

Albumin binding and ligand-exchange processes of the Ru(III) anticancer agent NAMI-A and its bis-DMSO analogue determined by ENDOR spectroscopy

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Supporting Information

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Table S1: g values and line widths, used in the simulation of all EPR spectra.

Compound	g values			Linewidths		
	g ₁	g ₂	g ₃	LW ₁	LW ₂	LW ₃
Ru-bis-DMSO (Ru-DMSO-H ₂ O)	2.43	2.43	1.77	100	100	150
Ru-bis-DMSO-D ₆ (Ru-DMSO-D ₆ -H ₂ O)	2.43	2.43	1.77	100	100	150
Ru-(H ₂ O) ₂ -eq	2.40	2.24	1.89	90	90	90
Ru-(H ₂ O) ₂ -ax	2.35	2.35	1.80	110	110	200
Ru-HSA-1	2.40	2.40	1.72	210	210	400
Ru-HSA-2	2.50	2.25	1.10	400	150	400
Ru-HSA-3	2.42	2.05	1.88	80	70	55
Ru-His-1	2.37	2.37	1.82	60	60	80
Ru-His-2	2.40	2.40	1.77	200	200	400
NAMI-A	2.47	2.47	1.72	105	105	180
NAMI-A-H ₂ O	2.30	2.30	1.88	75	75	90
NAMI-A-D ₄ , D ₆ , D ₁₀	2.47	2.47	1.72	100	100	170
NAMI-A-HSA-1	2.43	2.43	1.76	300	300	300
NAMI-A-HSA-2	2.54	2.26	1.76	450	175	350
NAMI-A-HSA-3	2.42	2.06	1.96	80	70	55
NAMI-A-His	2.52	2.29	1.78	175	175	400

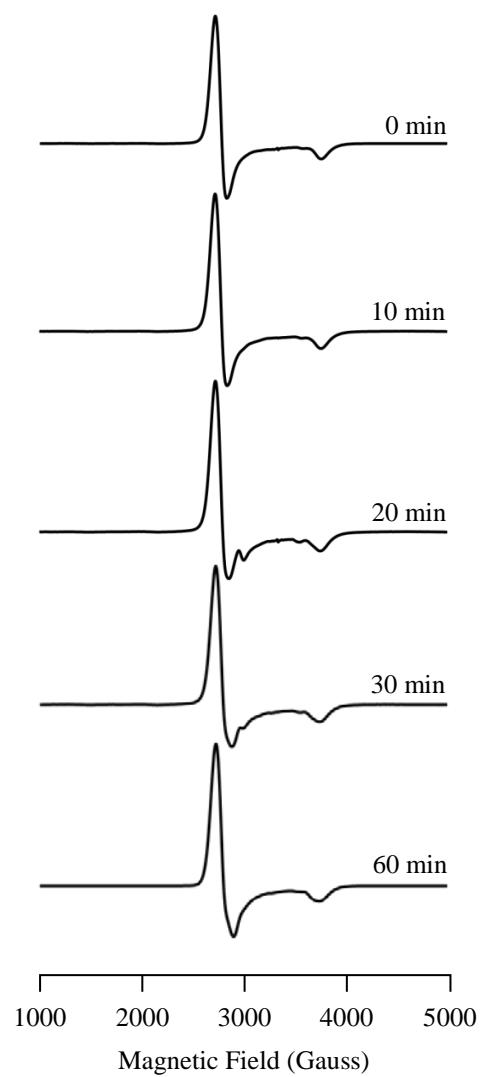


Figure S1 EPR spectra of Ru-bis-DMSO after incubation in PBS for 0, 10, 20, 30, and 60 minutes at 37 °C.
For spectral parameters see experimental section in main text.

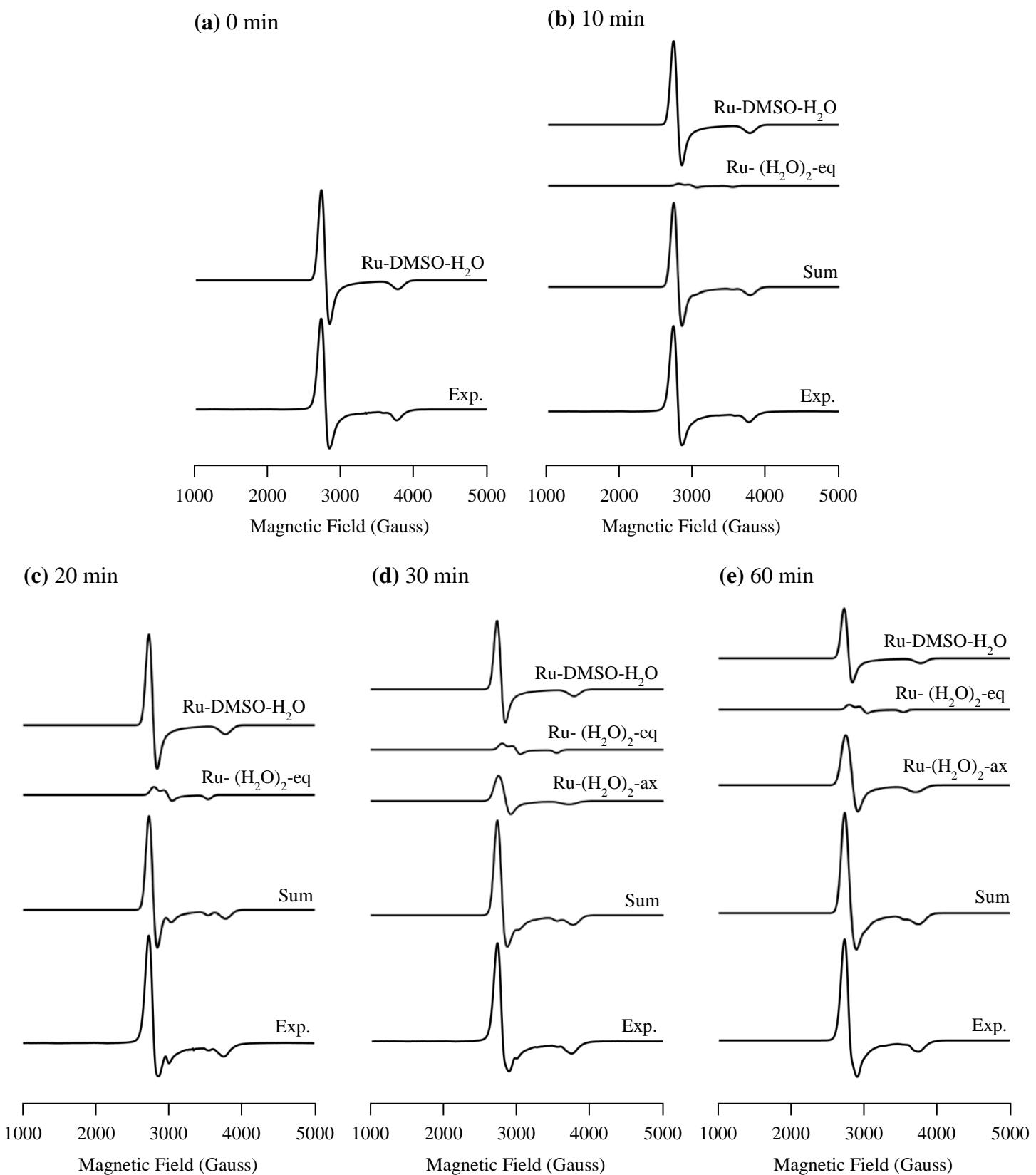


Figure S2 (a-e) Deconvolution of EPR spectra from Ru-bis-DMSO following incubation in PBS for 0, 10, 20, 30, and 60 minutes at 37 °C. For g values and linewidths used in simulations see Table S1.

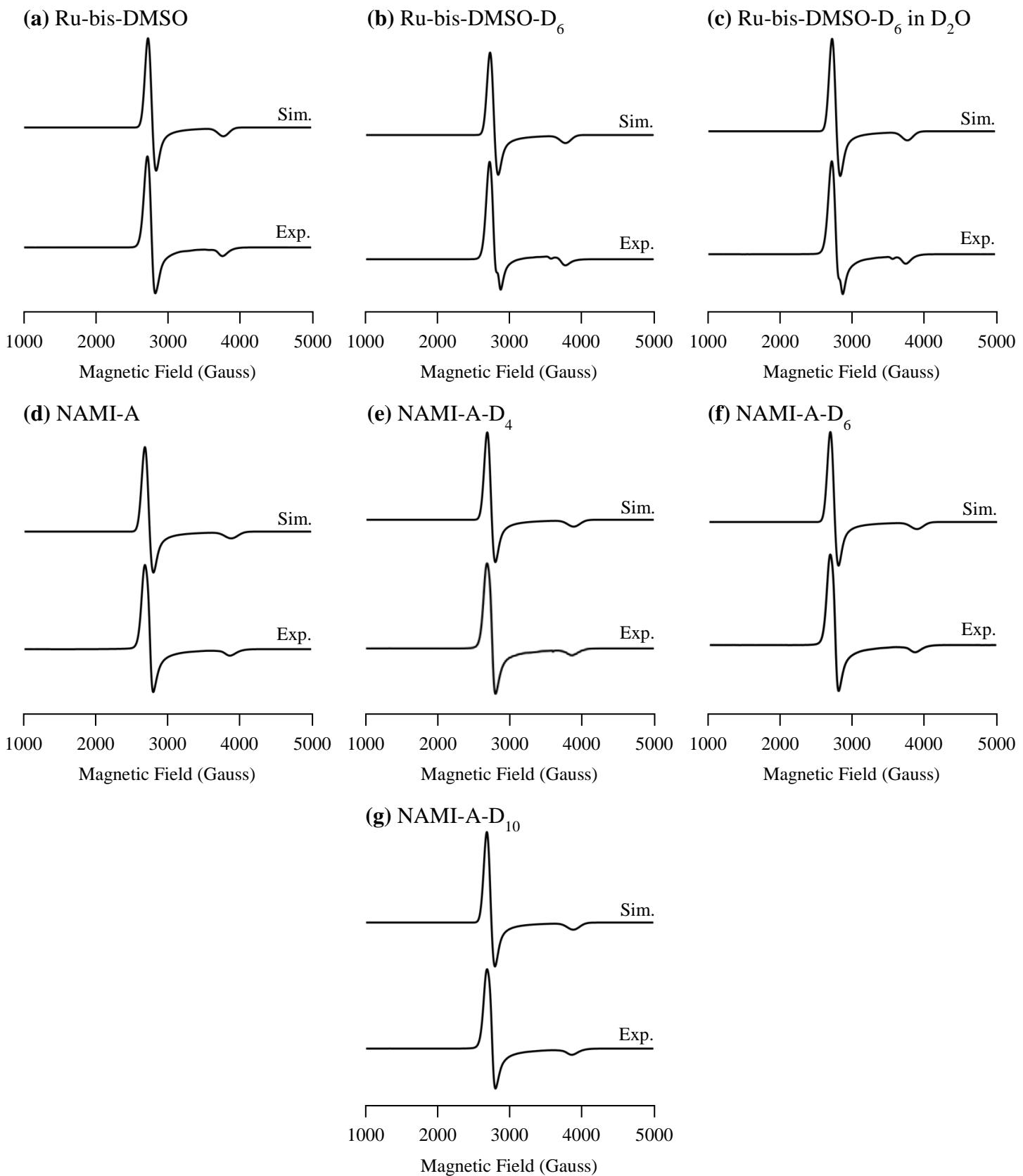


Figure S3 (a-g) EPR spectra from all of the complexes studied in this report in PBS prior to incubation at 37 °C and simulations. For g values and linewidths used in simulations see **Table S-1**.

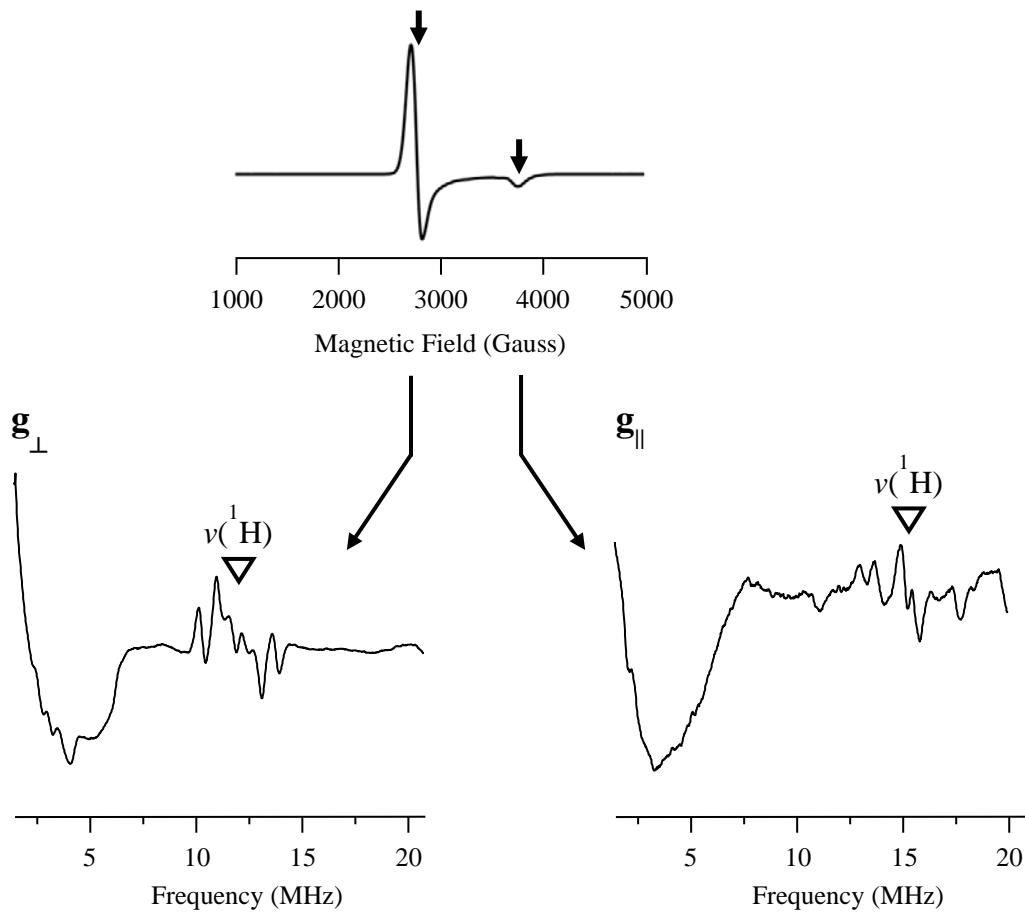


Figure S4 ENDOR spectra of Ru-bis-DMSO in PBS at g_{\perp} and g_{\parallel} prior to incubation at 37 °C.

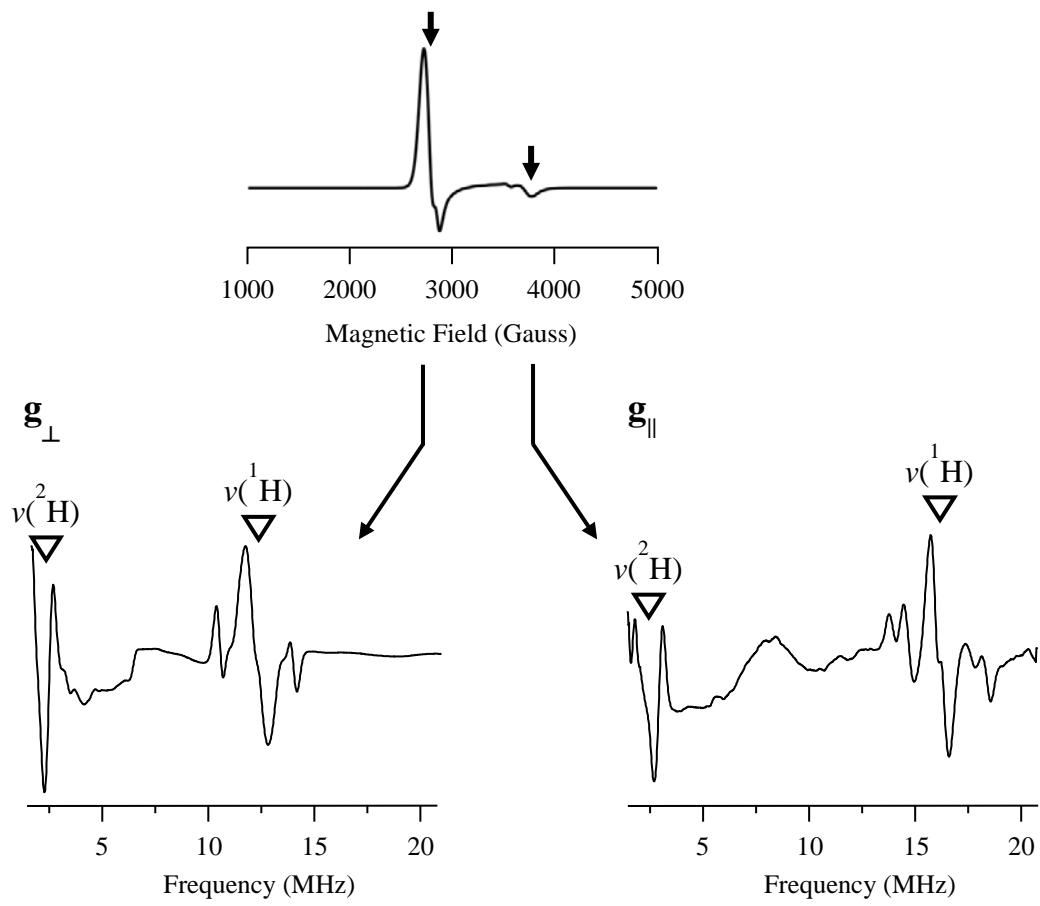


Figure S5 ENDOR spectra of Ru-bis-DMSO-D₆ in PBS at g_{\perp} and g_{\parallel} prior to incubation at 37 °C.

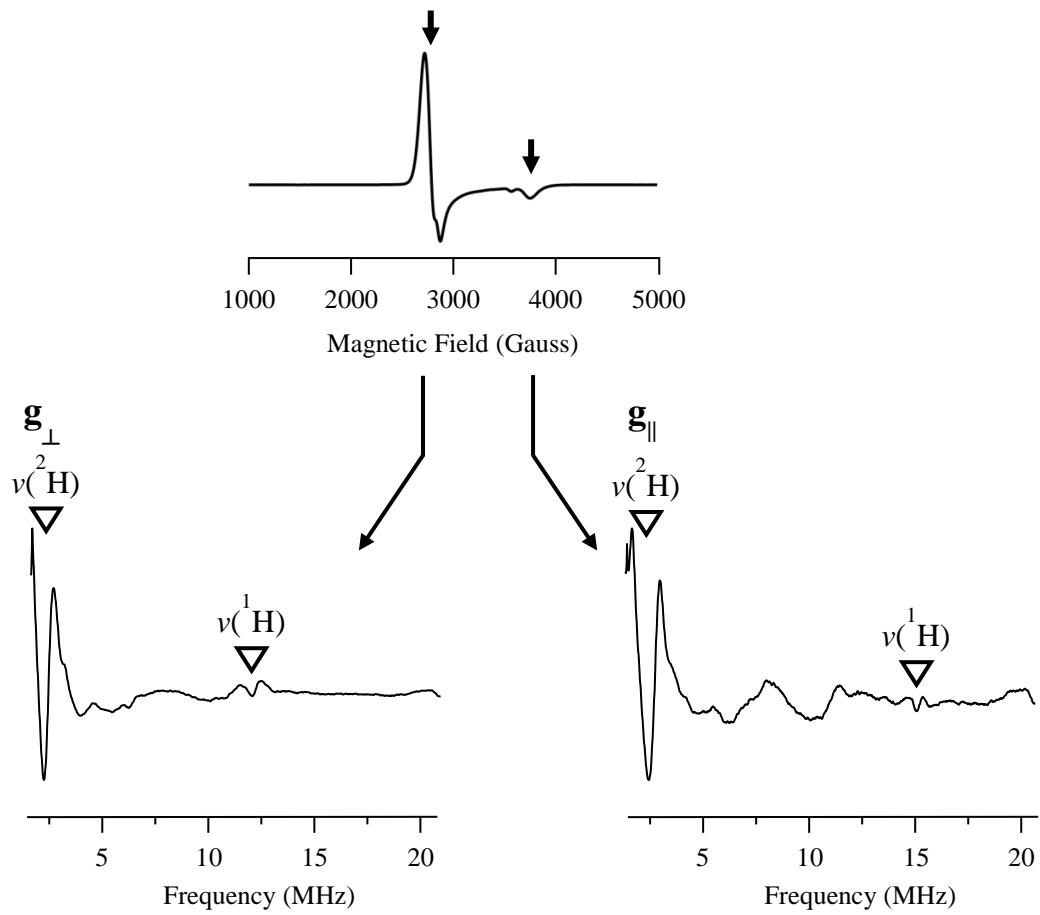


Figure S6 ENDOR spectra of Ru-bis-DMSO-D₆ in D₂O PBS at \mathbf{g}_{\perp} and \mathbf{g}_{\parallel} prior to incubation at 37 °C.

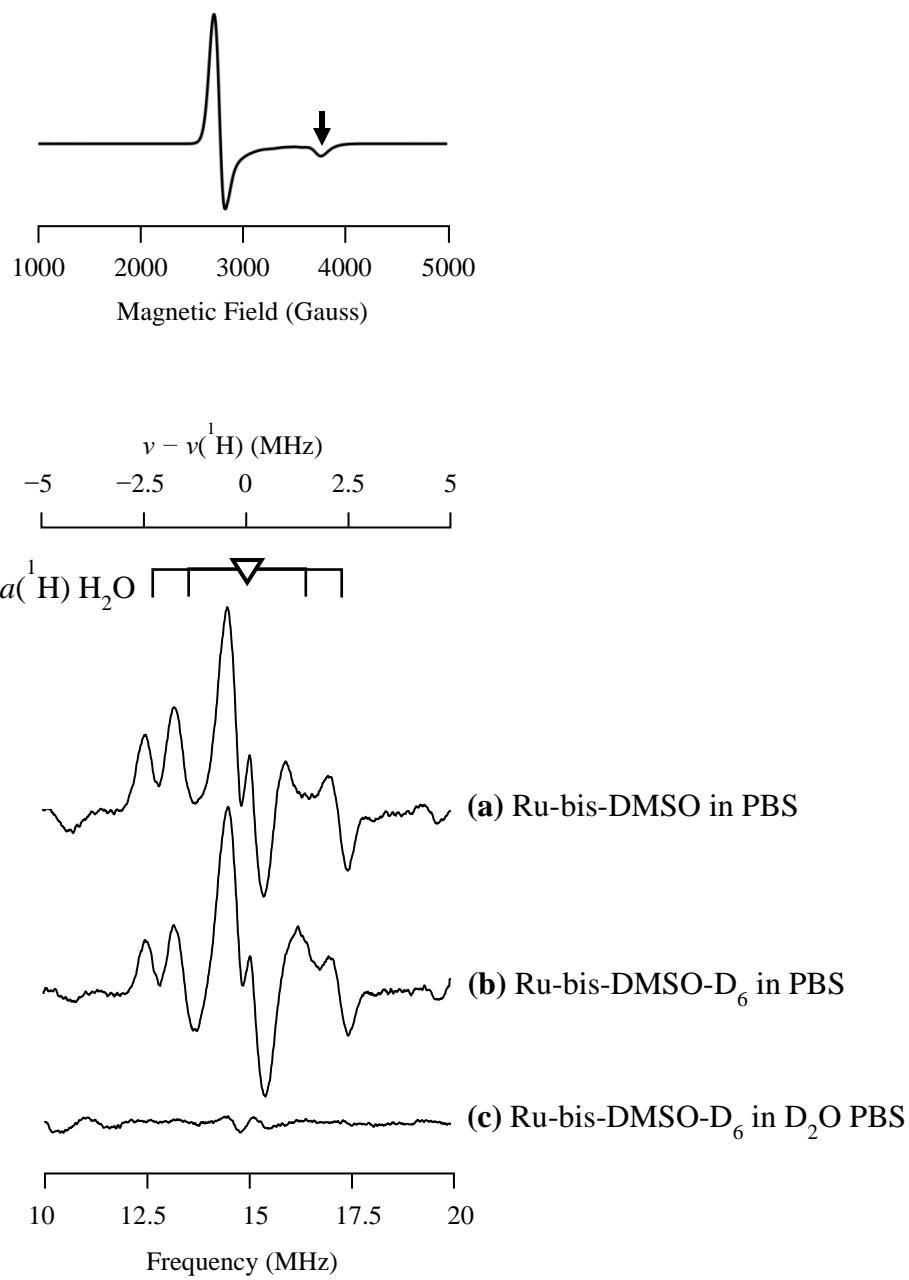


Figure S7 ${}^1\text{H}$ ENDOR spectra at g_{\parallel} of (a) Ru-bis-DMSO in PBS, (b) Ru-bis-DMSO- D_6 in PBS, (c) Ru-bis-DMSO- D_6 in D_2O PBS prior to incubation at 37 °C.

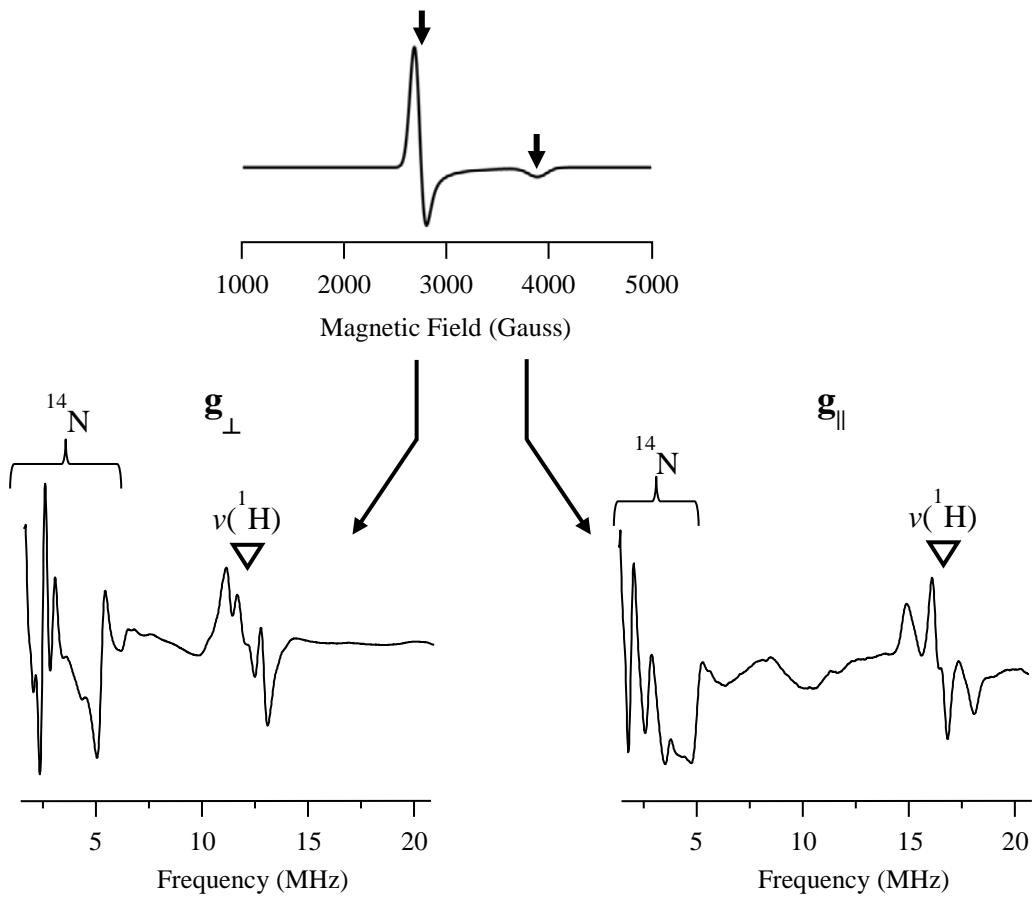


Figure S8 ENDOR spectra of NAMI-A in PBS at g_{\perp} and g_{\parallel} prior to incubation at 37 °C.

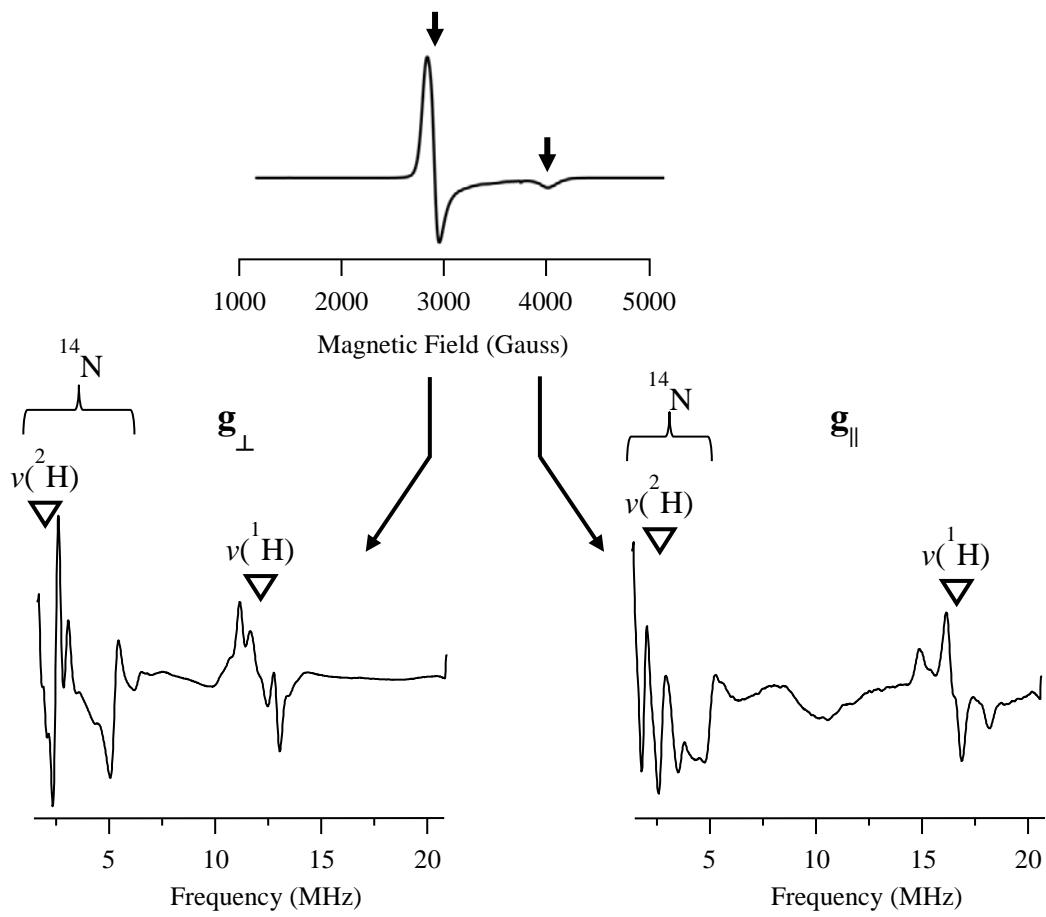


Figure S9 ENDOR spectra of NAMI-A-D₄ in PBS at g_{\perp} and g_{\parallel} prior to incubation at 37 °C.

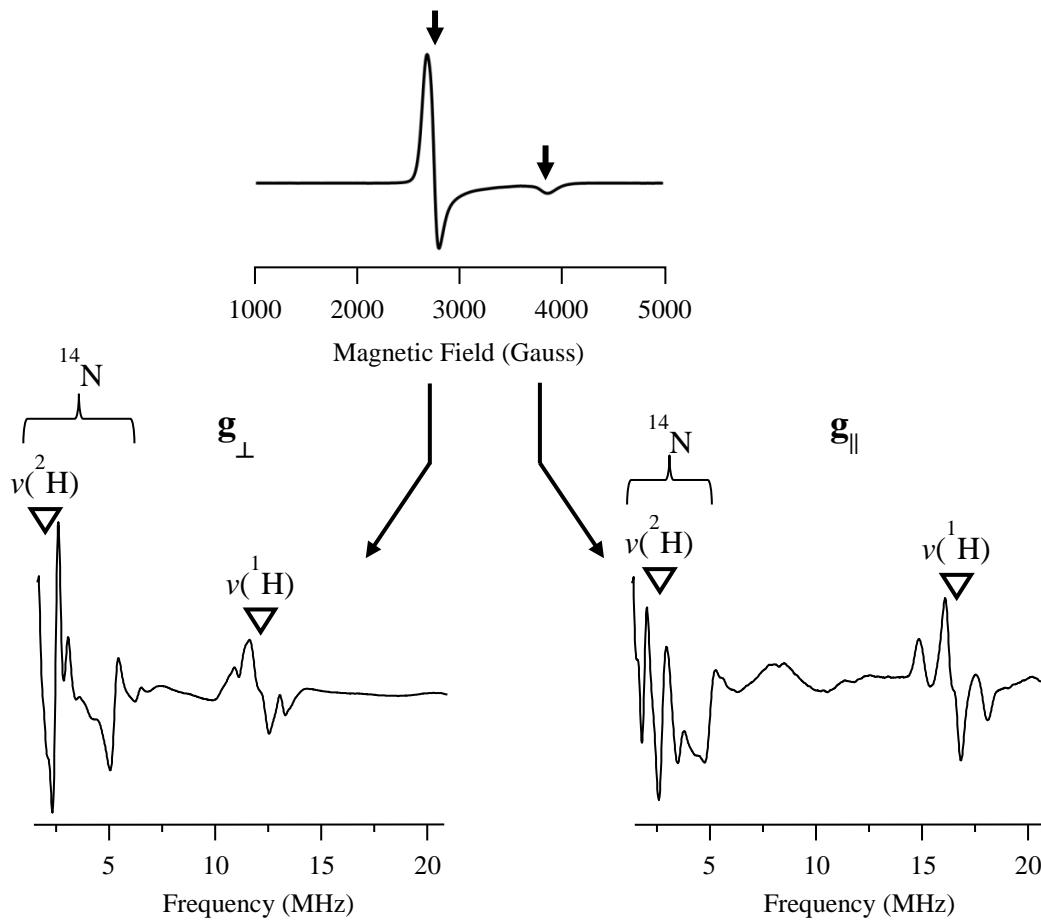


Figure S10 ENDOR spectra of NAMI-A-D₆ in PBS at \mathbf{g}_{\perp} and \mathbf{g}_{\parallel} prior to incubation at 37 °C.

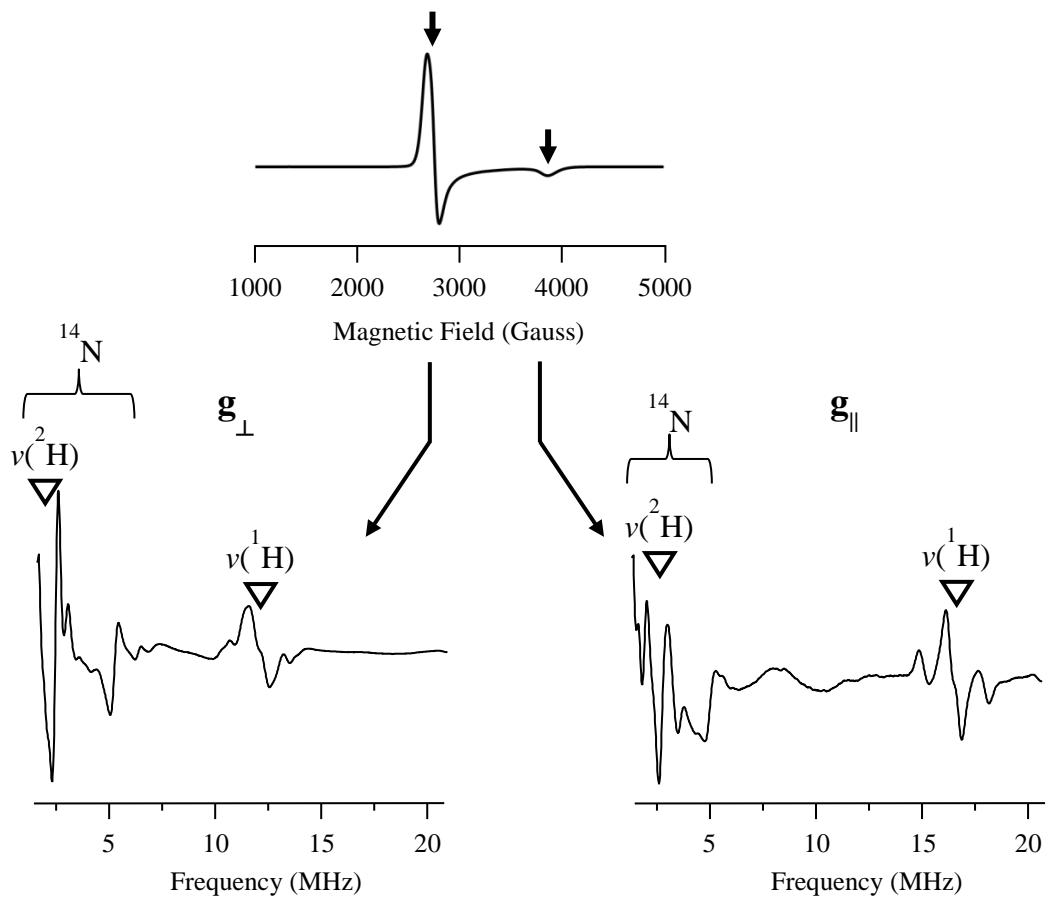


Figure S11 ENDOR spectra of NAMI-A-D₁₀ in PBS at g_{\perp} and g_{\parallel} prior to incubation at 37 °C.

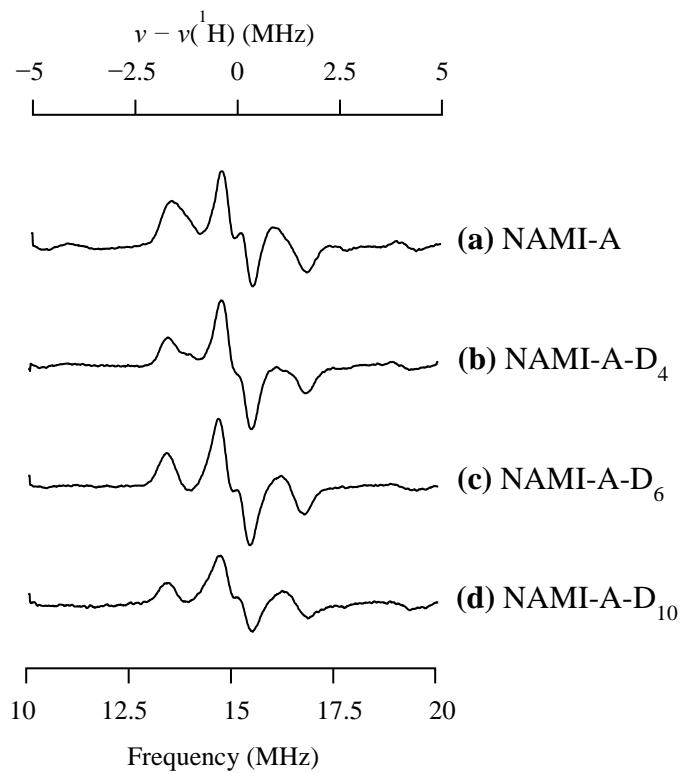
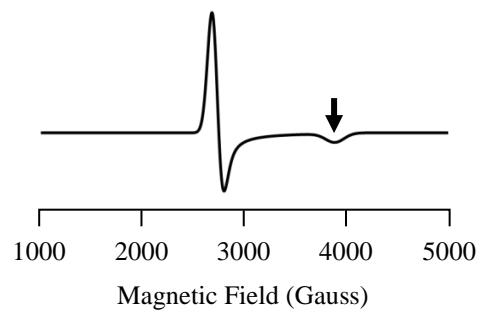


Figure S12 ^1H ENDOR spectra at g_{\parallel} of (a) NAMI-A, (b) NAMI-A-D₄, (c) NAMI-A-D₆, (d) NAMI-A-D₁₀.

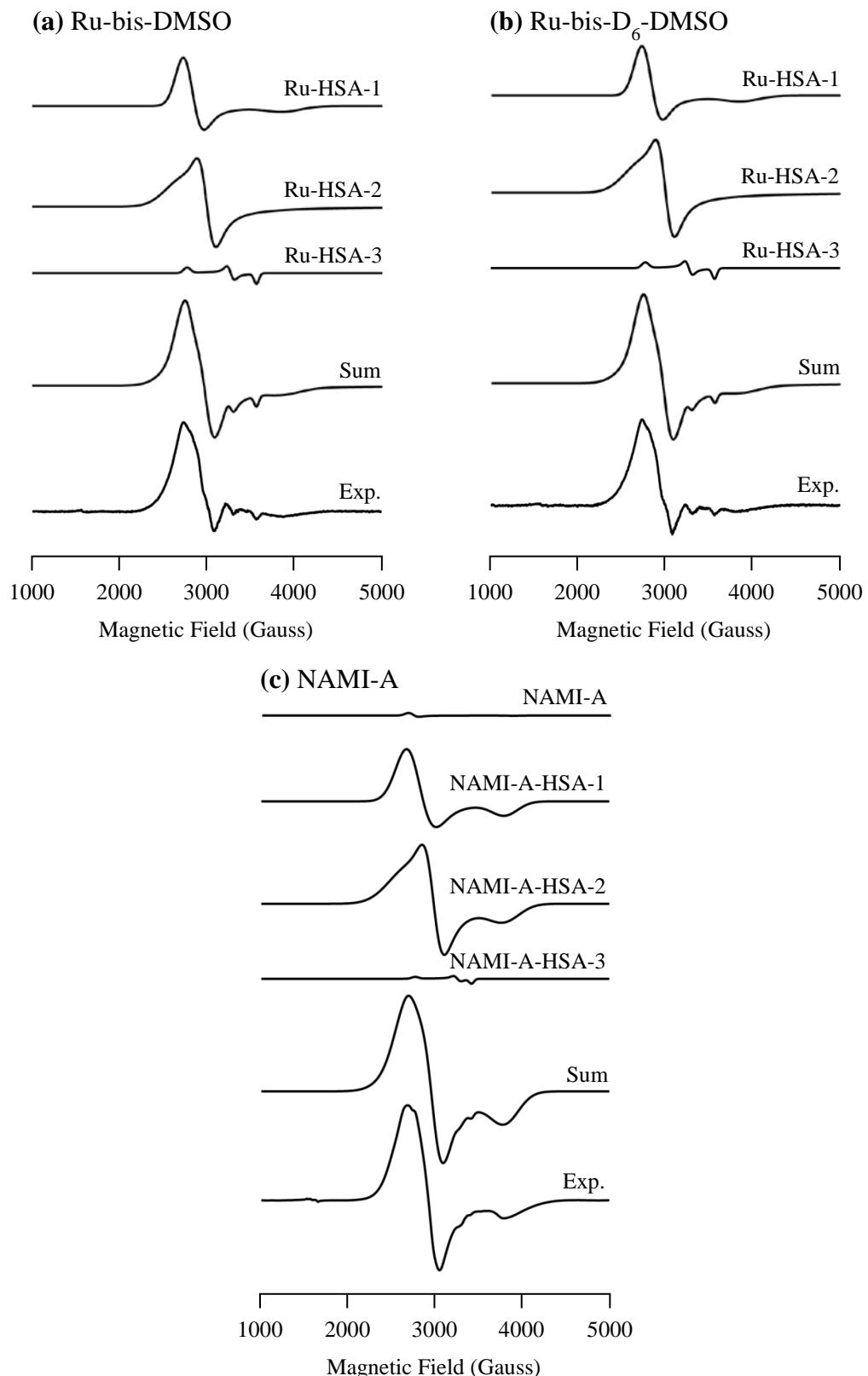


Figure S13 (a-c) Deconvolution of the EPR spectra from **(a)** Ru-bis-DMSO, **(b)** Ru-bis-DMSO- D_6 , and **(c)** NAMI-A in PBS with HSA after incubation for 30 minutes at 37 °C. For g values and linewidths used in simulations see **Table S1**.

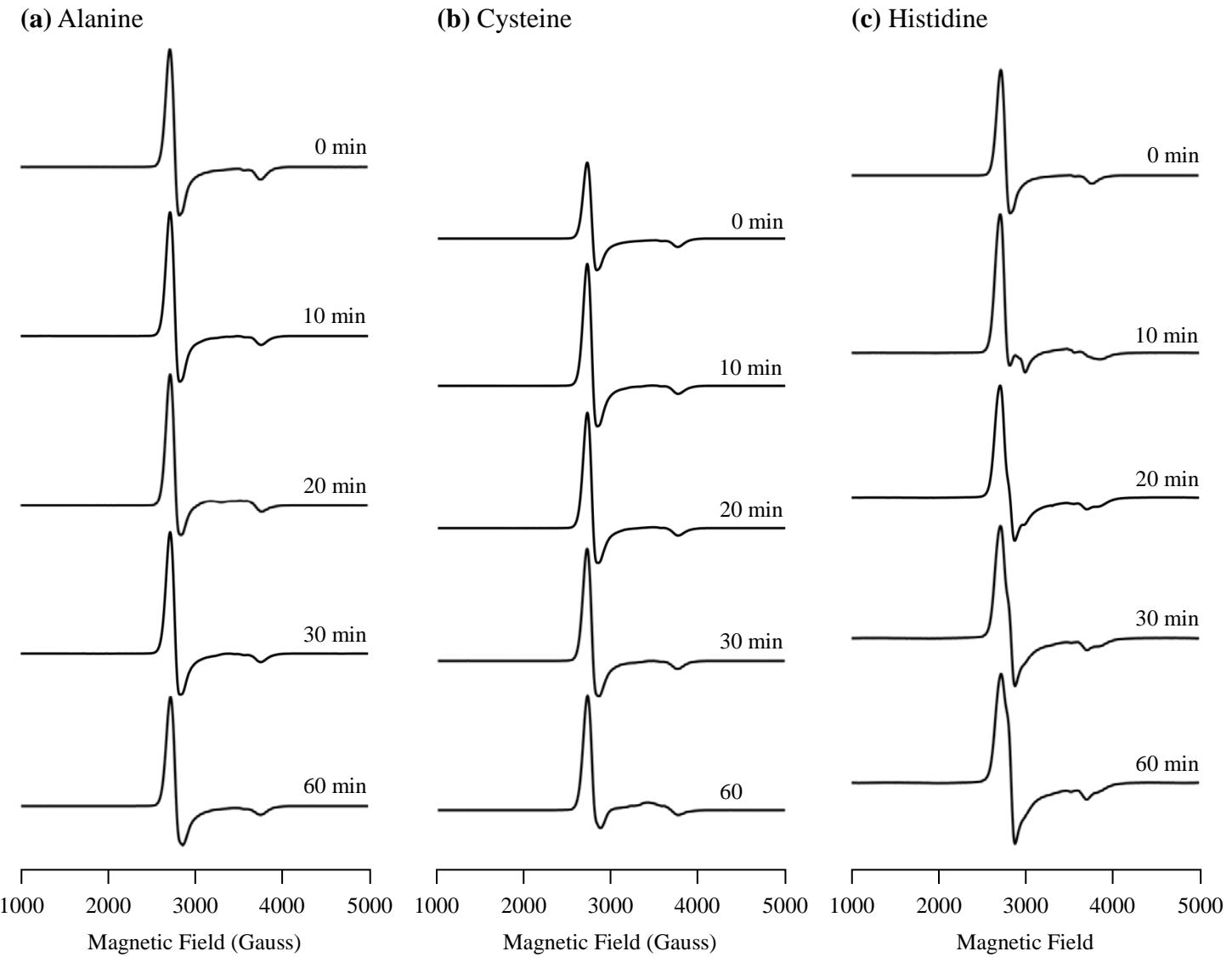


Figure S14 (a-c) EPR spectra of Ru-bis-DMSO with (a) alanine, (b) cysteine, and (c) histidine following incubation in PBS for 0, 10, 20, 30, and 60 minutes at 37 °C.

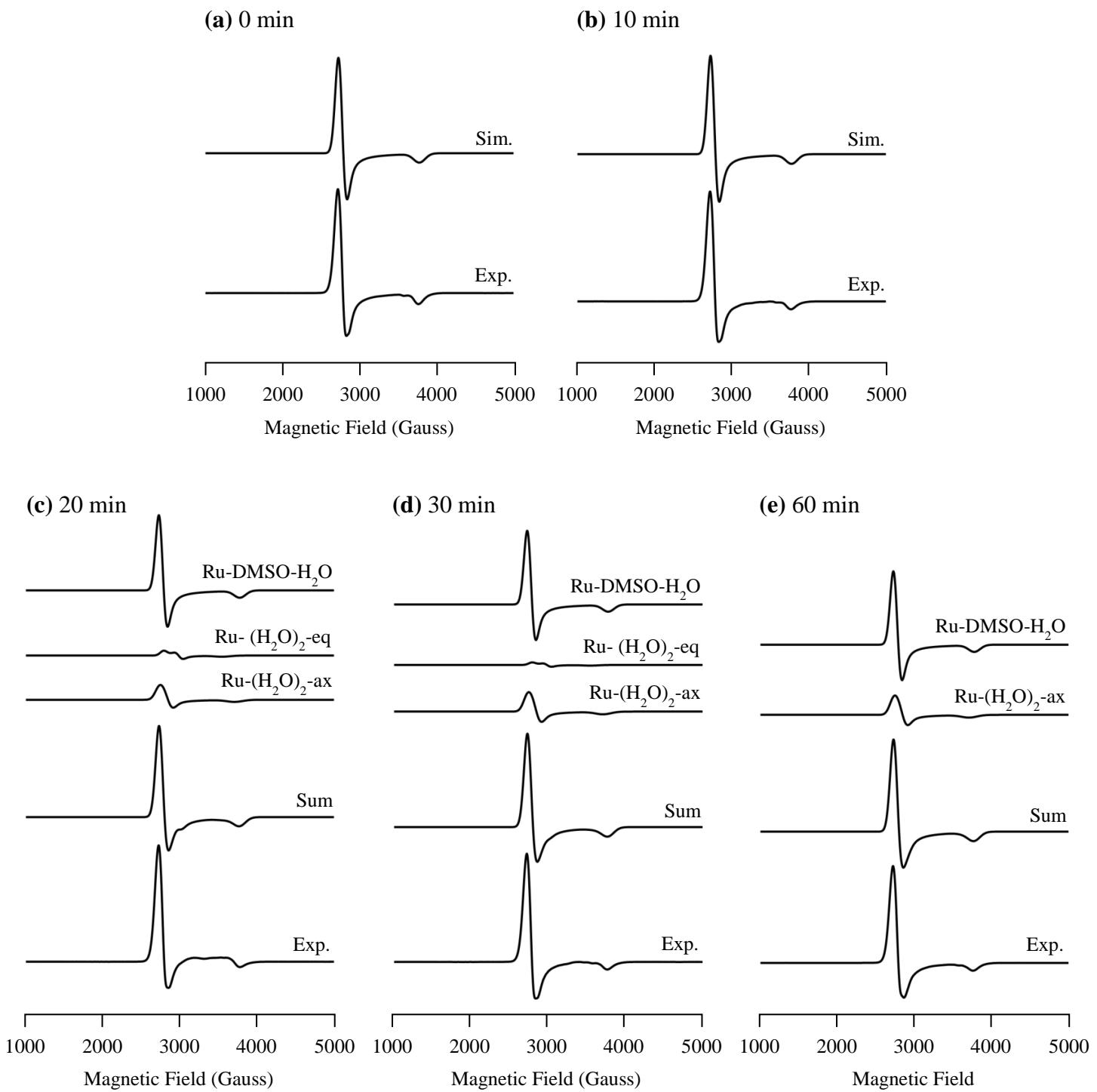


Figure S15 (a-e) Deconvolution of EPR spectra from Ru-bis-DMSO with alanine after incubation in PBS for 0, 10, 20, 30, and 60 minutes at 37 °C. For g values and linewidths used in simulations see **Table S1**.

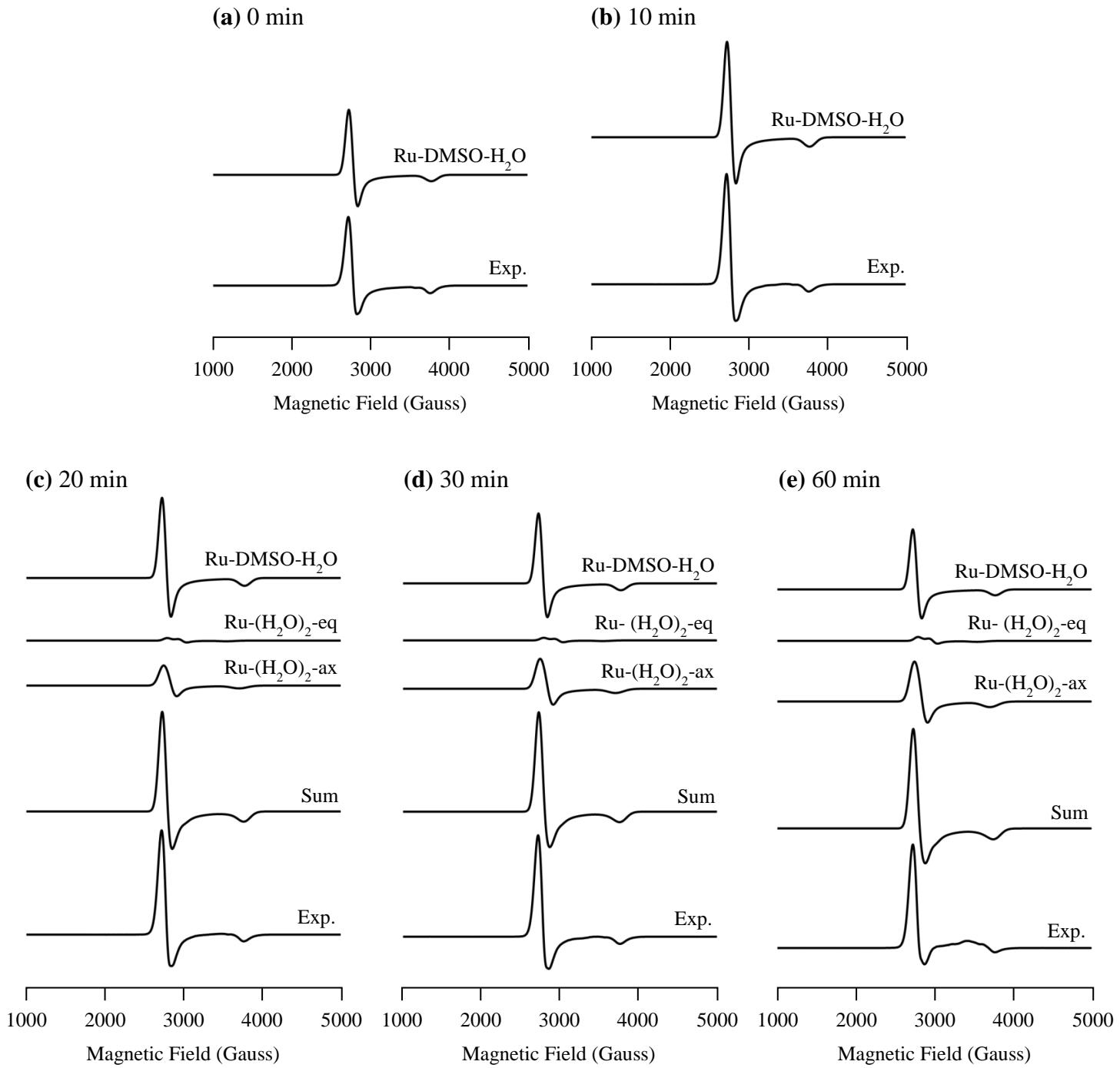


Figure S16 (a-e) Deconvolution of EPR spectra from Ru-bis-DMSO with cysteine after incubation in PBS for 0, 10, 20, 30, and 60 minutes at 37 °C. For g values and linewidths used in simulations see **Table S1**.

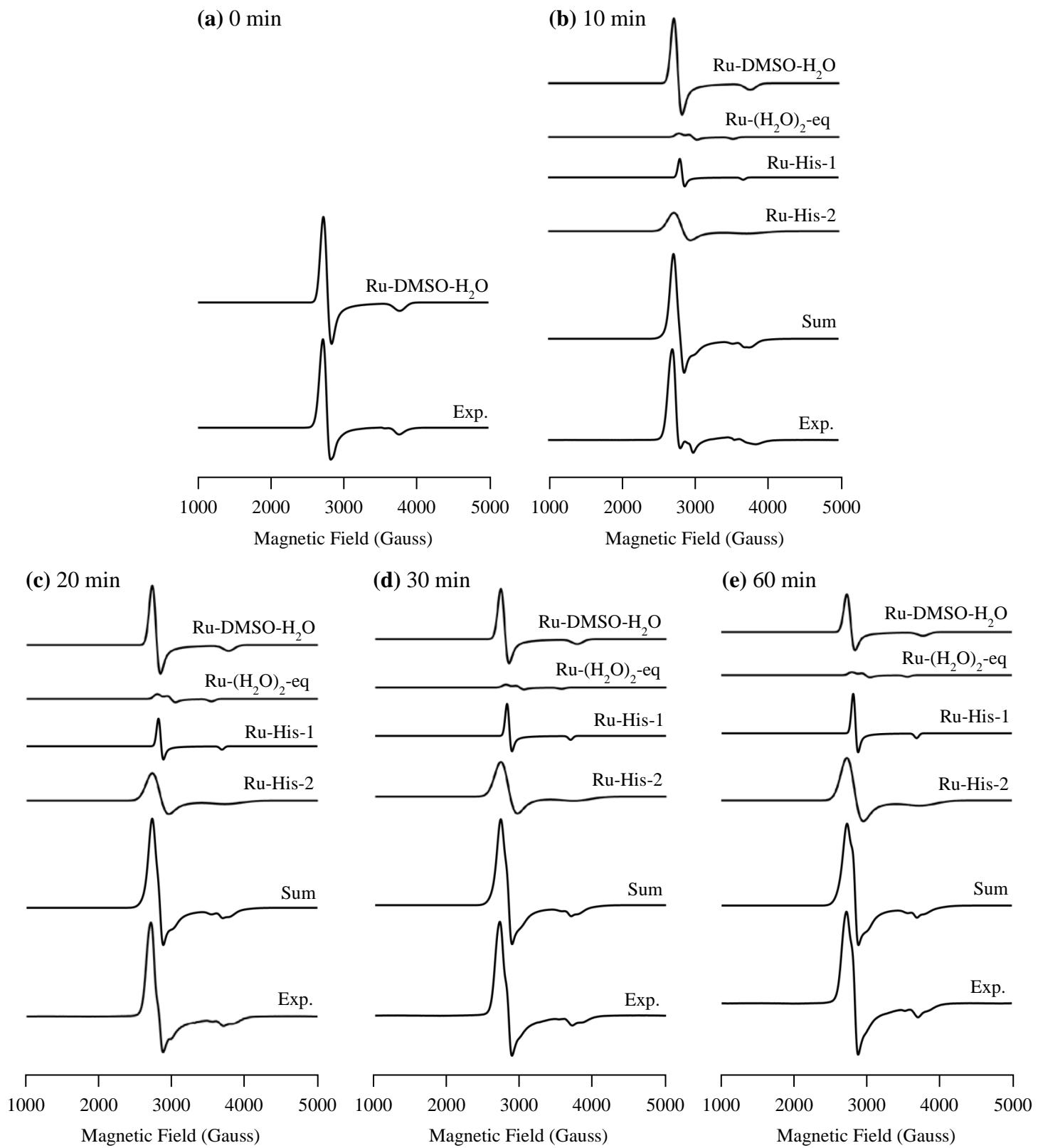


Figure S17 (a-e) Deconvolution of EPR spectra from Ru-bis-DMSO with histidine after incubation in PBS for 0, 10, 20, 30, and 60 minutes at 37 °C. For g values and linewidths used in simulations see **Table S1**.

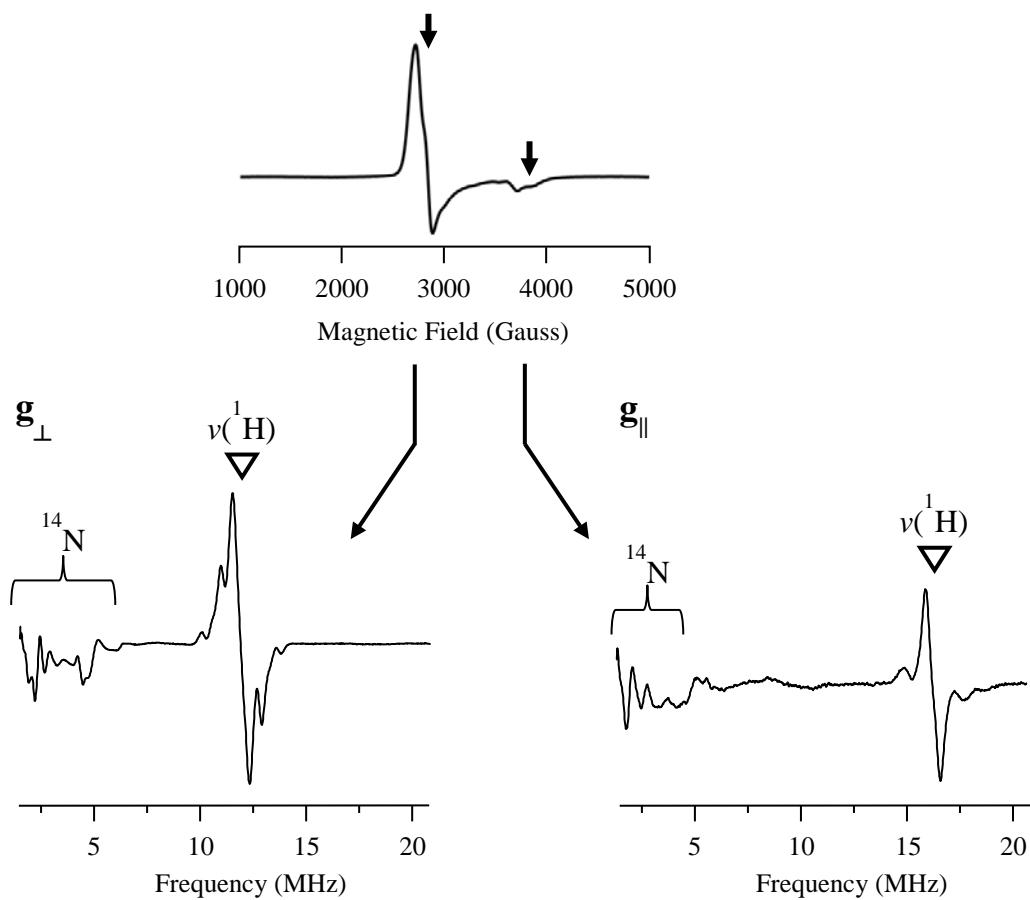


Figure S18 ENDOR spectra of Ru-bis-DMSO with histidine in PBS at g_{\perp} and g_{\parallel} after incubation for 30 minutes at 37 °C.

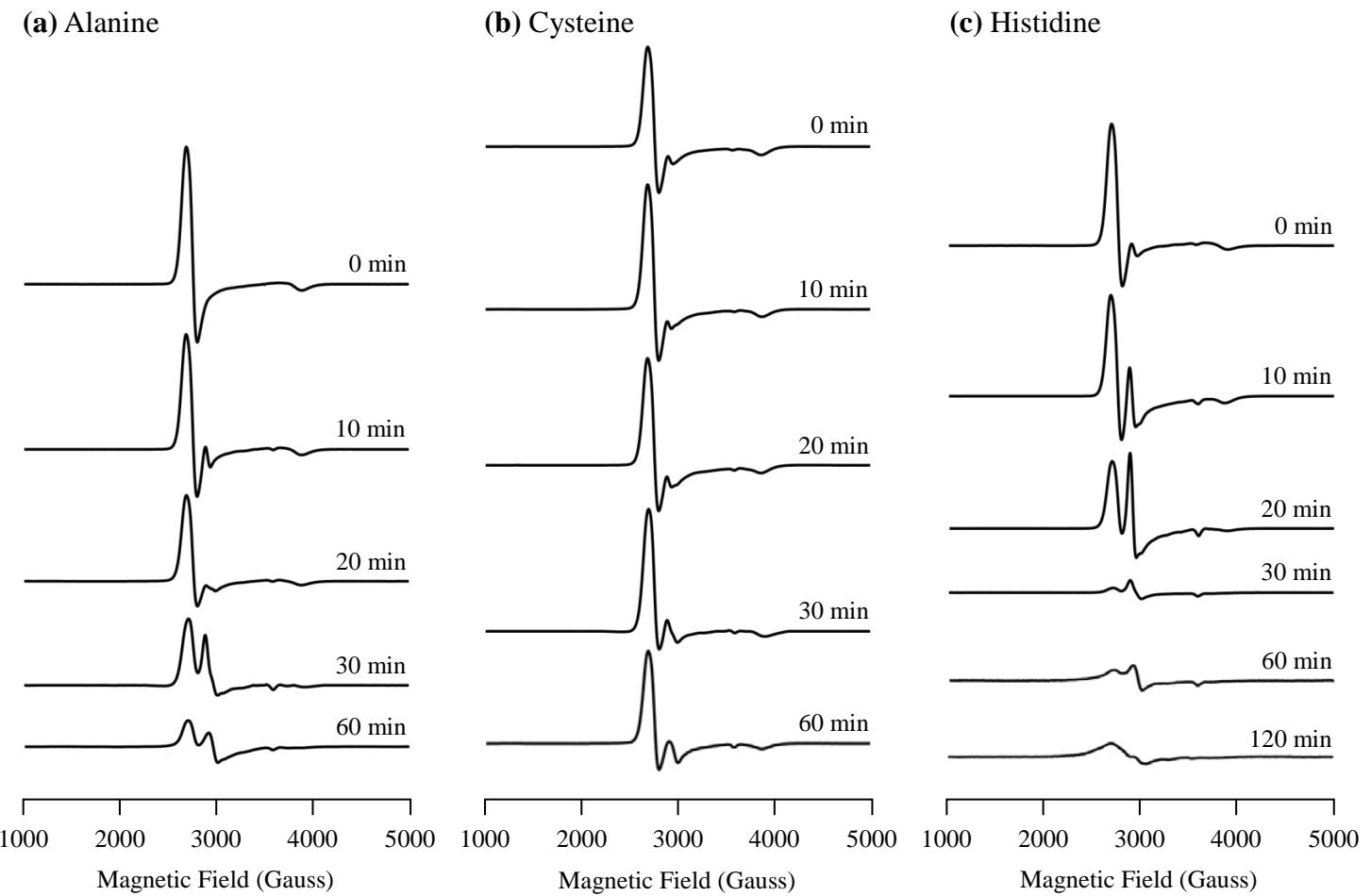


Figure S19 (a-c) EPR spectra of NAMI-A with: (a) alanine and (b) cysteine following 0 – 60 minutes of incubation in PBS at 37 °C, and (c) histidine following 0 – 120 minutes of incubation; 60, 120 min spectra scaled by factor of 10.

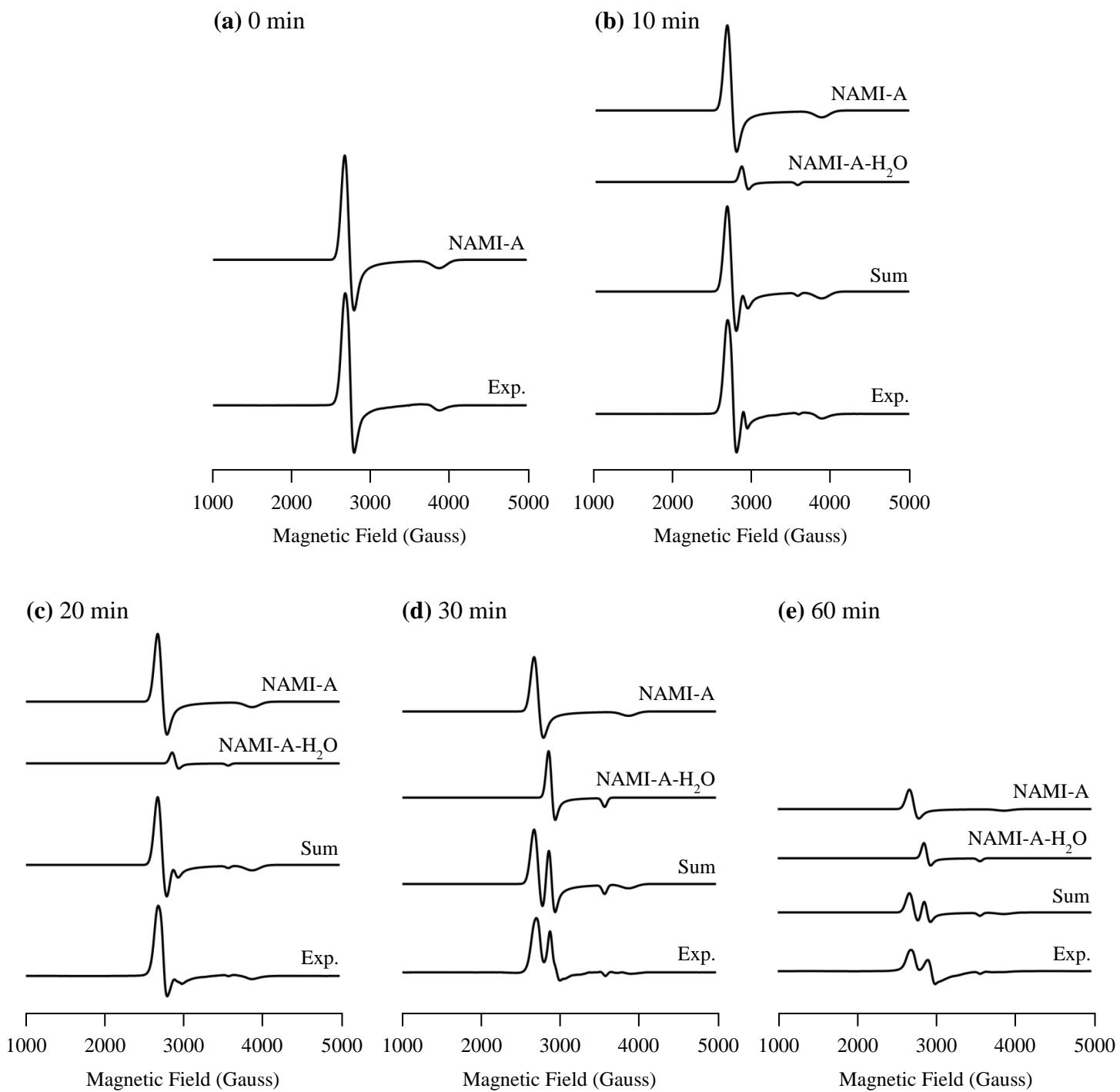


Figure S20 (a-e) Deconvolution of EPR spectra from NAMI-A with alanine after incubation in PBS for 0, 10, 20, 30, and 60 minutes at 37 °C. For g values and linewidths used in simulations see **Table S1**.

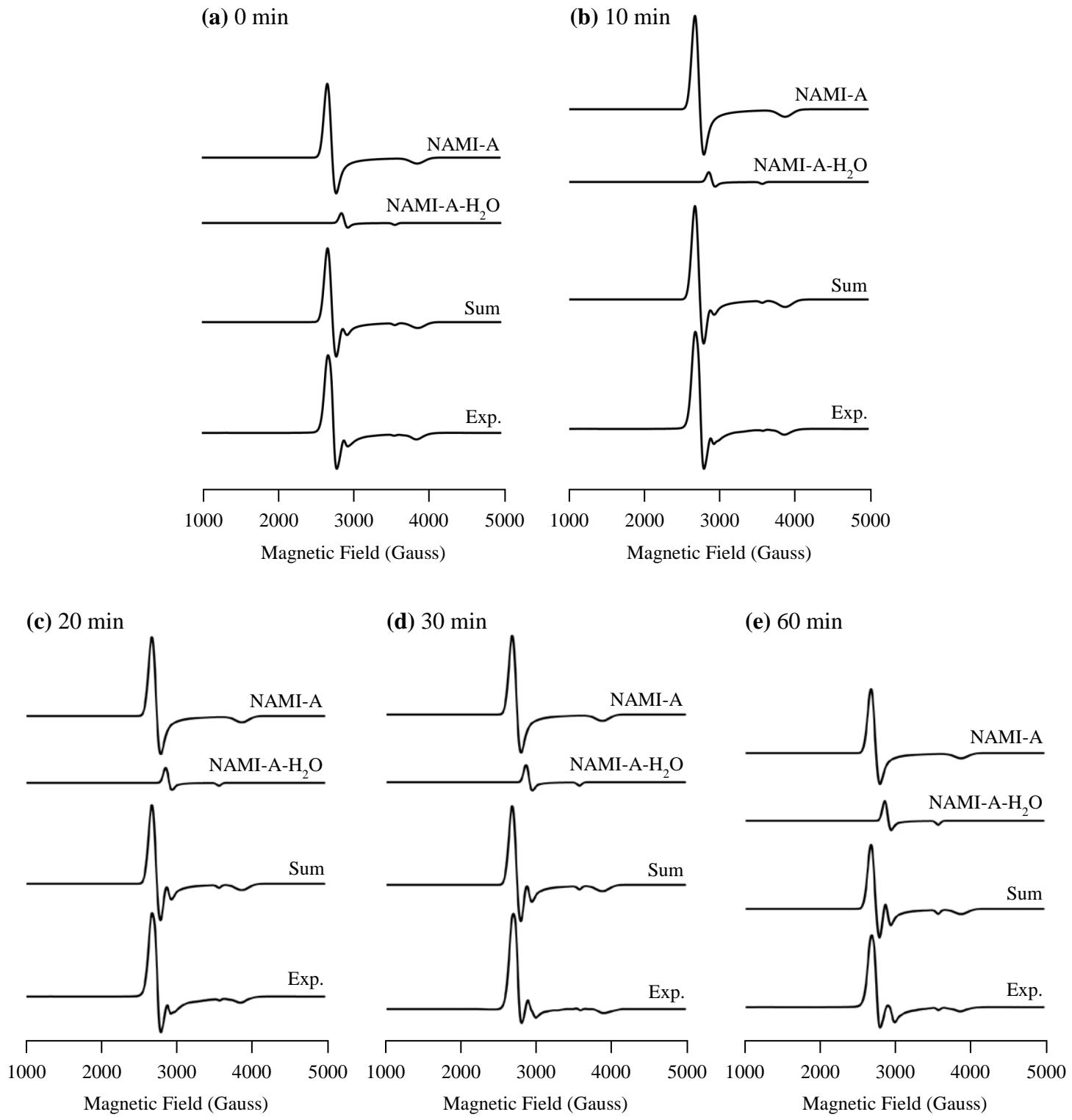


Figure S21 (a-e) Deconvolution of EPR spectra from NAMI-A with cysteine after incubation in PBS for 0, 10, 20, 30, and 60 minutes at 37 °C. For g values and linewidths used in simulations see **Table S1**.

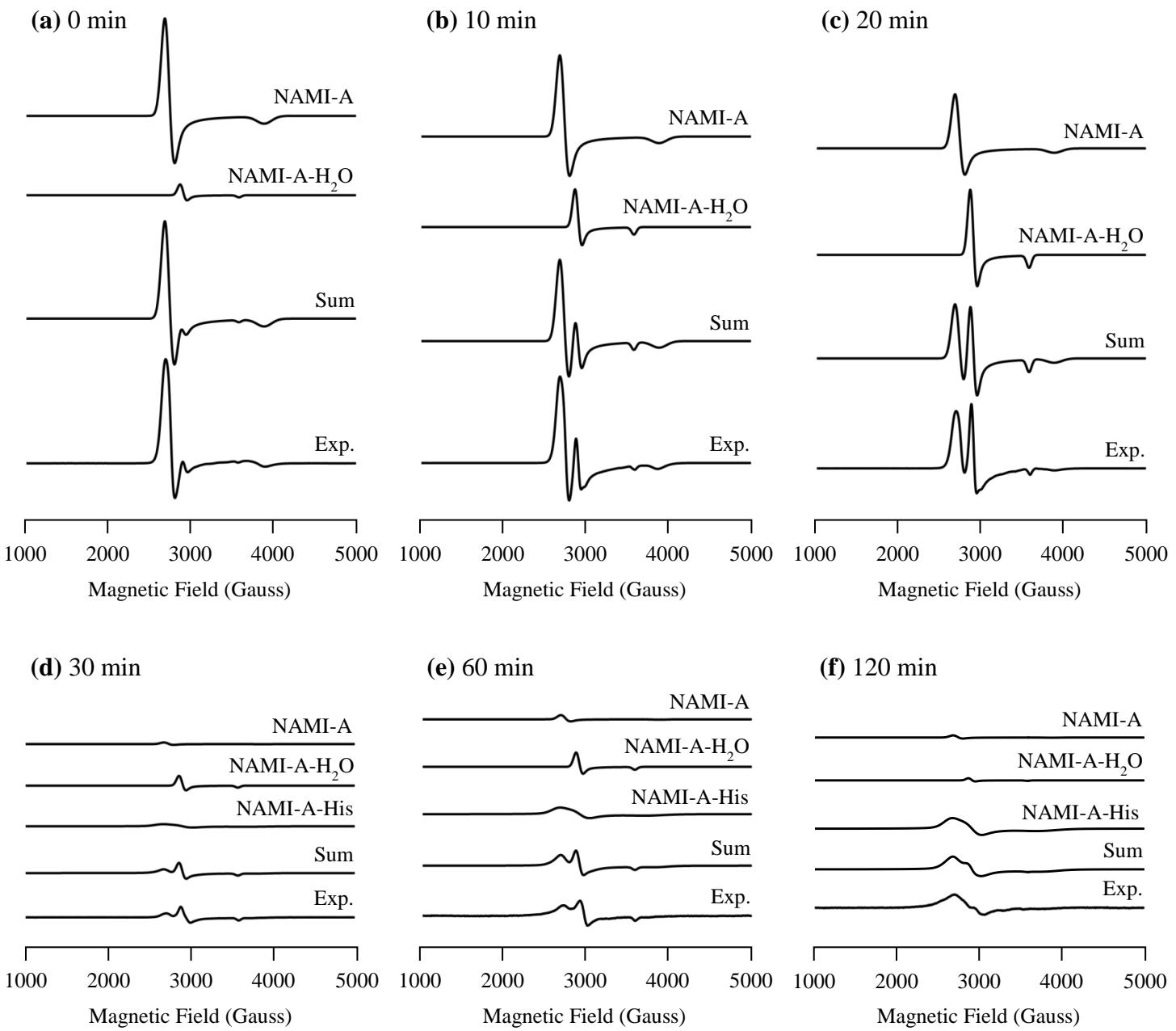


Figure S12 (a-f) Deconvolution of EPR spectra from NAMI-A with histidine after incubation in PBS for 0, 10, 20, 30, 60, and 120 minutes at 37 °C. For g values and linewidths used in simulations see **Table S1**.