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ELECTRONIC SUPPLEMENTARY INFORMATION

Interconversion between [2Fe-2S] and [4Fe-4S] cluster glutathione complexes

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1. Experimental Procedures

1.1 Methods and materials

All reagents were from Sigma Aldrich and used without any further purification. Degassed MilliQ water or degassed D_2O were used as solvents. The preparation of the reagent solutions, the synthesis of the Fe-S complexes and their transfer to the NMR tubes, EPR tubes and quartz cuvettes, was carried out in an anaerobic glovebox. The samples in the NMR and EPR tubes were maintained anaerobic using rubber septa and sealed with parafilm. Anaerobic sealed Hellma quartz cuvettes were used for UV-vis spectroscopy. Cyclic voltammetry was performed directly in glovebox.

1.2 Sample preparation

X μL of 1 M Na_2S (78.05 g/mol) was added to 5 mL of an aqueous solution of 0.24 M GSH at pH 8.6 in a 10 mL falcon. Y μL of 1 M $FeCl_3$ anhydrous (162.20 g/mol) was then added to obtain GSH-Fe-S complexes. When X = 10 μL and Y = 50 μL, GSH:Na_2S:$FeCl_3$ molar ratio of 120:1:5 (solution 1) is obtained and the $[Fe^{II}Fe^{III}S_2(GS)_4]^{3-}$ complex is the main species present in solution. When X = 100 μL and Y = 10 μL, GSH:Na_2S:$FeCl_3$ molar ratio of 120:10:1 (solution 2) is obtained and the $[Fe^{II}_2Fe^{III}_2S_4(GS)_4]^{2-}$ complex is the main species present in solution. For pH dependent experiments, Tris-HCl 50 mM were used as buffer and the pH was adjusted using NaOH 10 M solution. NMR samples contained 10% (v/v) of D_2O. EPR samples contained 10% (v/v) glycerol. CV samples contained 0.05 M NaCl as supporting electrolyte.

1.3 NMR spectroscopy

NMR spectra were recorded at 298 K using a 600 MHz Bruker Avance III spectrometer equipped with a selective 5mm ^1H probe without pulsed field gradients. Diamagnetic NMR experiments were performed using 0.85 s and 1 s, respectively, as acquisition time and recycle delay, over a 16 ppm spectral window. Water presaturation was achieved by applying a selective pulse during the recycle delay. Each experiment consisted of 32 scans. To identify signals sensing the hyperfine interaction with a paramagnetic center, the experiments were performed using a superWEFT experiment, recorded with interpulse delay of 30 ms and using 50 ms as acquisition and recycle delay. Squared cosine and exponential multiplications were applied prior to Fourier transformation. Manual baseline correction was performed, using polynomial functions. The spectral window was set to 298 ppm. Each experiment consisted of 5120 scans. Longitudinal relaxation rates (R_1) of paramagnetic signals were measured via inversion recovery experiments. All spectra were processed using the Bruker software TopSpin.

1.4 Cluster complex interconversion experiment by NMR

Samples containing 500 μL of 0.24 M GSH and variable amounts of 1.0 M $FeCl_3$ and 1.0 M Na_2S in D_2O at 298K (see Tables S1-S4 for details) were analyzed by paramagnetic superWEFT NMR spectra (see section 1.2).

Table S1. Experimental conditions used in the cluster complex interconversion from $[Fe^{II}Fe^{III}S_2(GS)_4]^{3-}$ to $[Fe^{II}_2Fe^{III}_2S_4(GS)_4]^{2-}$, at pH 7.9.

N.[a]	Na_2S [mM]	$FeCl_3$ [mM]	$FeCl_3/Na_2S$ ratio
1	1.99	5.95	3.0
2	2.98	5.95	2.0
3	3.96	5.95	1.5
4	5.90	5.95	1.0
5	9.85	5.90	0.6
6	15.6	5.85	0.38
7	23.3	5.80	0.25

[a] Number of experiments

Table S2. Experimental conditions used in the cluster complex interconversion from $[Fe^{II}_2Fe^{III}_2S_4(GS)_4]^{2-}$ to $[Fe^{II}Fe^{III}S_2(GS)_4]^{3-}$, at pH 7.9.

N.[a]	Na_2S [mM]	$FeCl_3$ [mM]	$FeCl_3/Na_2S$ ratio
1	5.95	1.98	0.33
2	5.95	2.97	0.50
3	5.95	3.96	0.67
4	5.95	5.92	1.00
5	5.95	7.89	1.33
6	5.95	9.84	1.67
7	5.95	23.3	4.00

[a] Number of experiments

Table S3. Experimental conditions used in the cluster complex interconversion from $[Fe^{II}Fe^{III}S_2(GS)_4]^{3-}$ to $[Fe^{II}_2Fe^{III}_2S_4(GS)_4]^{2-}$, at pH 8.9.

N.[a]	$FeCl_3$ [mM]	Na_2S [mM]	$FeCl_3/Na_2S$ ratio
1	6.0	2.0	3.0
2	6.0	3.0	2.0
3	6.0	4.0	1.5
4	6.0	6.0	1.0
5	6.0	10.0	0.6
6	6.0	16.0	0.4
7	6.0	24.0	0.25

[a] Number of experiments

Table S4. Experimental conditions used in the cluster complex interconversion from $[Fe^{II}_2Fe^{III}_2S_4(GS)_4]^{2-}$ to $[Fe^{II}Fe^{III}S_2(GS)_4]^{3-}$, at pH 8.6.

N.[a]	Na_2S [mM]	$FeCl_3$ [mM]	$FeCl_3/Na_2S$ ratio
1	10.0	1.0	0.1
2	10.0	2.0	0.2
3	10.0	3.0	0.3
4	10.0	4.0	0.4
5	10.0	5.0	0.5
6	10.0	6.0	0.6
7	10.0	7.0	0.7
8	10.0	8.0	0.8
9	10.0	10.0	1.0
10	10.0	12.0	1.2
11	10.0	16.0	1.6
12	10.0	20.0	2.0
13	10.0	30.0	3.0

[a] Number of experiments

1.5 EPR and UV-vis spectroscopy

EPR spectra were recorded at 45 K and 10 K, using a Bruker Elexsys E500 spectrometer working at a microwave frequency of ca. 9.39 GHz, equipped with a SHQ cavity and a continuous cryostat He flow (ESR900, Oxford instruments) for temperature control. Acquisition parameters were as follows: microwave power 2 mW, modulation frequency 100 kHz, modulation amplitude 10 G, sampling time 163.84 ms, number of points 1024. UV-vis absorption spectra were recorded with Cary 50 spectrophotometer with anaerobic sealed Hellma quartz cuvettes (path length: 1 cm).

1.6 Electrochemistry

Cyclic voltammetry experiments were carried out with a portable potentiostat/galvanostat PalmSens electrochemical analyser (PalmSens BV, Houten, The Netherlands), using screen-printed electrodes consisting in a graphite working electrode (3 mm diameter), a silver pseudo-reference electrode and a graphite counter electrode (GSPEs). The results were analysed by PSTrace 5.8 software. The experiments were performed by dropping 50 µL of the sample on the electrode. The potential was scanned from −1000 mV to +500 mV for 5 cycles at 50 mV/s scan rate.

2. Supplementary figures

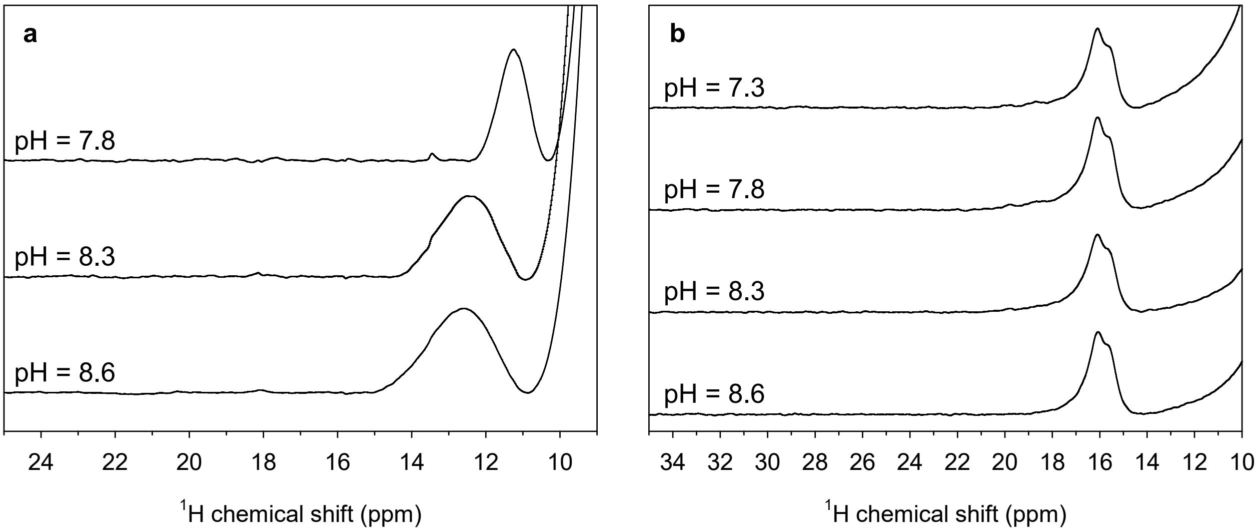

Figure S1. NMR spectra recorded at different pH of solution **1** containing $[Fe^{II}Fe^{III}S_2(GS)_4]^{3-}$ (**a**) and solution **2** containing $[Fe^{II}_2Fe^{III}_2S_4(GS)_4]^{2-}$ (**b**). $[Fe^{II}Fe^{III}S_2(GS)_4]^{3-}$ is stable up to pH=7.8. $[Fe^{II}_2Fe^{III}_2S_4(GS)_4]^{2-}$ remain is stable up to pH 7.3. Lower pH values produce black precipitate.

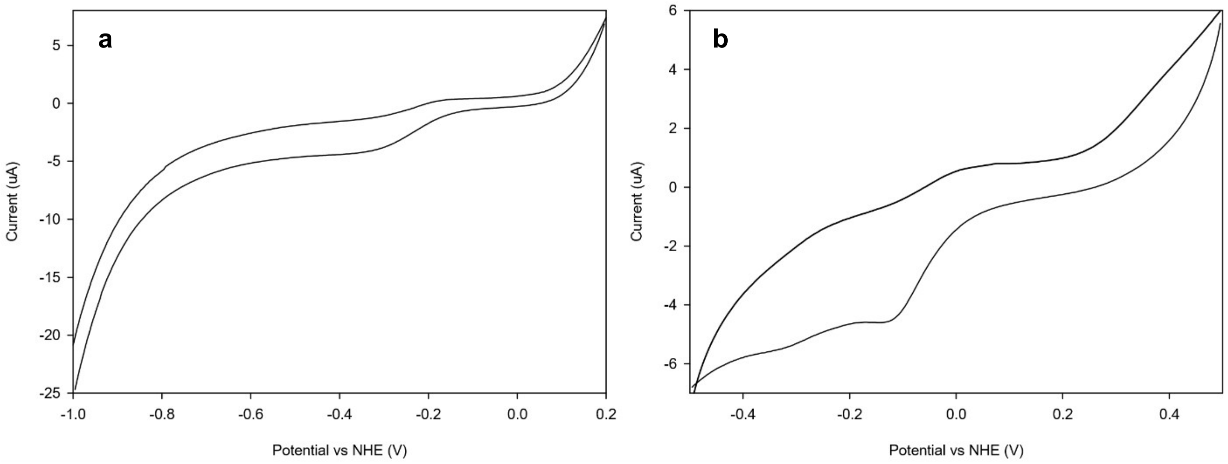

Figure S2. Full cyclic voltammograms recorded on solutions **1** (**a**) and **2** (**b**).

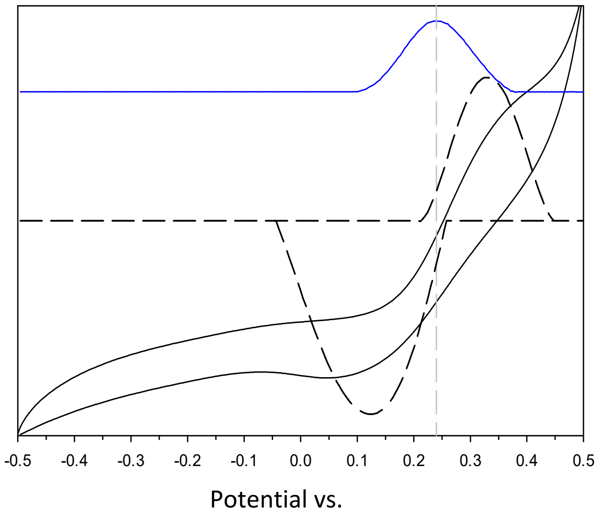

Figure S3. Cyclic (solid black line) and differential pulse (solid blue line) voltammogram of a solution containing 240 mM of GSH and 2 mM of $FeCl_3$. The dashed line shows baseline subtracted cyclic voltammogram. The cathodic and anodic current fall at $E_{1/2}$ = +0.24 mV due to the following redox event: $[Fe^{II}(H_2O)_5(GS)]^{+} \rightleftharpoons [Fe^{III}(H_2O)_5(GS)]^{2+} + e^{-}$.

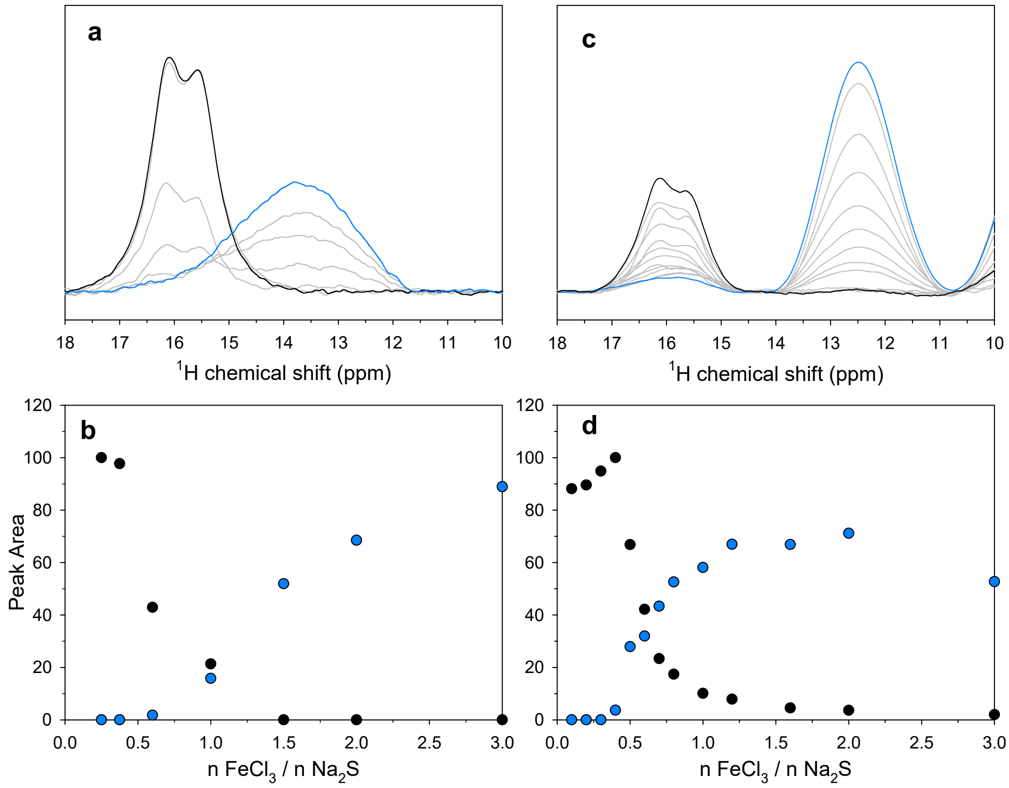

Figure S4. Interconversion between dinuclear $[Fe^{II}Fe^{III}S_2(GS)_4]^{3-}$ and tetranuclear $[Fe^{II}_2Fe^{III}_2S_4(GS)_4]^{2-}$ complexes. (**a**) Paramagnetic ^{1}H NMR spectra of a solution containing: 240 mM GSH and 11 mM $FeCl_3$, with increasing amounts of Na_2S, at pH 8.9. Initial and final spectra of the titration are represented, respectively, as solid blue and solid black lines. (**b**) Peak areas for the signals at 16 ppm (black circles) and 13.6 ppm (blue circles) observed in titration (**a**). (**c**) Paramagnetic ^{1}H NMR spectra of a solution containing 240 mM GSH and 6 mM Na_2S, with increasing amounts of $FeCl_3$, at pH 8.6. The initial spectrum is in solid black line and the final spectrum is in solid blue line. (**d**): Peak area for the signals at 16 ppm (black circles) and 12.5 ppm (blue circles) observed in titration (**c**). In panel (**d**), all areas are normalized according to the amount of $FeCl_3$ added, while in panel (**b**) the original signal intensity is shown.