) was added to ammonium carbonate (192 mg, 2.0 mmol) in D_2O (0.8 ml) in an NMR tube and heated to 60 °C until full conversion to the disulfide (18 h). The solution was added to Amberlite IR 120 (strongly acidic, 13 g), equilibrated for 1 h and washed until pH = 5 of the washing. The combined aqueous parts were evaporated under high vacuum. The Amberlite step repeated once and the combined aqueous parts dried under high vacuum. The residue was dissolved in a small amount of water and NH_3 (aq., conc. 0.5 ml) and activated carbon (50 mg) were added. The suspension was treated in the ultrasonic bath and centrifuged after sitting for 30 min. The supernatant was filtered through cotton and dried under high vacuum. The residue was dissolved in methanol (10 ml) and filtered through cotton three times to remove all activated carbon. Evaporation and drying under high vacuum gave 158 mg of a colorless solid. This crude product was suspended and refluxed in ethanol (8 ml) and slowly

cooled down to room temperature. The mother liquor was removed and the residue washed with ethanol (2 ml). Drying the residue under high vacuum gave the product as a colorless powder (109 mg, 0.342 mmol, 68% yield).

[2-^{13}C]-Mercaptoethane sulfonate ammonium salt (7)

[2,2'-$^{13}C_2$]-2,2'-Dithioethane sulfonate diammonium salt (**6**) (30 mg, 0.095 mmol) and dithiothreitol (23 mg, 0.15 mmol) were dissolved under nitrogen in D_2O (1.5 ml) in an NMR tube. ND_3 (2 µl, 27% in D_2O) was added and full reduction to coenzyme M (thiol form) was confirmed via NMR spectroscopy. The solution was concentrated under reduced pressure to about 2 drops. The product was precipitated by addition of pure acetone (ca. 10 ml) and centrifuged. The pellet was dissolved in a small amount of H_2O and filtrated. Lyophilization of the filtrate gave **7** as a white powder (21 mg, 0.130 mmol, 69%).

NMR: ^{1}H-NMR (500 MHz, D_2O, ref. dioxane = 3.7 ppm) 3.122 m, 2H; 2.58 dxm, 2H, $^1J_{CH}$ = 142.7 Hz). $^{13}C\{^1H\}$-NMR (125 MHz, D_2O, ref. dioxane = 67.0 ppm): 54.78 d, $^1J_{CC}$ = 35.2 Hz, rel. integral 1.06), 32.2, rel. integral: 100.

Alternative (non-optimal) simulations for HdrB

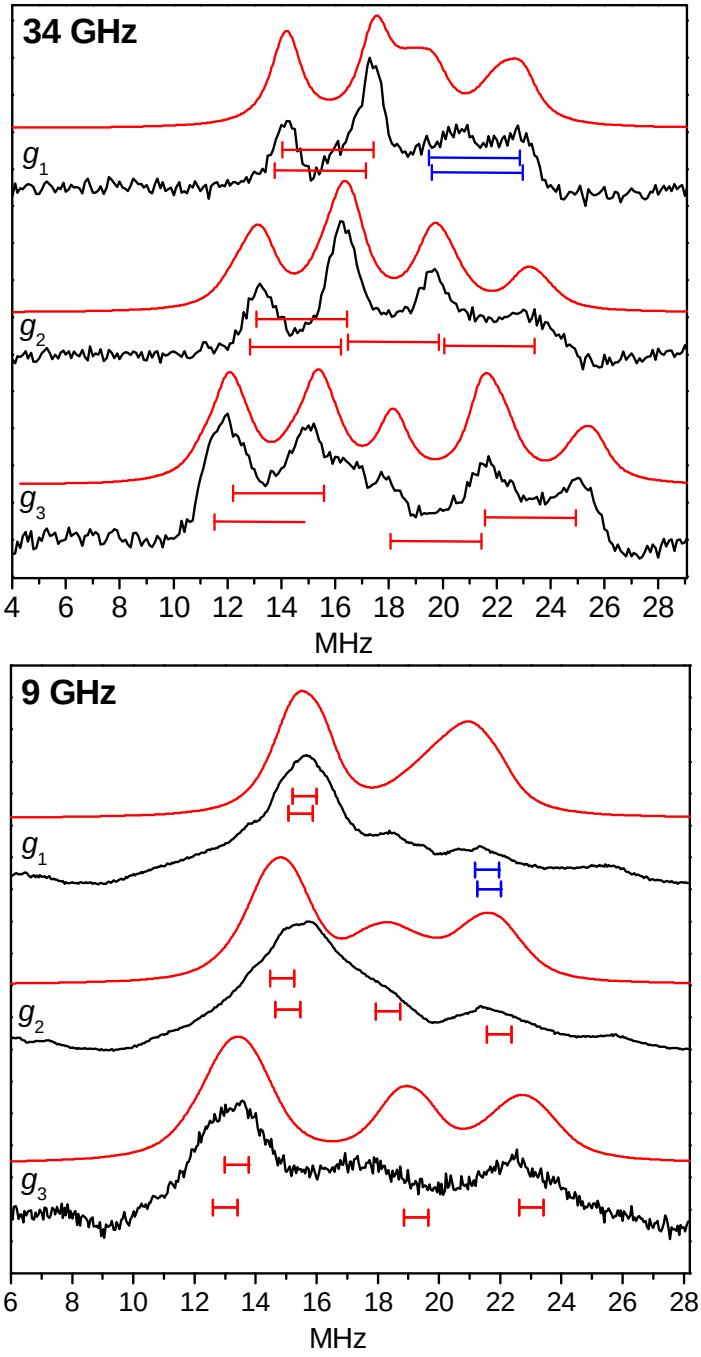


Figure S1. Davies ENDOR spectra of ^{57}Fe-enriched $HdrB_{OXID}$ recorded at 34 GHz (top) and 9 GHz (bottom) at different positions of the EPR line according to g_1, g_2 and g_3. Simulations are displayed in red and use: g_1 = [32.0 (Fe_1), 32.5 (Fe_2), 42.0 (Fe_3), 42.2 (Fe_4)], g_2 = [29.5 (Fe_1), 29.7 (Fe_2), 36.2 (Fe_3), 43.7 (Fe_4)], g_3 = [24.9 (Fe_1), 25.9 (Fe_2), 39.9 (Fe_3), 48.4 (Fe_4)]. The simulations give a non-optimal fit with these parameters.

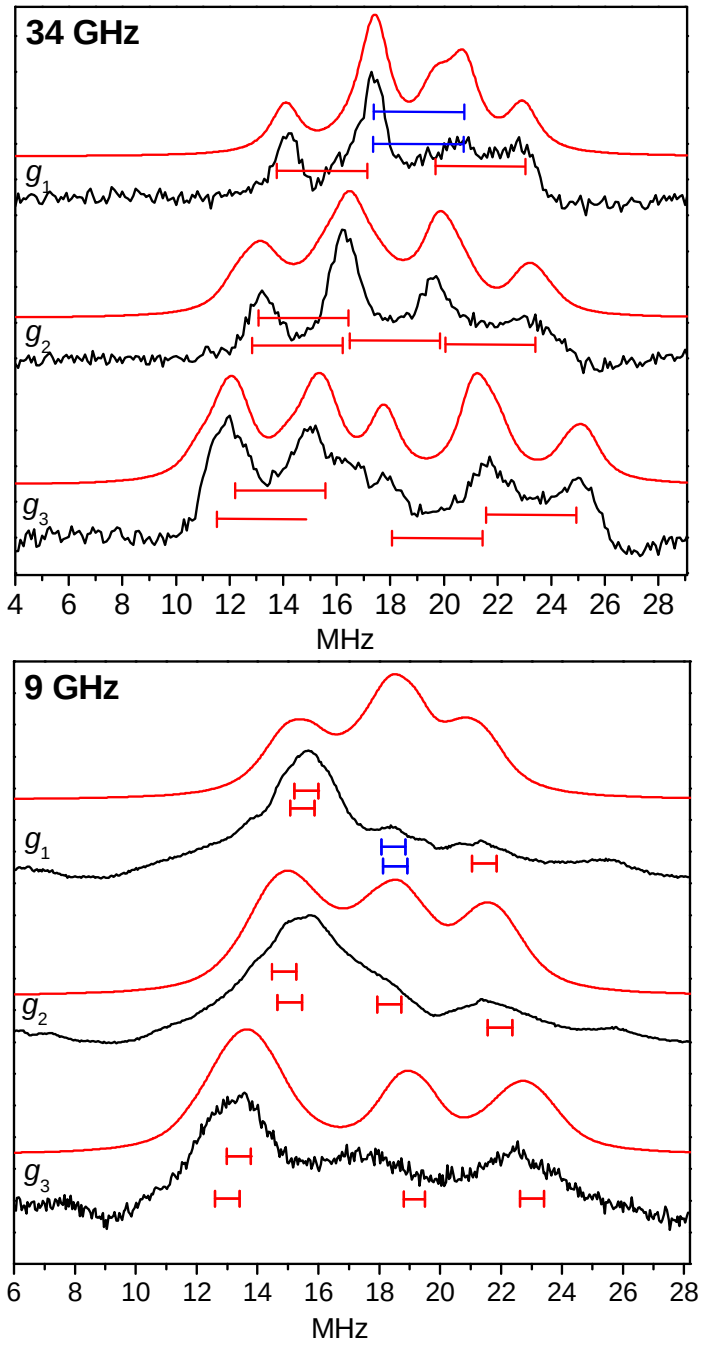


Figure S2. Davies ENDOR spectra of ^{57}Fe-enriched $HdrB_{OXID}$ recorded at 34 GHz (top) and 9 GHz (bottom) at different positions of the EPR line according to g_1, g_2 and g_3. Simulations are displayed in red and use: g_1 = [32.0 (Fe_1), 38.8 (Fe_2), 38.8 (Fe_3), 42.2 (Fe_4)], g_2 = [29.5 (Fe_1), 29.7 (Fe_2), 36.2 (Fe_3), 43.7 (Fe_4)], g_3 = [24.9 (Fe_1), 25.9 (Fe_2), 39.9 (Fe_3), 48.4 (Fe_4)]. The simulations give a non-optimal fit with these parameters.

^{14}N ESEEM Spectra

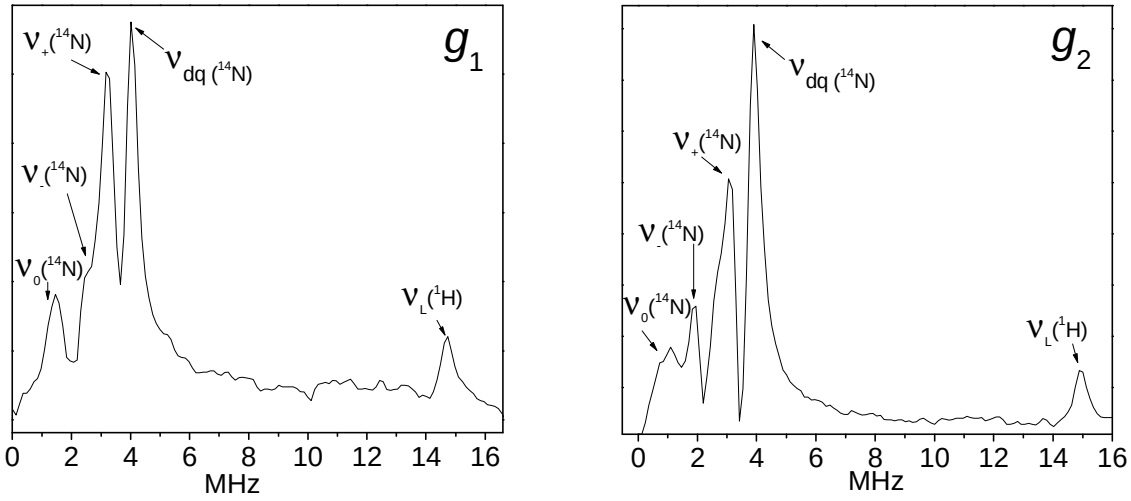


Figure S3. 3 Pulse ESEEM spectra of ^{57}Fe-enriched HdrABC at 9 GHz at different positions of the EPR line according to g_1, and g_2. Nuclear quadrupole resonances (NQR) $\nu_{0/-/+}$ (^{14}N), double quantum ν_{dq} (^{14}N) and proton Larmor ν_L (^{1}H) frequency transitions are marked. The NQR lines are typical of nitrogen weakly coupled to an electron spin in orientationally disordered systems in the so-called cancellation condition (ref 26).

^{13}C Mims ENDOR spectra of CoM-HdrB$_{oxid}$

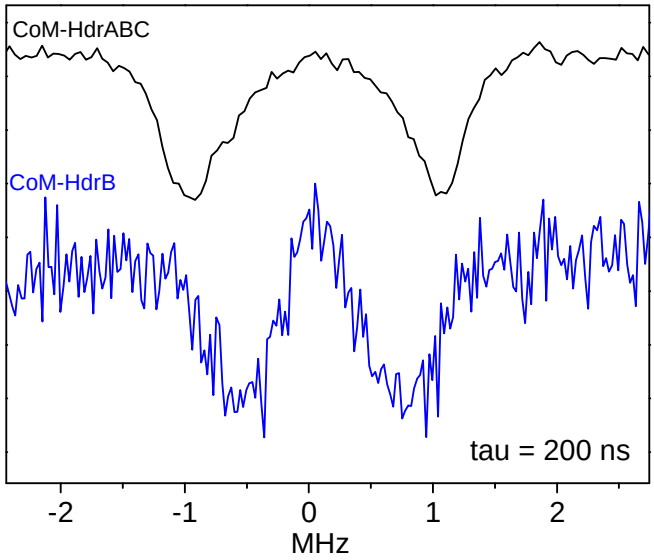


Figure S4. Mims ^{13}C ENDOR spectra of CoM-HdrABC (black) and CoM-HdrB (blue) with ^{13}C labelled CoM ($HS^{13}CH_2{}^{12}CH_2SO_3^-$) recorded at 34 GHz and 10 K at g_2 using a tau value of 200 ns.