EPR as a probe of the intracellular speciation of ruthenium(III) anticancer compounds

Michael I. Webb, Charles J. Walsby

Supplementary Material

Table of contents:

- **Table S1**: g values and line widths used in EPR simulations of Ru(III) species.
- **Figures S1 and S2**: Deconvolution of EPR spectra from NAMI-A in buffer after incubation with yeast cells.
- **Figures S3 and S4**: Deconvolution of EPR spectra from KP1019 in buffer after incubation with yeast cells.
- **Figures S5 and S6**: Deconvolution of EPR spectra from NAMI-A and KP1019 in buffer after incubation with yeast cell walls.
- **Figures S7 and S8**: Deconvolution of EPR spectra from NAMI-A and KP1019 in buffer after incubation with yeast mitochondria.
- **Figures S9 and S10**: Deconvolution of EPR spectra from NAMI-A and KP1019 in buffer after incubation with yeast nuclear fraction.
- **Figures S11 and S12**: Deconvolution of EPR spectra from NAMI-A and KP1019 in buffer after incubation with yeast cytoplasmic protein fraction
- **Figures S13 and S14**: Concentration calibration curve for NAMI-A.
- **Figures S15 and S16**: Concentration calibration curve for KP1019.
- **Figures S17 and S18**: EPR spectra of NAMI-A and KP1019 with yeast cells at 4 °C.

Table S1 g values and line widths used in EPR simulations of Ru(III) species interaction with *Saccharomyces cerevisiae* whole cells and cell components.

Species	g_1	g_2	g_3	L_1	L_2	L_3
NAMI-A						
NAMI-A	2.47	2.47	1.72	105	105	180
NAMI-A-H_2O	2.32	2.32	1.87	75	75	60
NAMI-A-Cell-Wall-1	2.45	2.21	1.10	300	80	200
NAMI-A-Cell-Wall-2	2.38	2.18	1.80	325	250	350
NAMI-A-Mitochondria-1	2.40	2.21	1.10	400	70	200
NAMI-A-Mitochondria-2	2.40	2.22	1.75	200	175	350
NAMI-A-Cyto.-Proteins-1	2.42	2.25	1.84	200	175	350
NAMI-A-Cyto.-Proteins-2	2.20	2.10	1.84	200	150	250
NAMI-A-Nuclear-Fraction-1	2.40	2.22	1.10	400	100	200
NAMI-A-Nuclear-Fraction-2	2.48	2.30	1.88	250	150	350
KP1019						
KP1019-Axial	2.64	2.64	1.20	120	120	500
KP1019-Rhombic	2.94	2.31	0.95	100	200	600
KP1019-Whole-Cells-1	2.40	2.18	1.10	400	80	500
KP1019-Cell-Wall-1	2.50	2.30	1.80	300	200	250
KP1019-Cell-Wall-2	2.40	2.20	1.88	250	100	350
KP1019-Mitochondria-1	2.36	2.19	1.84	175	85	250
KP1019-Mitochondria-2	2.40	2.20	1.80	275	225	350
KP1019-Cyto.-Proteins-1	2.35	2.25	1.88	300	250	300
KP1019-Cyto.-Proteins-2	2.20	2.10	1.80	250	200	250
KP1019-Nuclear-Fraction-1	2.41	2.20	1.80	100	150	250
KP1019-Nuclear-Fraction-2	2.40	2.20	1.84	250	150	350

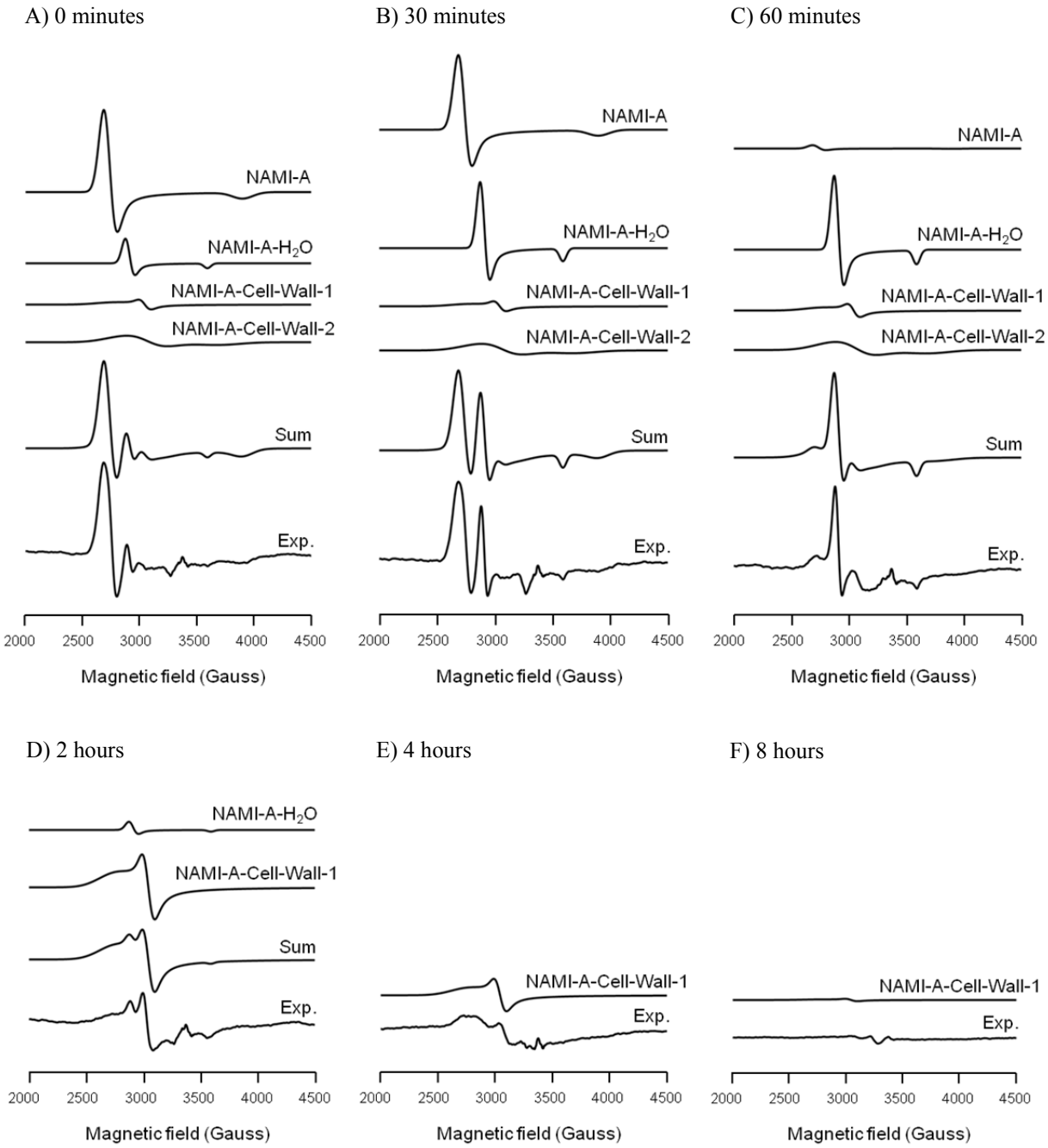


Figure S1 Deconvolution of EPR spectra from NAMI-A in buffer after incubation with yeast cells for 0 minutes, 30 minutes, 1, 2, 4, and 8 hours at 30 °C.

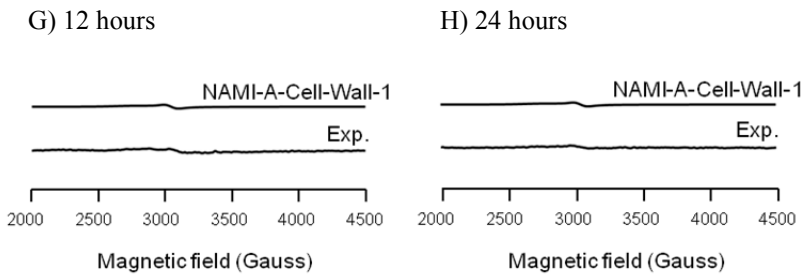


Figure S2 Deconvolution of EPR spectra from NAMI-A in buffer after incubation with yeast cells for 12 and 24 hours at 30 °C.

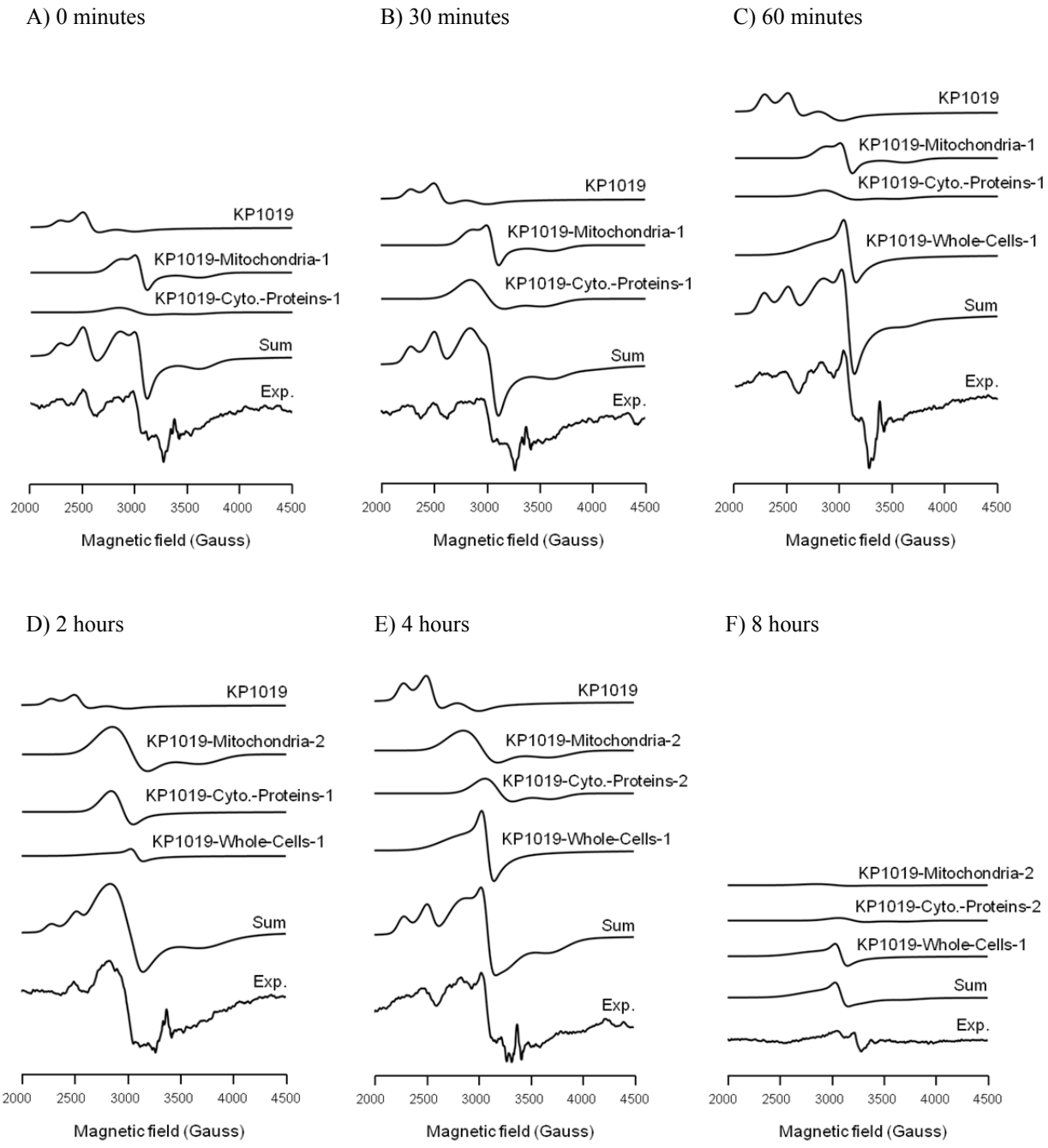
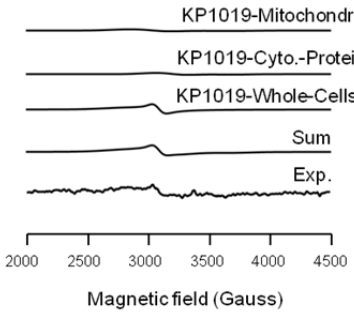


Figure S3 Deconvolution of EPR spectra from KP1019 in PBS after incubation with yeast cells for 0 minutes, 30 minutes, 1, 2, 4, and 8 hours at 30 °C.

F) 12 hours



G) 24 hours

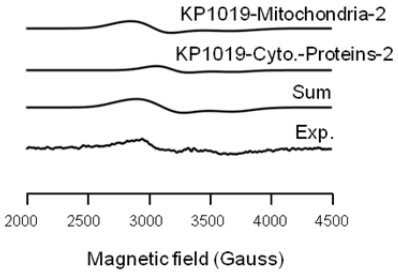


Figure S4 Deconvolution of EPR spectra from KP1019 in PBS after incubation with yeast cells for 12 and 24 hours at 30 °C.

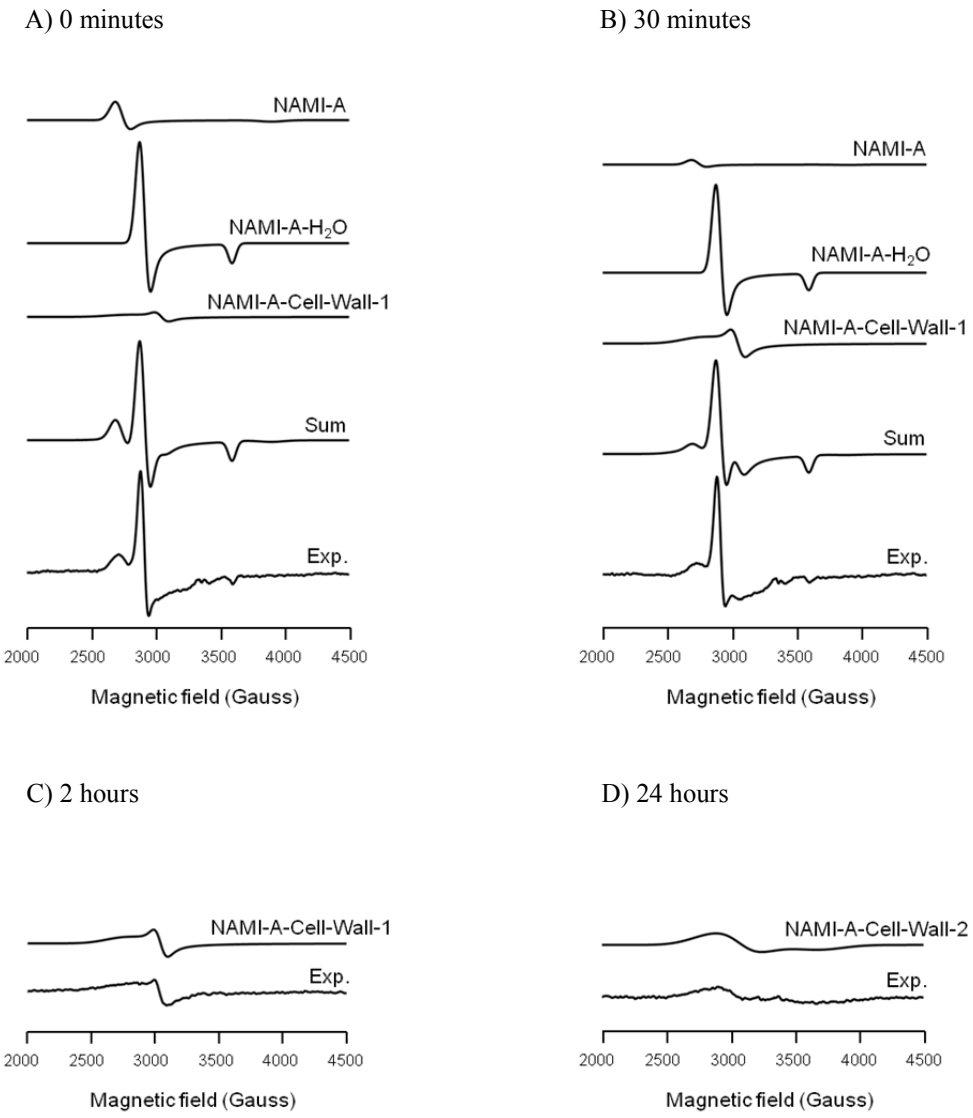


Figure S5 Deconvolution of EPR spectra from NAMI-A in PBS after incubation with yeast cell walls for 0 minutes, 30 minutes, 2, and 24 hours at 30 °C.

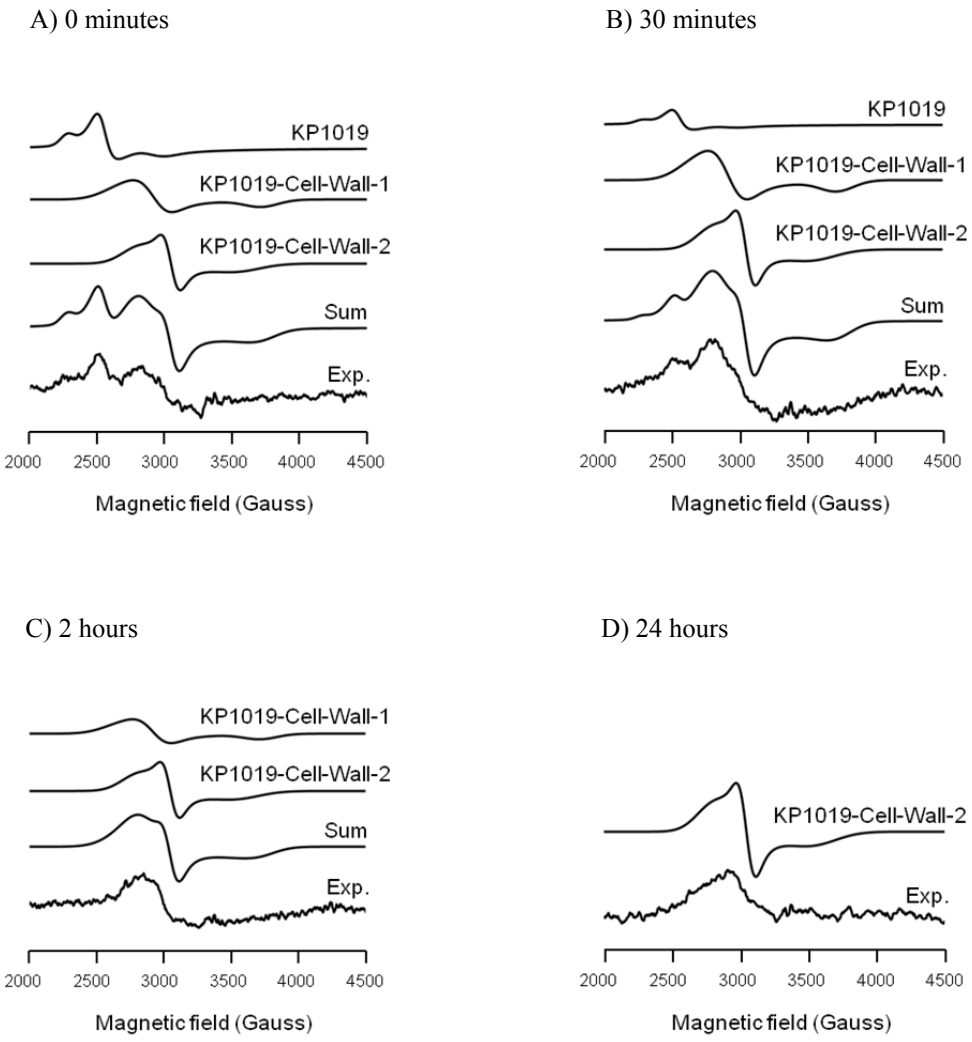


Figure S6 Deconvolution of EPR spectra from KP1019 in PBS after incubation with yeast cell walls for 0 minutes, 30 minutes, 2, and 24 hours at 30 °C.

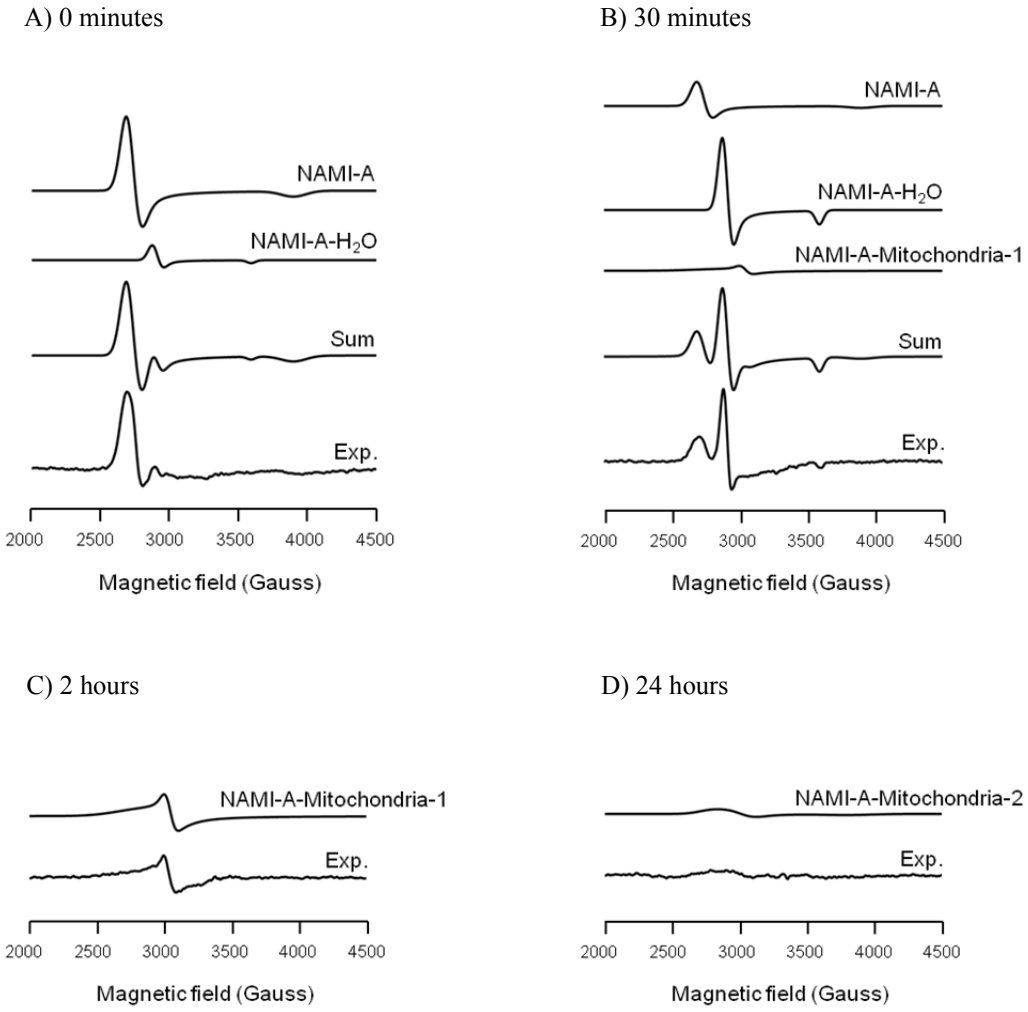


Figure S7 Deconvolution of EPR spectra from NAMI-A in PBS after incubation with yeast cell mitochondria for 0 minutes, 30 minutes, 2, and 24 hours at 30 °C.

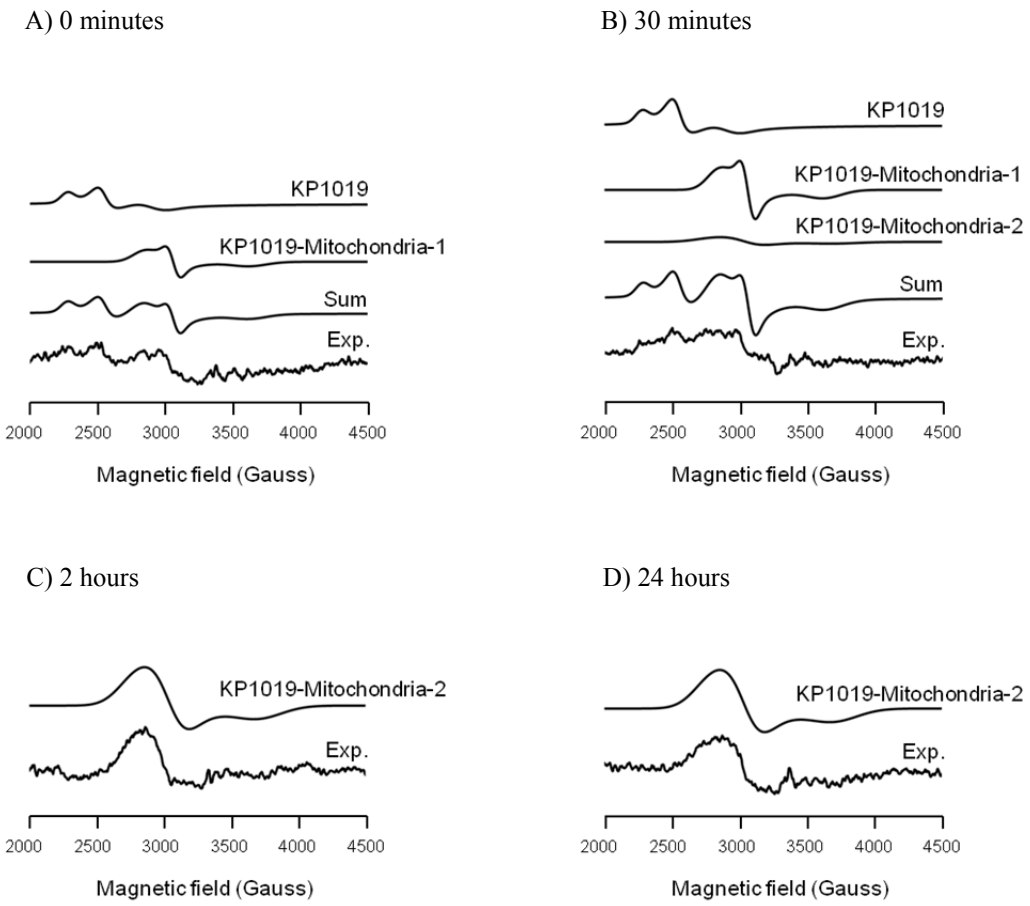


Figure S8 Deconvolution of EPR spectra from KP1019 in PBS after incubation with yeast cell mitochondria for 0 minutes, 30 minutes, 2, and 24 hours at 30 °C.

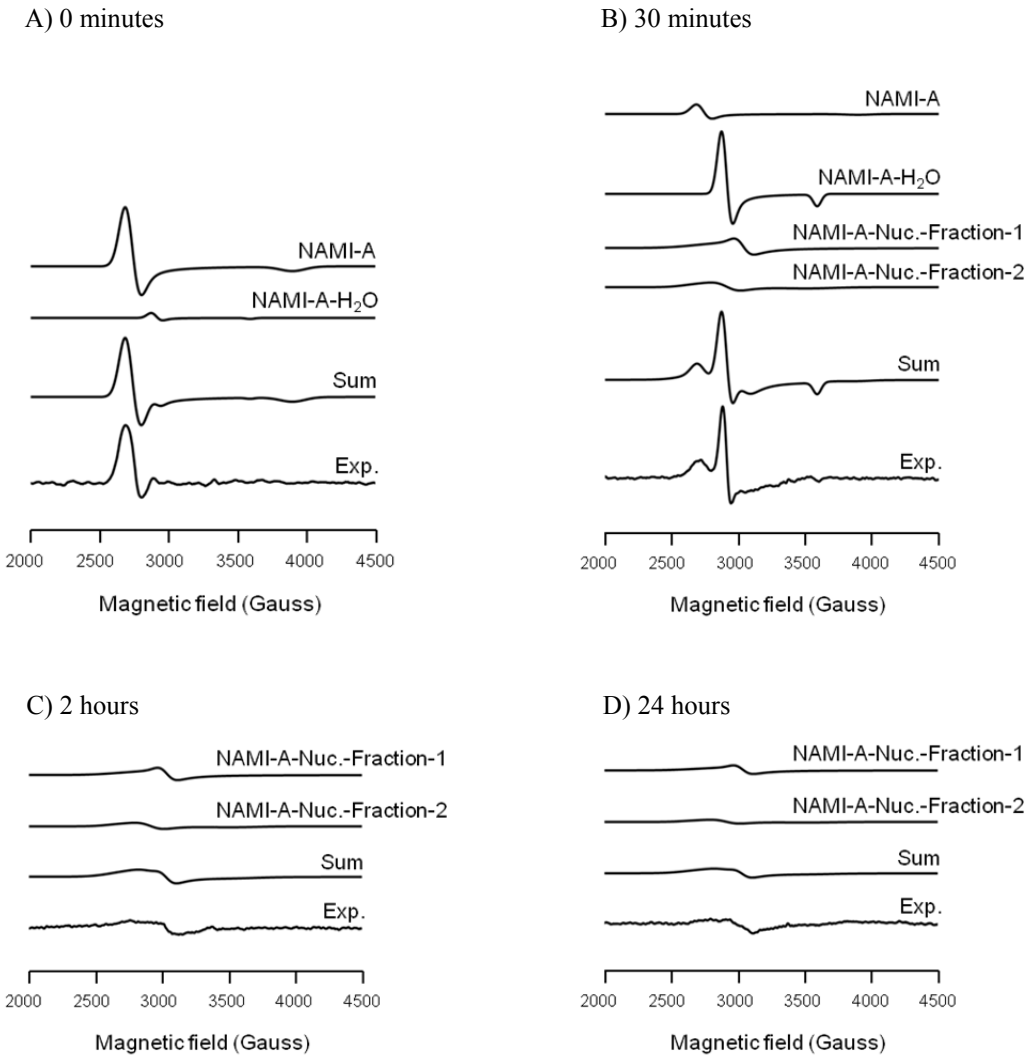


Figure S9 Deconvolution of EPR spectra from NAMI-A in PBS after incubation with yeast cell nuclear fraction for 0 minutes, 30 minutes, 2, and 24 hours at 30 °C.

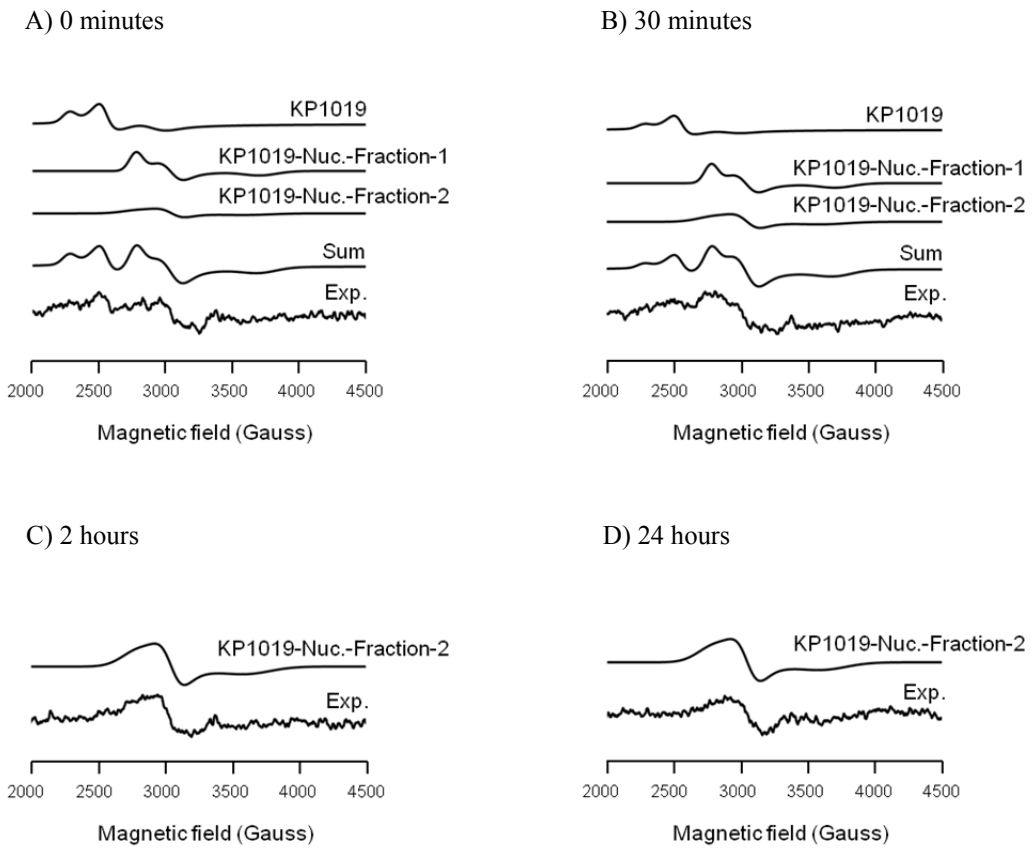


Figure S10 Deconvolution of EPR spectra from KP1019 in PBS after incubation with yeast cell nuclear fraction for 0 minutes, 30 minutes, 2, and 24 hours at 30 °C.

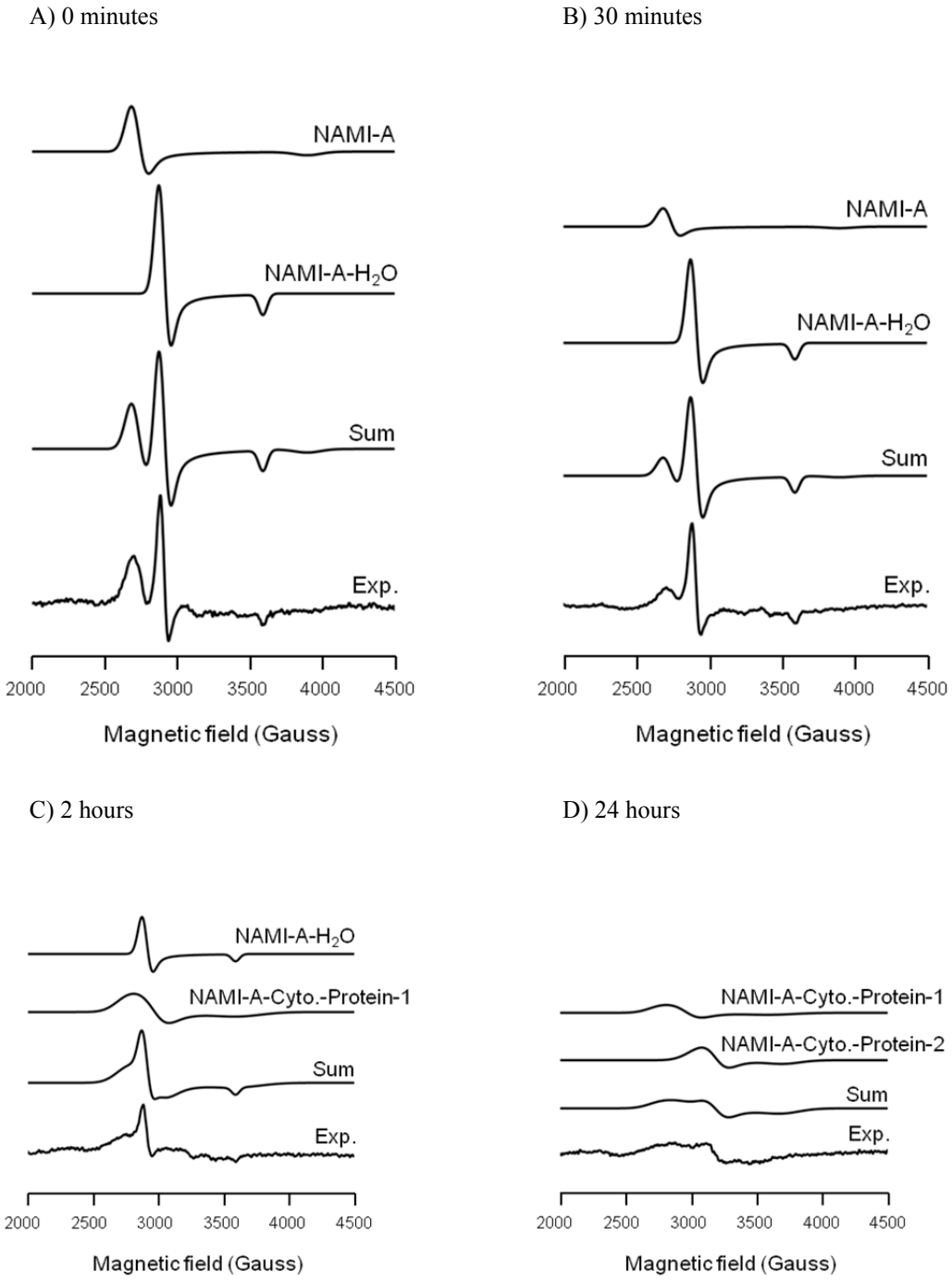


Figure S11 Deconvolution of EPR spectra from NAMI-A in PBS after incubation with yeast cell cytoplasmic protein fraction for 0 minutes, 30 minutes, 2, and 24 hours at 30 °C.

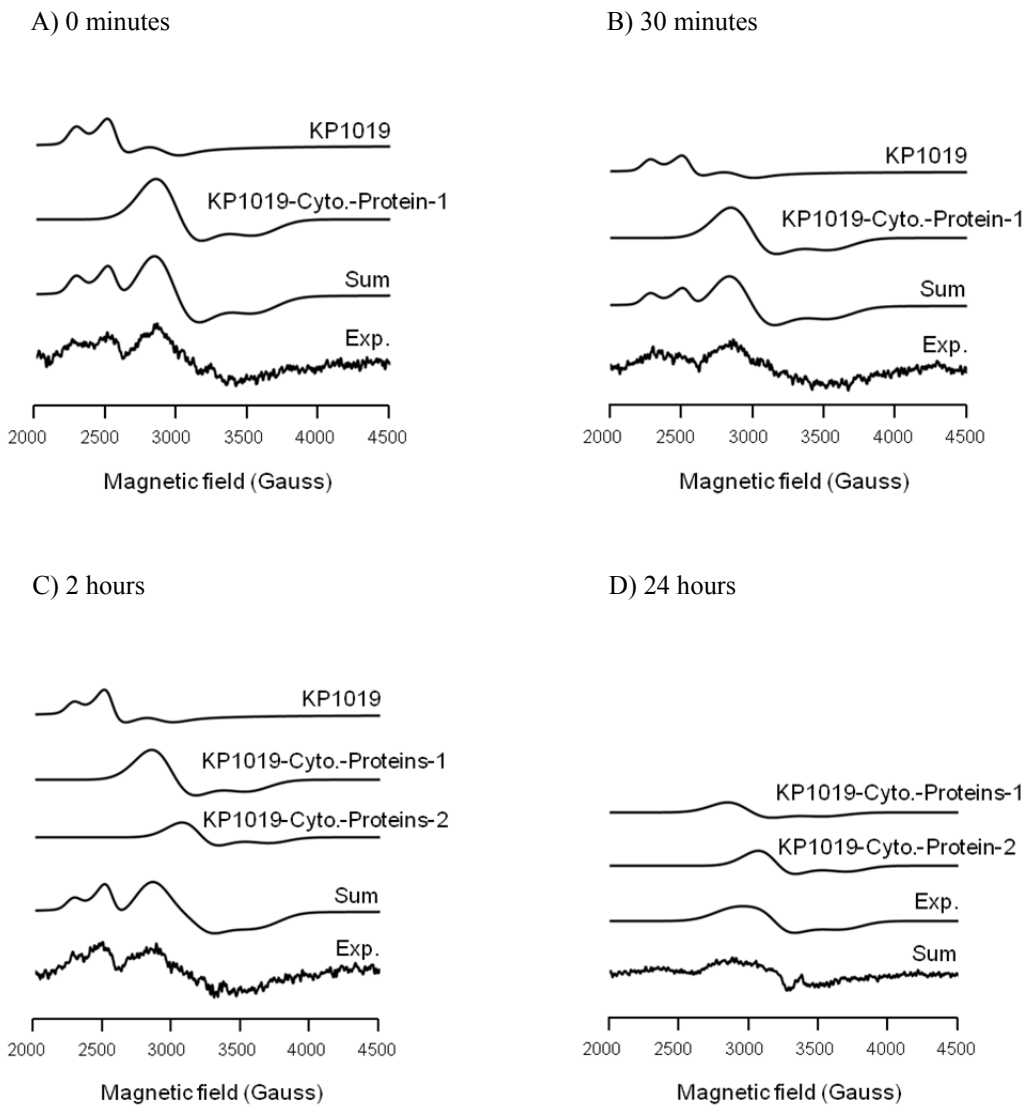


Figure S12 Deconvolution of EPR spectra from KP1019 in PBS after incubation with yeast cell cytoplasmic protein fraction for 0 minutes, 30 minutes, 2, and 24 hours at 30 °C.

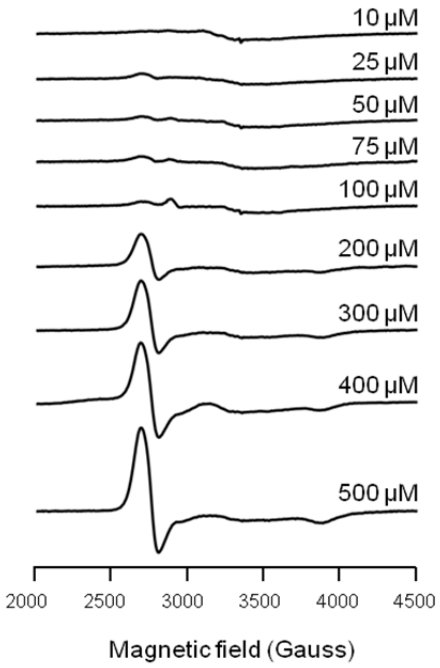


Figure S13 Serial dilution of NAMI-A in PBS to determine the calibration curve.

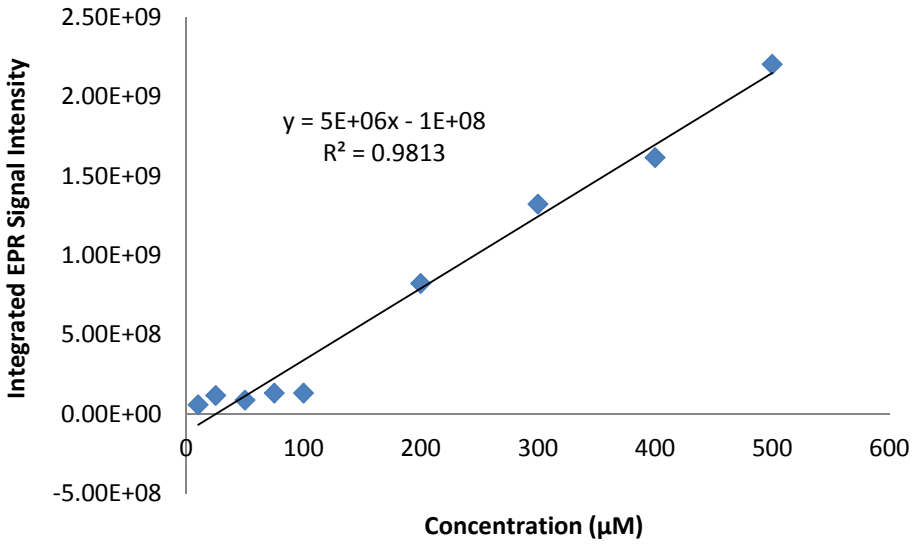


Figure S14 Plot of the integrated signal intensities determined by the titration of NAMI-A in PBS to determine the detection limit of the EPR spectrometer.

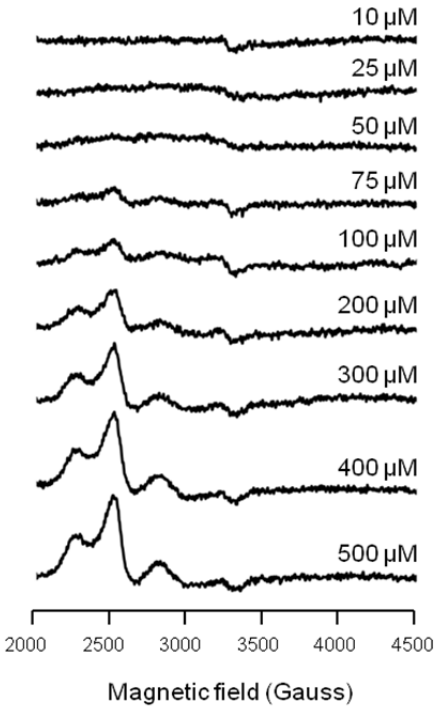


Figure S15 Serial dilution of KP1019 in PBS to determine the detection calibration curve.

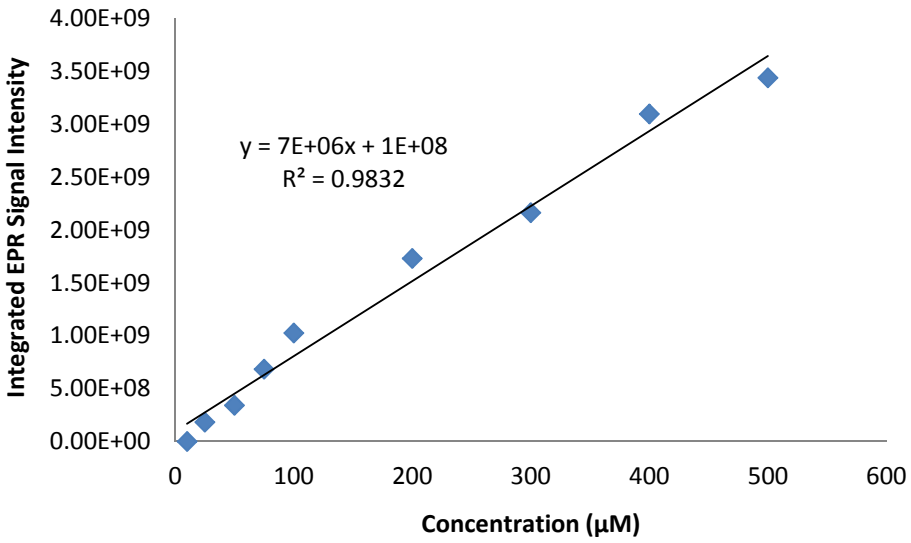


Figure S16 Plot of the integrated signal intensities determined by the titration of KP1019 in PBS to determine the detection limit of the EPR spectrometer.

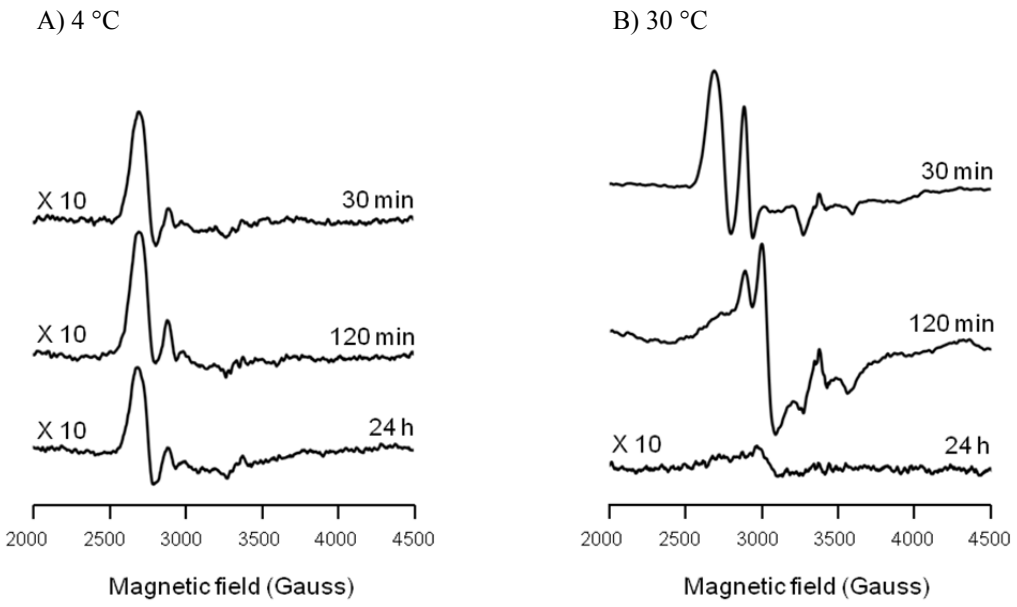


Figure S17 EPR spectra of NAMI-A in PBS after incubation with whole yeast cells for 30 minutes, 2, and 24 hours at 4 and 30 °C.

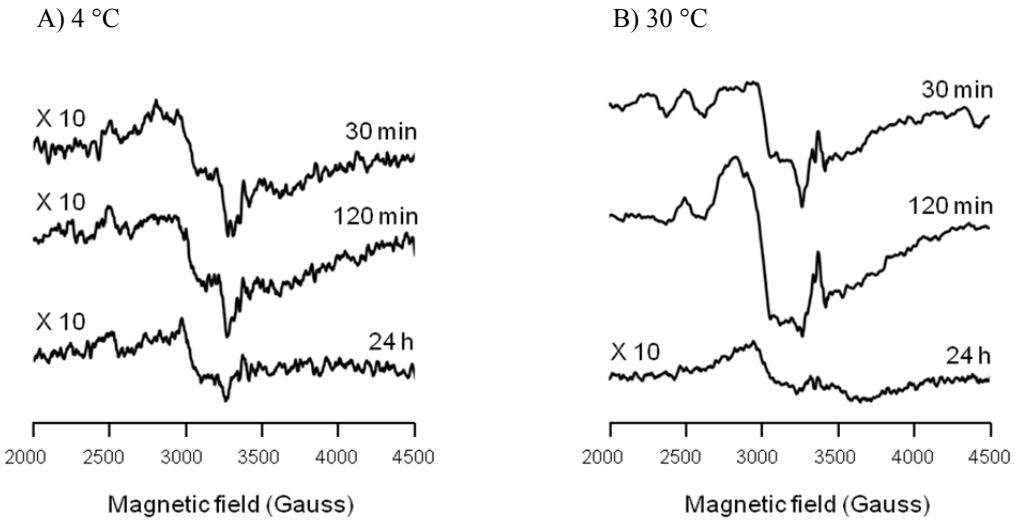


Figure S18 EPR spectra of KP1019 in PBS after incubation with whole yeast cells for 30 minutes, 2, and 24 hours at 4 and 30 °C.