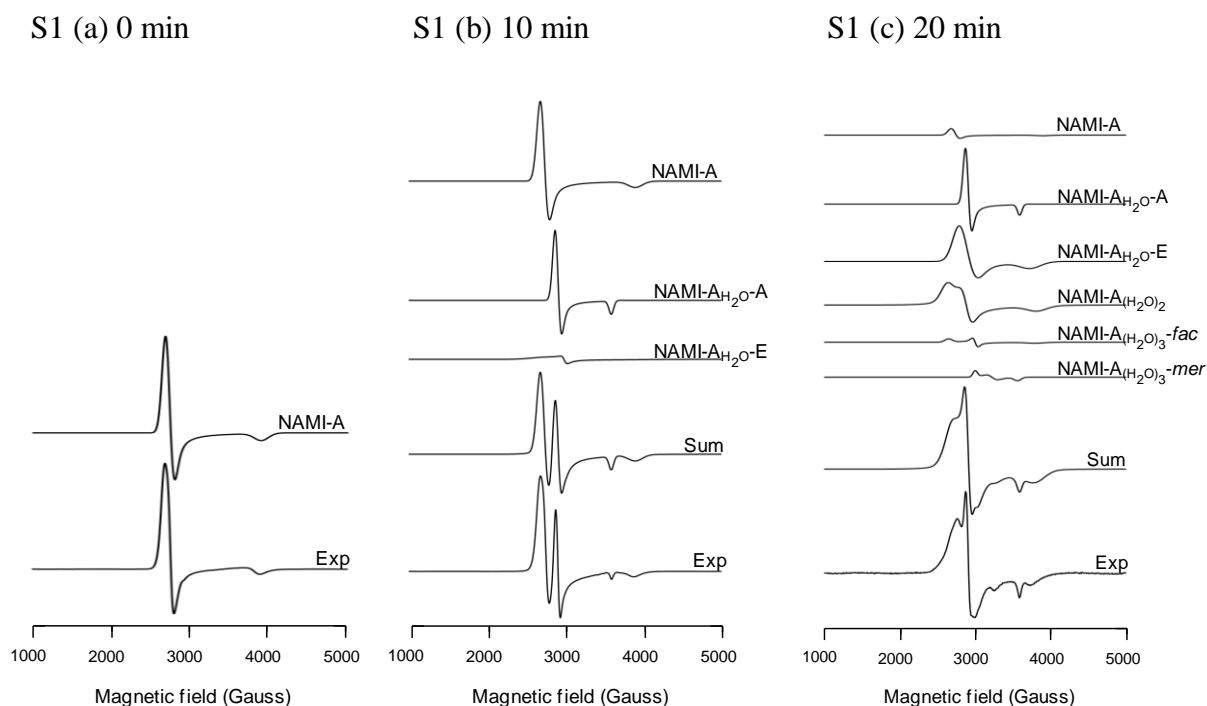


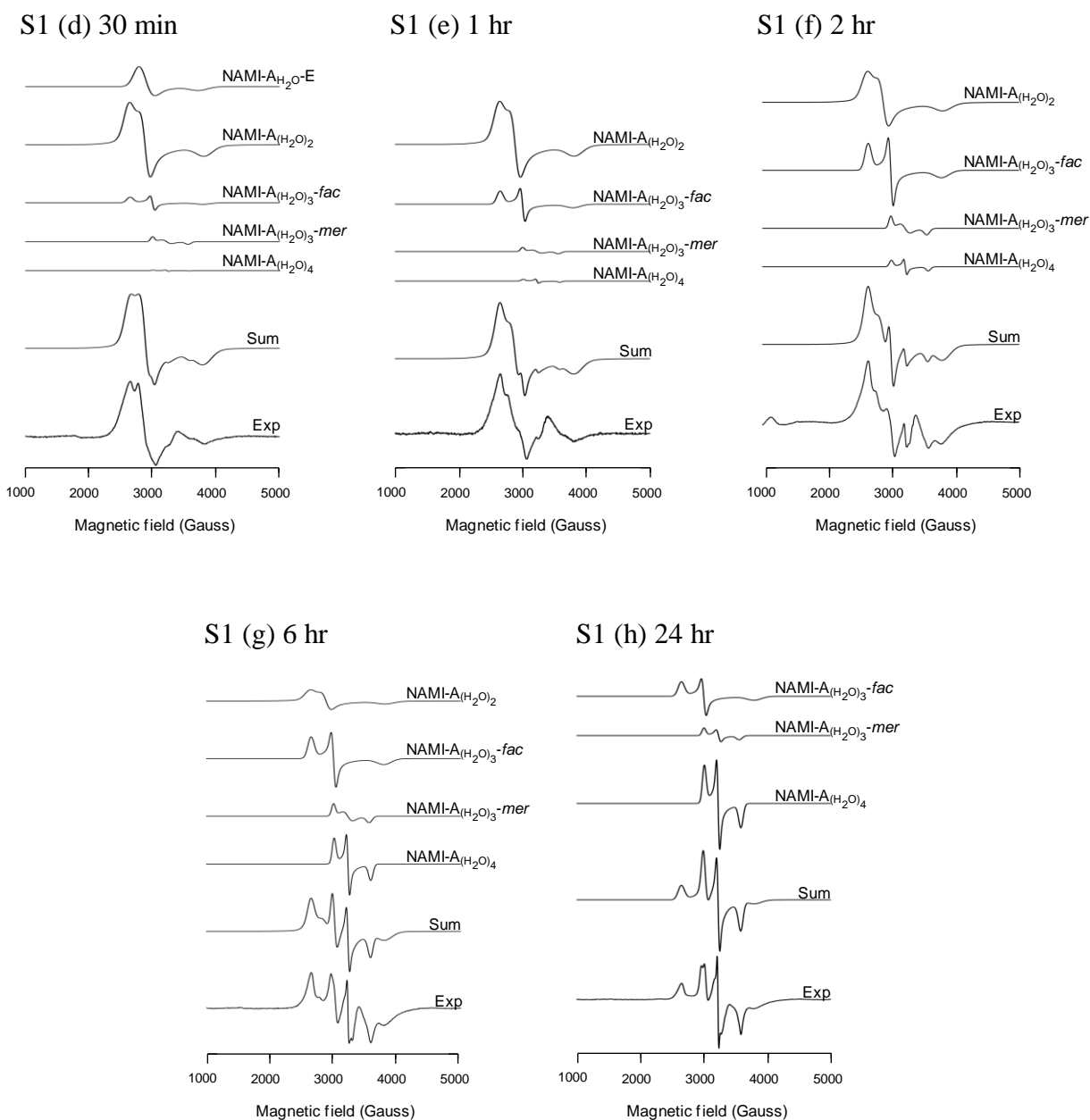
# Control of Ligand-exchange Processes and the Oxidation State of the Antimetastatic Ru(III) Complex NAMI-A by Interactions with Human Serum Albumin

Michael I. Webb, Charles J. Walsby\*

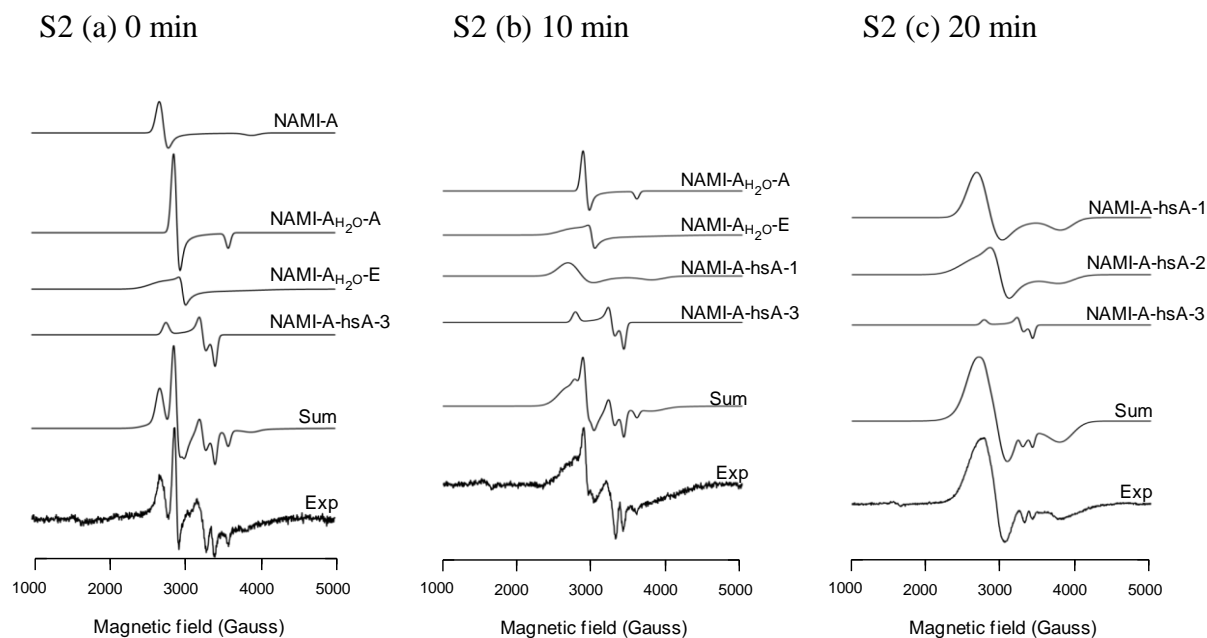
Supplementary Material



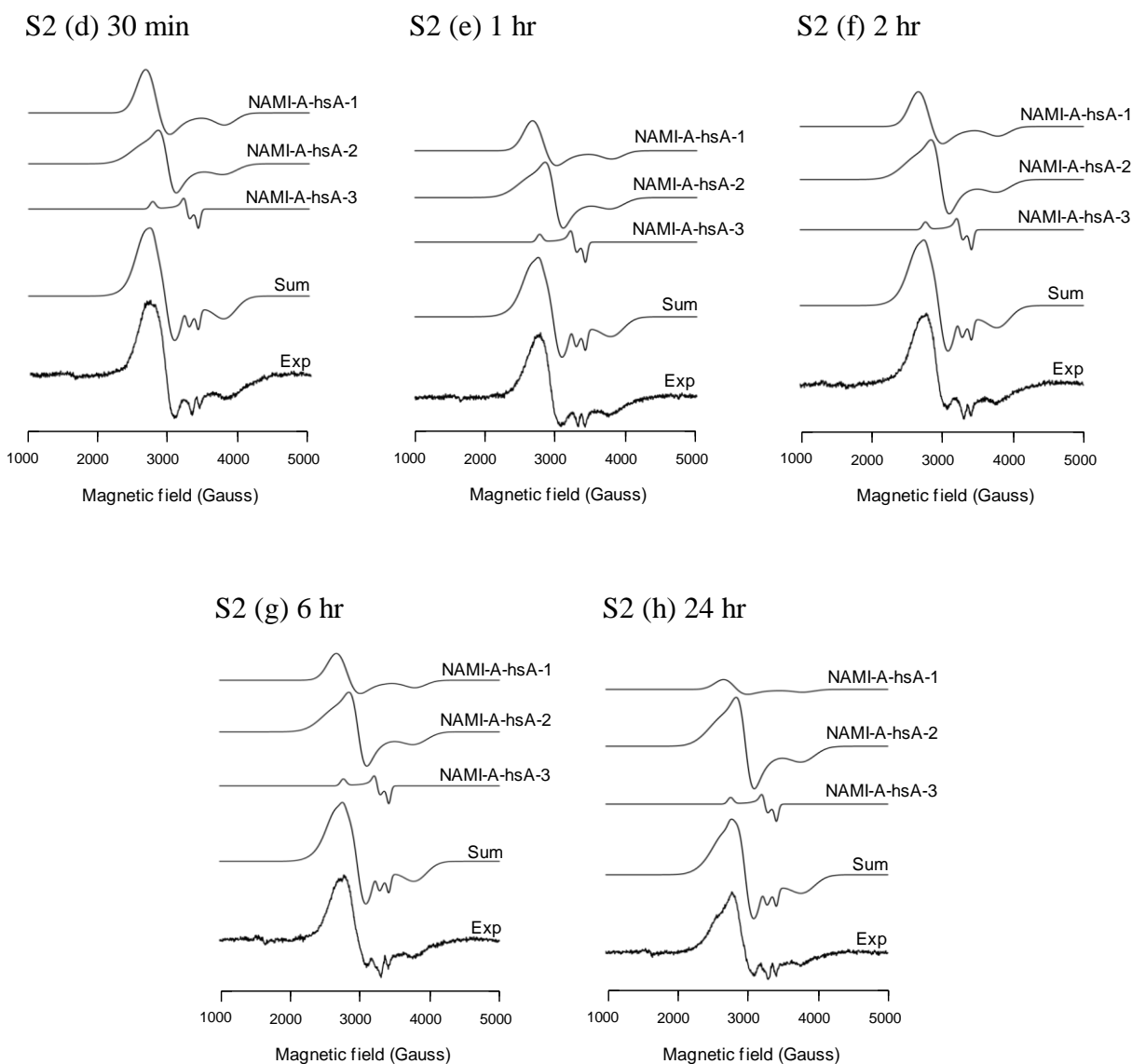
**Fig. S1 (a-c)** Deconvolution of EPR spectra from NAMI-A in buffer after incubation for 0, 10 and 20 minutes at 37 °C. **Experimental conditions:** frequency = 9.376 GHz, microwave power = 2.0 mW, time constant = 40.96 ms, modulation amplitude = 6 G, average of five 2-minute scans. **Simulation parameters:** For g values and line widths, see main text. Relative intensities used in simulations, NAMI-A : NAMI-A<sub>H<sub>2</sub>O</sub>-A : NAMI-A<sub>H<sub>2</sub>O</sub>-E : NAMI-A<sub>(H<sub>2</sub>O)<sub>2</sub></sub> : NAMI-A<sub>(H<sub>2</sub>O)<sub>3</sub></sub>-*fac* : NAMI-A<sub>(H<sub>2</sub>O)<sub>3</sub></sub>-*mer* : NAMI-A<sub>(H<sub>2</sub>O)<sub>4</sub></sub>, **(a)** 0 mins, 1.0 : 0 : 0 : 0 : 0 : 0 : 0, **(b)** 10 mins, 0.63 : 0.28 : 0.09 : 0 : 0 : 0 : 0, **(c)** 20 mins, 0.03 : 0.12 : 0.39 : 0.39 : 0.04 : 0.03 : 0.



**Fig. S1 (d-h)** Deconvolution of EPR spectra from NAMI-A in buffer after incubation for: 30 minutes and; 1, 2, 6 and 24 hours, at 37 °C. Experimental conditions as above. **Simulation parameters:** For g values and line widths, see main text. Relative intensities used in simulations, NAMI-A : NAMI-A<sub>H<sub>2</sub>O</sub>-A : NAMI-A<sub>H<sub>2</sub>O</sub>-E : NAMI-A<sub>(H<sub>2</sub>O)<sub>2</sub></sub> : NAMI-A<sub>(H<sub>2</sub>O)<sub>3</sub>-fac</sub> : NAMI-A<sub>(H<sub>2</sub>O)<sub>3</sub>-mer</sub> : NAMI-A<sub>(H<sub>2</sub>O)<sub>4</sub></sub>, **(d)** 30 mins, 0 : 0 : 0.209 : 0.699 : 0.067 : 0.022 : 0.002, **(e)** 1 h, 0 : 0 : 0 : 0.796 : 0.174 : 0.022 : 0.008, **(f)** 2 h, 0 : 0 : 0 : 0.56 : 0.35 : 0.06 : 0.03, **(g)** 6 h, 0 : 0 : 0 : 0.30 : 0.42 : 0.09 : 0.18, **(h)** 24 h, 0 : 0 : 0 : 0 : 0.47 : 0.09 : 0.44.

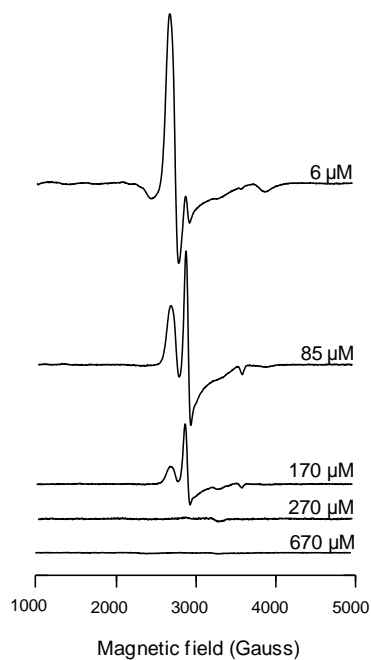


**Fig. S2 (a-c)** Deconvolution of EPR spectra from NAMI-A in buffer with hsA after incubation for 0, 10 and 20 minutes at 37 °C. **Experimental conditions:** frequency = 9.376 GHz, microwave power = 2.0 mW, time constant = 40.96 ms, modulation amplitude = 6 G, average of five 2-minute scans. **Simulation parameters:** For g values and line widths, see main text. Relative intensities used in simulations, NAMI-A : NAMI-A<sub>H<sub>2</sub>O-A</sub> : NAMI-A<sub>H<sub>2</sub>O-E</sub> : NAMI-A-hsA-1 : NAMI-A-hsA-2 : NAMI-A-hsA-3 **(a)** 0 mins, 0.20 : 0.50 : 0.10 : 0 : 0 : 0.20, **(b)** 10 mins, 0 : 0.43 : 0.14 : 0.14 : 0 : 0.29, **(c)** 20 mins, 0 : 0 : 0 : 0.51 : 0.31 : 0.18.

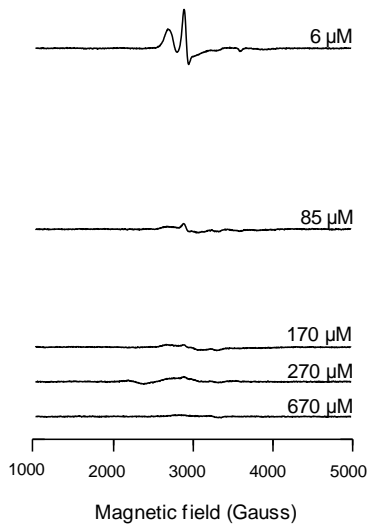


**Fig. S2 (d-h)** Deconvolution of EPR spectra from NAMI-A in buffer with hsA for: 30 minutes and; 1, 2, 6 and 24 hours, at 37 °C. Experimental conditions as above. **Simulation parameters:** Relative intensities used in simulations, NAMI-A : NAMI-A<sub>H<sub>2</sub>O</sub>-A : NAMI-A<sub>H<sub>2</sub>O</sub>-E : NAMI-A-hsA-1 : NAMI-A-hsA-2 : NAMI-A-hsA-3 **(d)** 30 mins, 0 : 0 : 0 : 0.45 : 0.35 : 0.20, **(e)** 1 h, 0 : 0 : 0 : 0.35 : 0.41 : 0.24, **(f)** 2 h, 0 : 0 : 0 : 0.37 : 0.42 : 0.21, **(g)** 6 h, 0 : 0 : 0 : 0.32 : 0.47 : 0.21, **(h)** 24 h, 0 : 0 : 0 : 0.13 : 0.64 : 0.23.

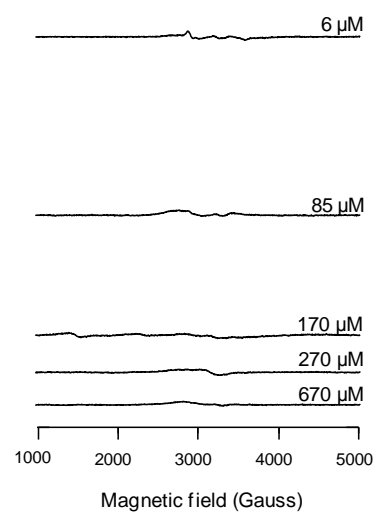
S3 (a) Immediately frozen.



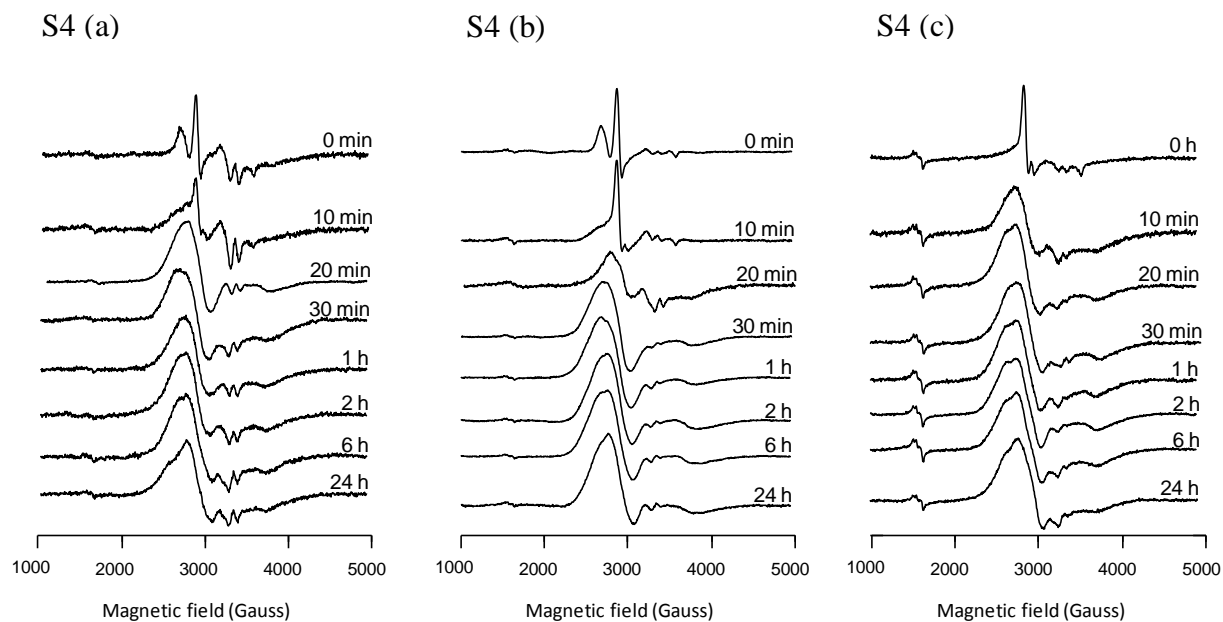
S3 (b) Incubated for 10 min at room temperature.



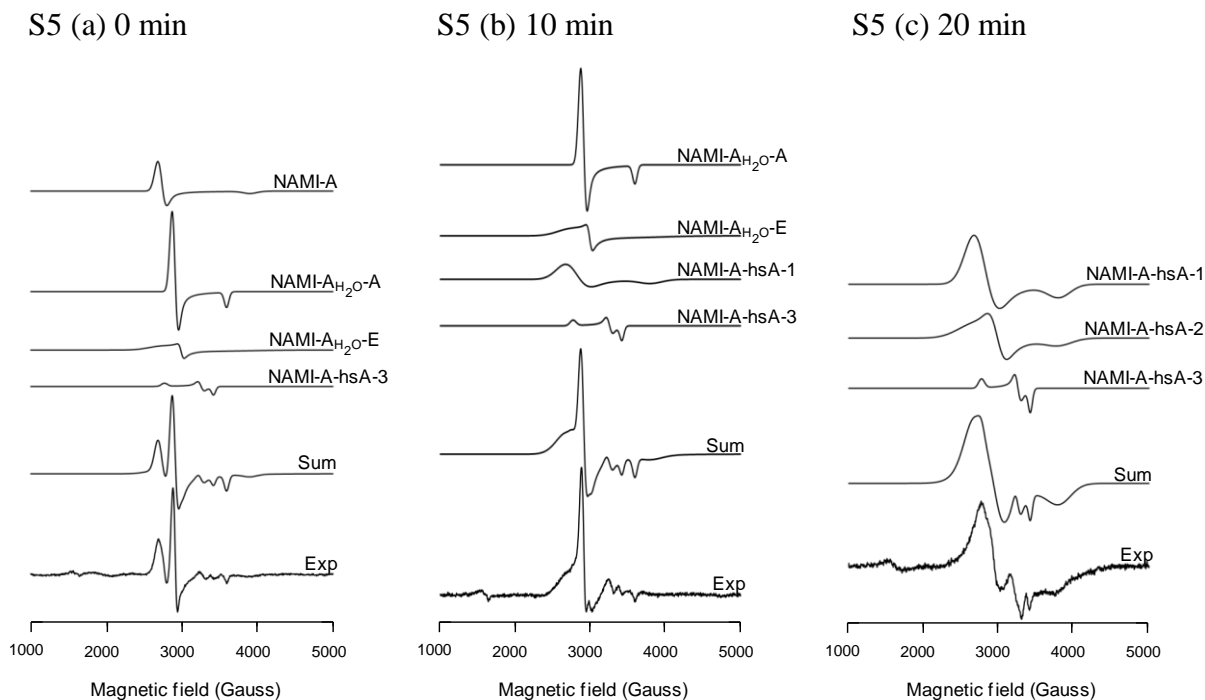
S3 (c) Incubated for 10 min at 37 °C.



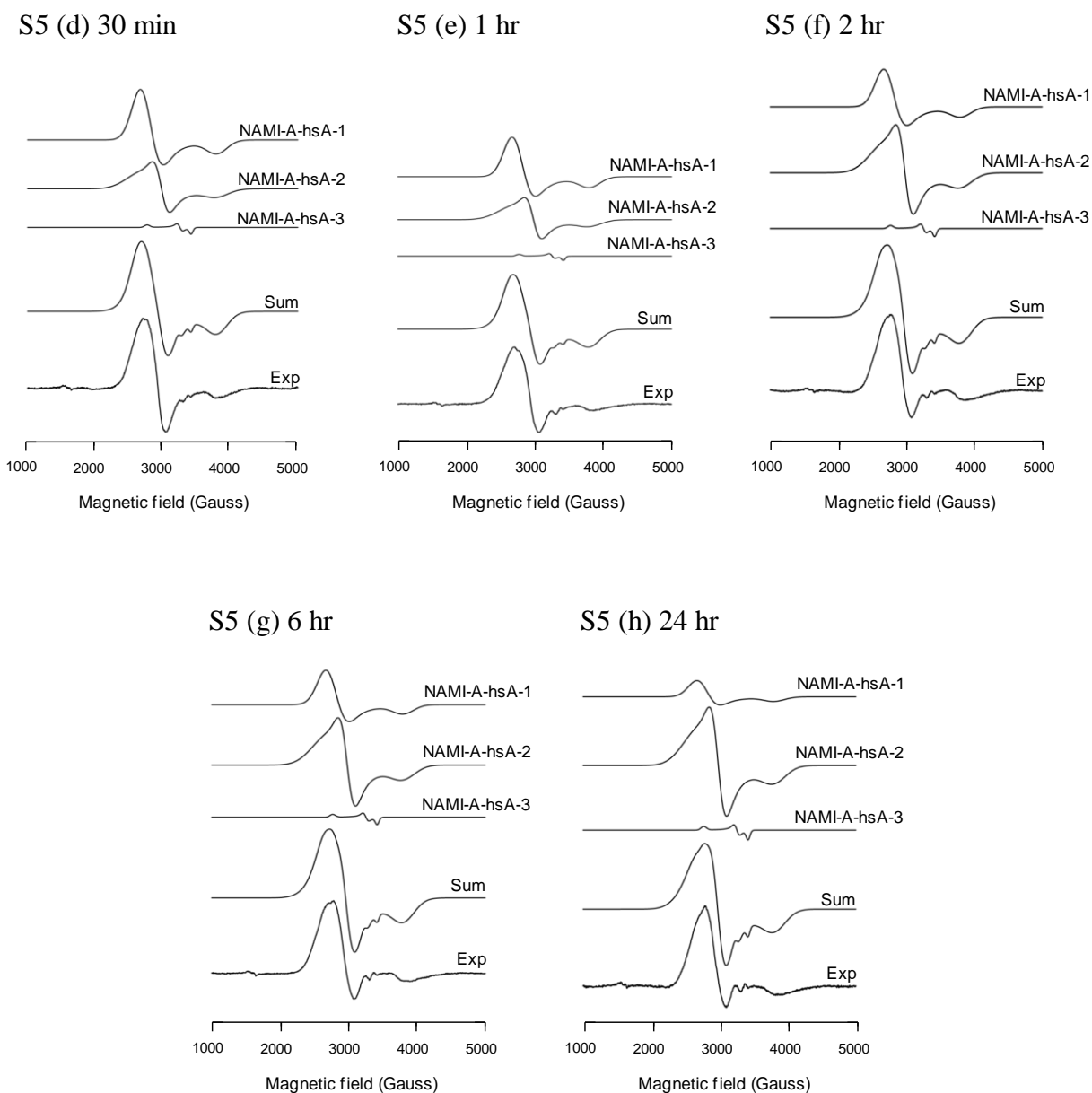
**Fig. S3 (a-c)** Effect of ascorbic acid concentration and incubation time on reduction of NAMI-A and aquated complexes as determined by EPR. **Experimental conditions:** frequency = 9.377 GHz, microwave power = 2.0 mW, time constant = 40.96 ms, modulation amplitude = 6 G, average of five 2-minute scans.



**Fig. S4 (a-c)** Comparison of EPR spectra from NAMI-A incubated with: (a) hsA, (b) hsA and ascorbic acid, (c) human serum. **Experimental conditions:** frequency = 9.378 GHz, microwave power = 2.0 mW, time constant = 40.96 ms, modulation amplitude = 6 G, average of five 2-minute scans.

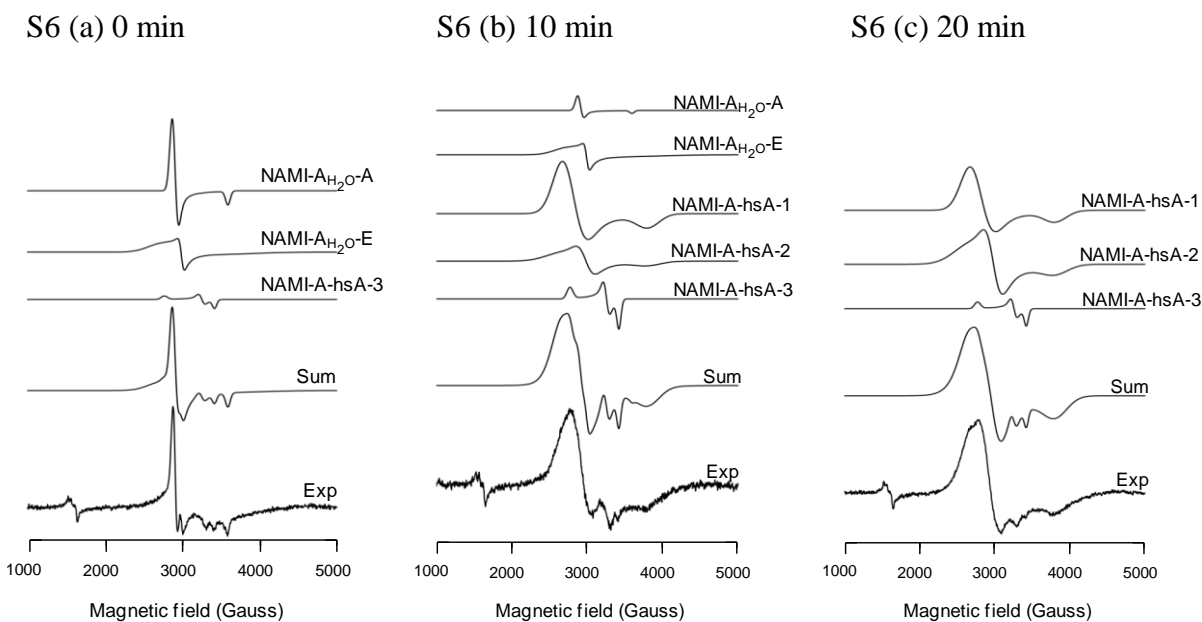


**Fig. S5 (a-c).** Deconvolution of EPR spectra from NAMI-A in buffer with hsA and ascorbic acid after incubation for 0, 10 and 20 minutes at 37 °C. **Experimental conditions:** frequency = 9.376 GHz, microwave power = 2.0 mW, time constant = 40.96 ms, modulation amplitude = 6 G, average of five 2-minute scans. **Simulation parameters:** For g values and line widths, see main text. Relative intensities used in simulations, NAMI-A : NAMI-A<sub>H<sub>2</sub>O<sup>-</sup></sub>A : NAMI-A<sub>H<sub>2</sub>O<sup>-</sup></sub>E : NAMI-A-hsA-1 : NAMI-A-hsA-2 : NAMI-A-hsA-3 **(a)** 0 mins, 0.23 : 0.63 : 0.07 : 0 : 0 : 0.07, **(b)** 10 mins, 0 : 0.67 : 0.11 : 0.11 : 0 : 0.11, **(c)** 20 mins, 0 : 0 : 0 : 0.50 : 0.25 : 0.25.

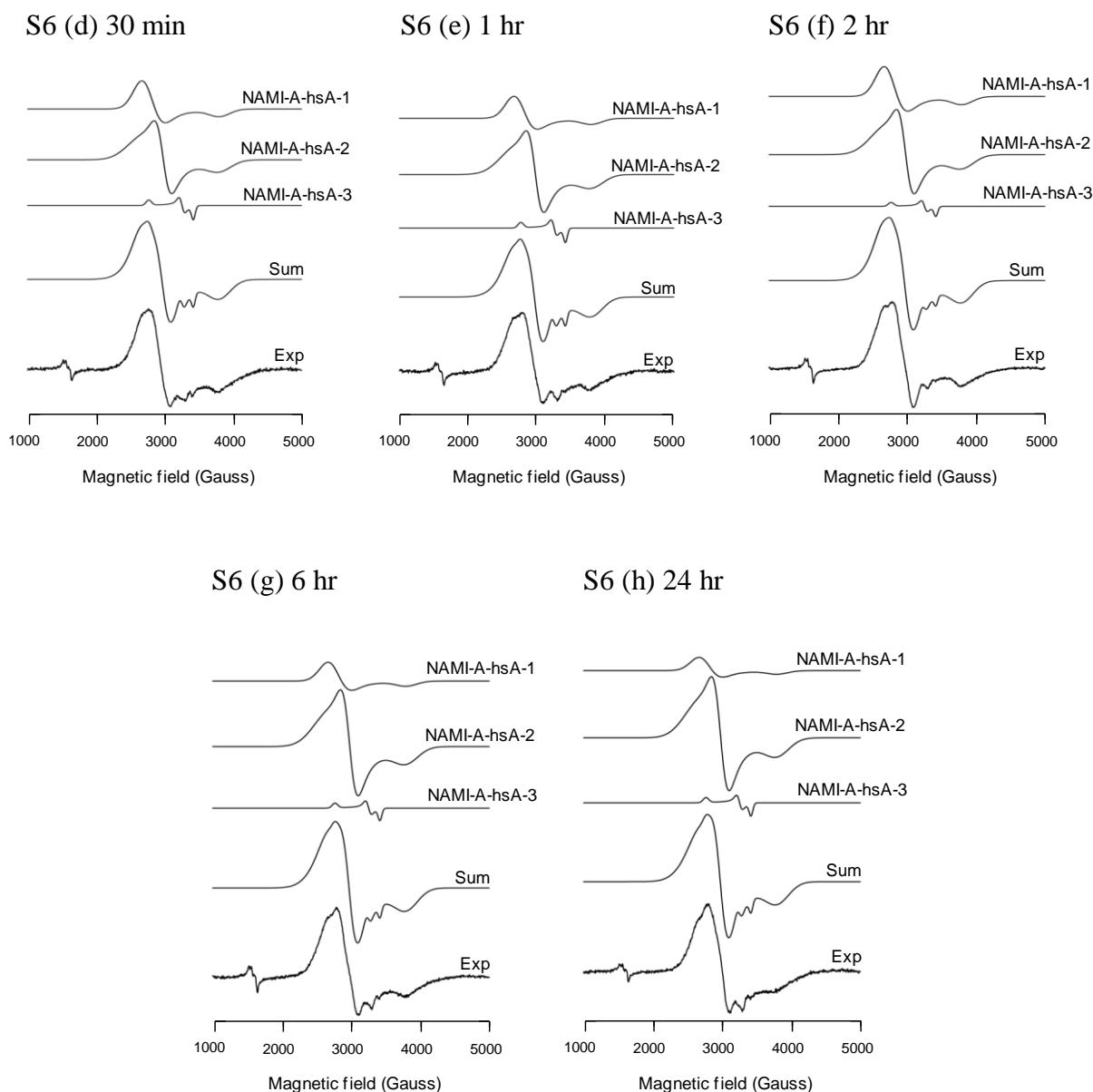


**Fig. S5 (d-h).** Deconvolution of EPR spectra from NAMI-A in buffer with hsA and ascorbic acid after incubation for: 30 minutes and; 1, 2, 6 and 24 hours, at 37 °C. Experimental conditions as above. **Simulation parameters:** Relative intensities used in simulations, NAMI-A : NAMI-A<sub>H<sub>2</sub>O</sub> -A : NAMI-A<sub>H<sub>2</sub>O</sub>-E : NAMI-A-hsA-1 : NAMI-A-hsA-2 : NAMI-A-hsA-3 **(d)** 30 mins, 0 : 0 : 0 : 0.60 : 0.32 : 0.08, **(e)** 1 h, 0 : 0 : 0 : 0.60 : 0.33 : 0.07, **(f)** 2 h, 0 : 0 : 0 : 0.40 : 0.51 : 0.09, **(g)** 6 h, 0 : 0 : 0 : 0.39 : 0.53 : 0.08, **(h)** 24 h, 0 : 0 : 0 : 0.19 : 0.69 : 0.12.





**Fig. S6 (a-c).** Deconvolution of EPR spectra from NAMI-A in human serum after incubation for 0, 10 and 20 minutes at 37 °C. **Experimental conditions:** frequency = 9.376 GHz, microwave power = 2.0 mW, time constant = 40.96 ms, modulation amplitude = 6 G, average of five 2-minute scans. **Simulation parameters:** For g values and line widths, see main text. Relative intensities used in simulations, NAMI-A : NAMI-A<sub>H<sub>2</sub>O</sub>-A : NAMI-A<sub>H<sub>2</sub>O</sub>-E : NAMI-A-hsA-1 : NAMI-A-hsA-2 : NAMI-A-hsA-3 **(a)** 0 mins, 0 : 0.73 : 0.18 : 0 : 0 : 0.09, **(b)** 10 mins, 0 : 0.12 : 0.12 : 0.41 : 0.12 : 0.23, **(c)** 20 mins, 0 : 0 : 0 : 0.46 : 0.36 : 0.18.



**Figures S6 (d-h).** Deconvolution of EPR spectra from NAMI-A in human serum after incubation after incubation for: 30 minutes and; 1, 2, 6 and 24 hours, at 37 °C. Experimental conditions as above. **Simulation parameters:** Relative intensities used in simulations, NAMI-A : NAMI-A<sub>H<sub>2</sub>O</sub> -A : NAMI-A<sub>H<sub>2</sub>O</sub>-E : NAMI-A-hsA-1 : NAMI-A-hsA-2 : NAMI-A-hsA-3 **(d)** 30 mins, 0 : 0 : 0 : 0.35 : 0.48 : 0.17, **(e)** 1 h, 0 : 0 : 0 : 0.27 : 0.55 : 0.18, **(f)** 2 h, 0 : 0 : 0 : 0.35 : 0.53 : 0.12, **(g)** 6 h, 0 : 0 : 0 : 0.22 : 0.64 : 0.14, **(h)** 24 h, 0 : 0 : 0 : 0.15 : 0.70 : 0.15.