Supporting Information

Detection, characterization, and decay kinetics of ROS and thiyl adducts of Mito-DEPMPO spin trap.

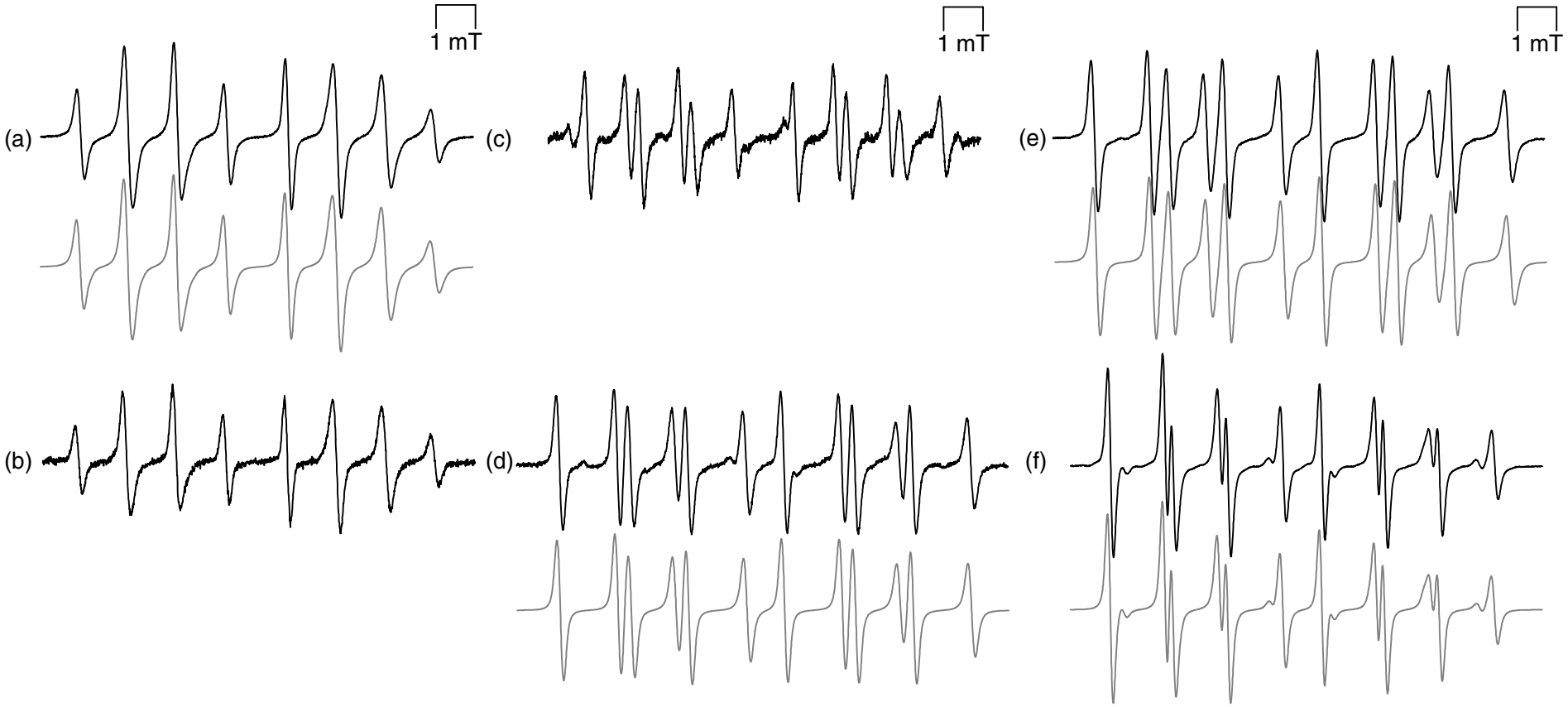

Figure 1S. Spin trapping of ROS with Mito-DEPMPO. (a) EPR spectrum obtained after 2 min incubation of a mixture containing KO_2/18-crown-6 system (10 mM) and Mito-DEPMPO (20 mM) in a phosphate buffer (0.1 M, pH 7.3); (b) Neuronal nitric oxide synthase (nNOS) (1.4 μg) was incubated with calcium (0.2 mM), calmodulin (20 μg/mL), Mito-DEPMPO (20 mM), and NADPH (0.1 mM) in a HEPES buffer (50 mM, pH 7.4) containing 0.1 mM DTPA after 1 min; (c) EPR spectrum obtained after 1 min incubation of a mixture containing Mito-DEPMPO (20 mM), H_2O_2 (2 mM), $FeSO_4$ (2 mM), DTPA (1 mM) in a phosphate buffer (0.1 M, pH 7.3); (d) as in (c) but in the presence of 10% of DMSO after 30 min and bubbling with Argon gas (1 min); (e) as in (c) but in the presence of 7% of MeOH after 30 min and (f) as in (c) but in the presence of 7% of HCOOH. Spectrometer settings: microwave power 10 mW (a-f); modulation amplitude, 0.5 G (a, d-f), 0.63 (b), 0.8 (c); time constant, 0.640 ms (a, c, e-f), 1.28 ms (b, d); gain 10^5 (a-f); sweep time, 335.54 s (a, c-f), 0.167 s (b); conversion time, 0.163 s (a, c-f), 0.8192 s (b).

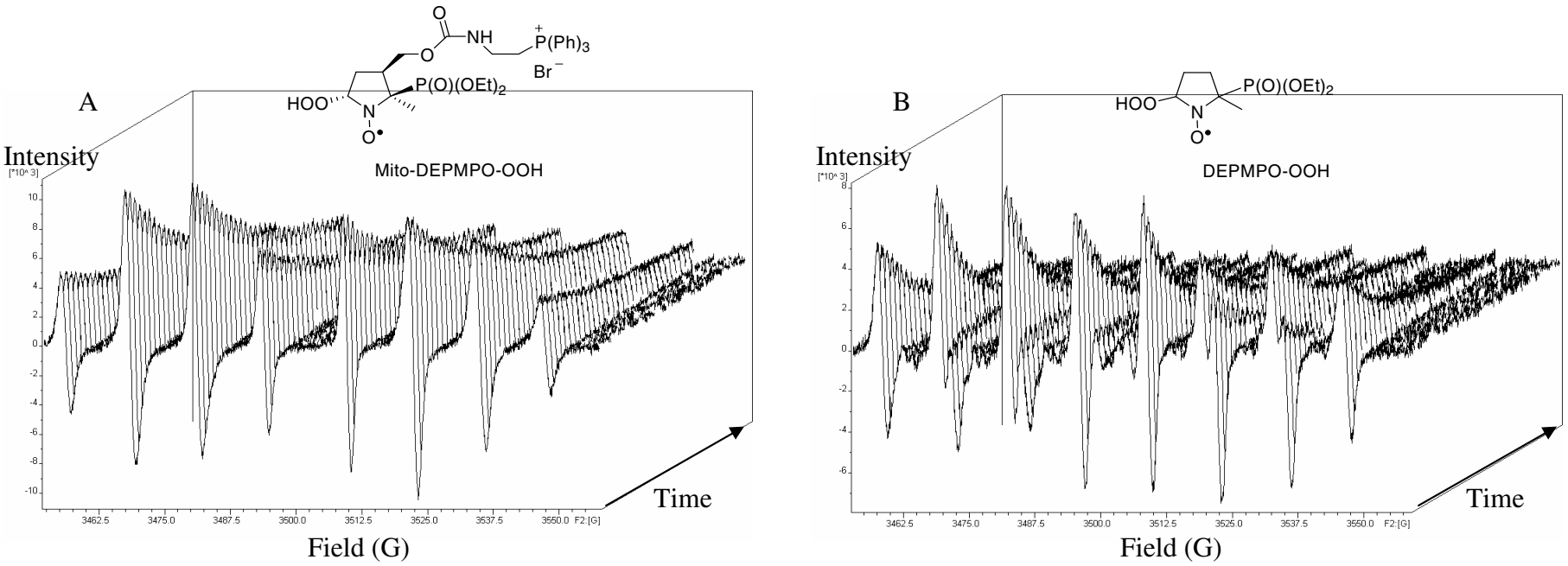

Figure 2S: Kinetics of the Decay of the Mito-DEPMPO-OOH and the DEPMPO-OOH at room temperature. (A) EPR spectra obtained after incubation of a mixture containing hypoxanthine (0.4 mM), xanthine oxidase (0.04 U mL^{-1}), DTPA (1 mM) and Mito-DEPMPO (20 mM) in a phosphate buffer (0.1 M, pH 7.3) at room temperature; (B) As in (A) but in the presence of DEPMPO. The production of superoxide radicals is terminated by addition of a large amount of SOD (1200 U mL^{-1}). Spectrometer settings: microwave power 10 mW; modulation amplitude, 0.63 G; time constant, 0.128 s; gain 10^5; sweep time, 0.163 s; conversion time, 0.8192 s.

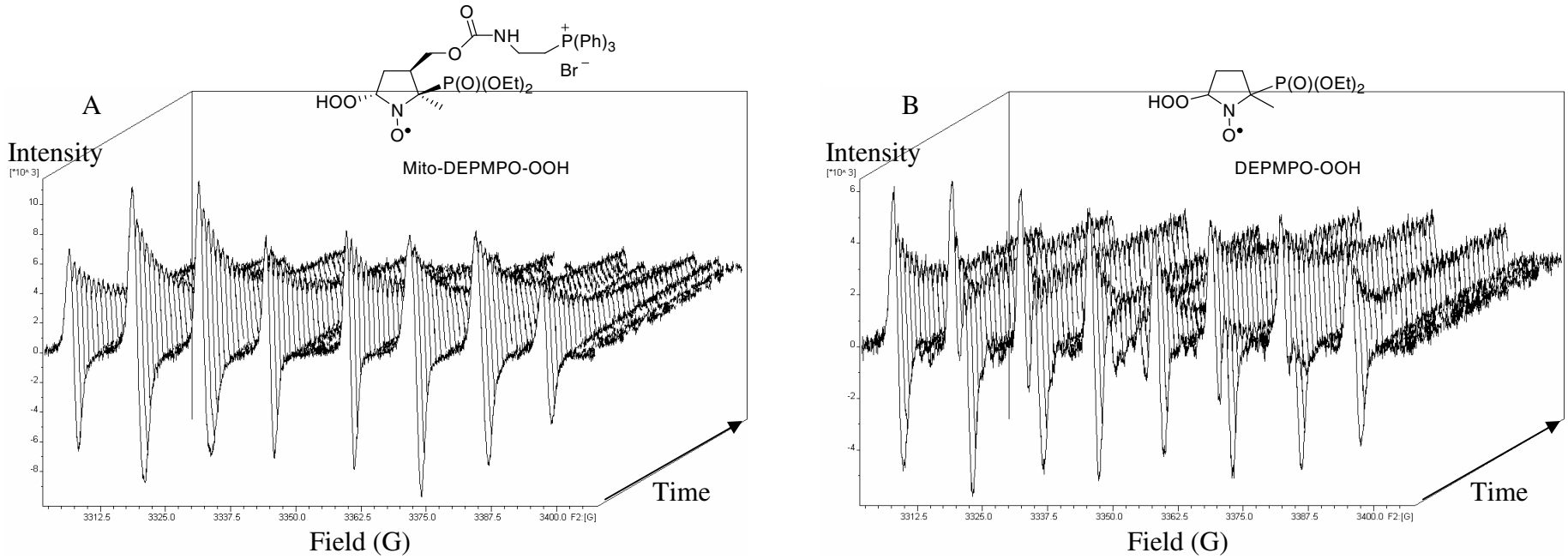

Figure 3S: Kinetics of the decay of the Mito-DEPMPO-OOH and the DEPMPO-OOH at 37°C. (A) EPR spectra obtained after incubation of a mixture containing hypoxanthine (0.4 mM), xanthine oxidase (0.04 U mL^{-1}), DTPA (1 mM) and Mito-DEPMPO (20 mM) in a phosphate buffer (0.1 M, pH 7.3) at 37°C; (B) As in (A) but in the presence of or DEPMPO. The production of superoxide radicals is terminated by addition of a large amount of SOD (1200 U mL^{-1}). Spectrometer settings: microwave power 10 mW; modulation amplitude, 0.63 G; time constant, 0.128 s; gain 10^5; sweep time, 0.163 s; conversion time, 0.8192 s.

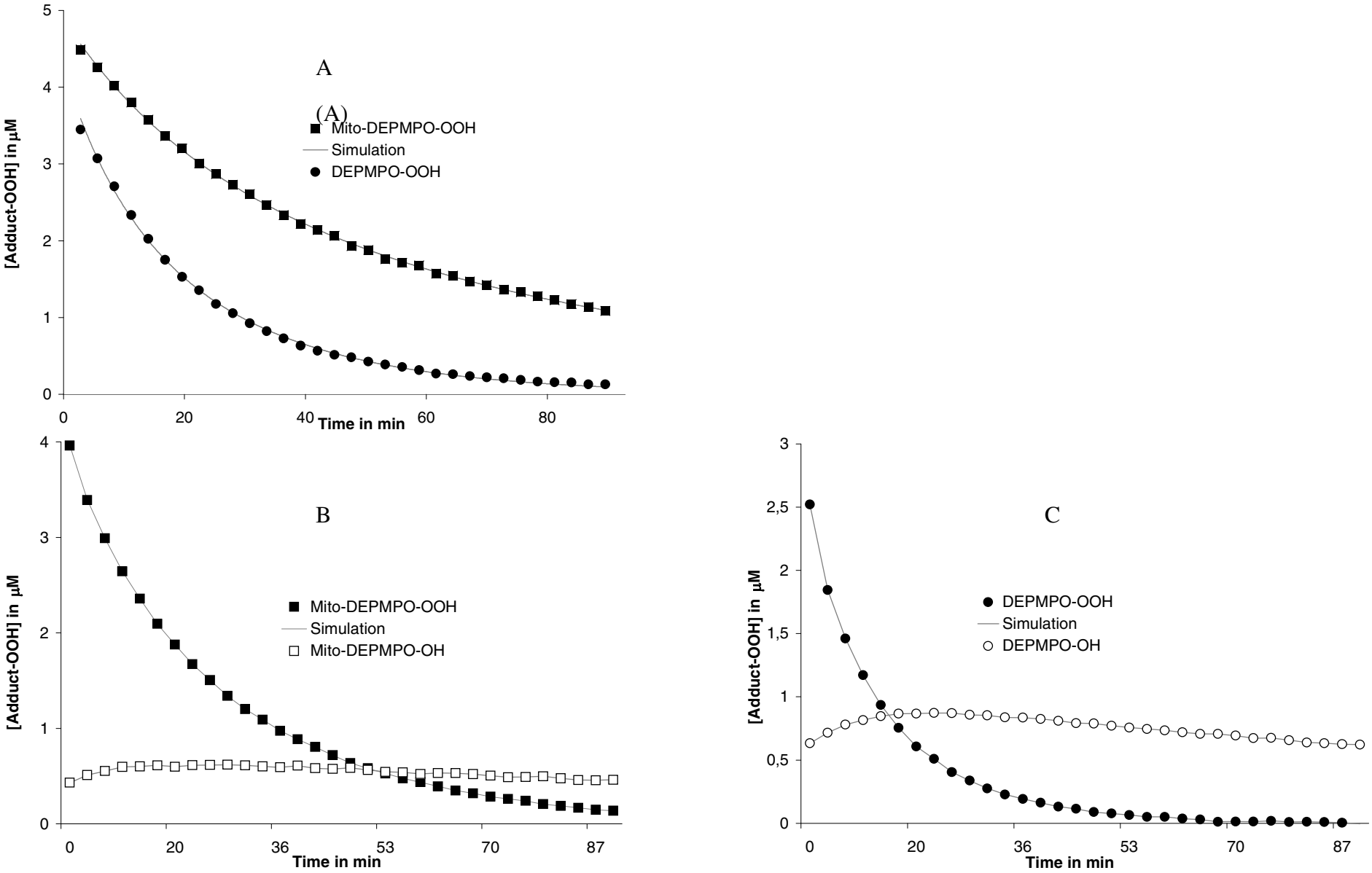

Figure 4S. Kinetics of the decay of the Mito-DEPMPO-OOH and the DEPMPO-OOH at room temperature and at 37°C. (A) Decay curves for the Mito-DEPMPO-OOH and DEPMPO-OOH adducts at room temperature; (B) Decay curves for the Mito-DEPMPO-OOH adduct at 37°C and (C) Decay curves for the DEPMPO-OOH adduct at 37°C. The gray line was computed by a combination of first and second order kinetics.

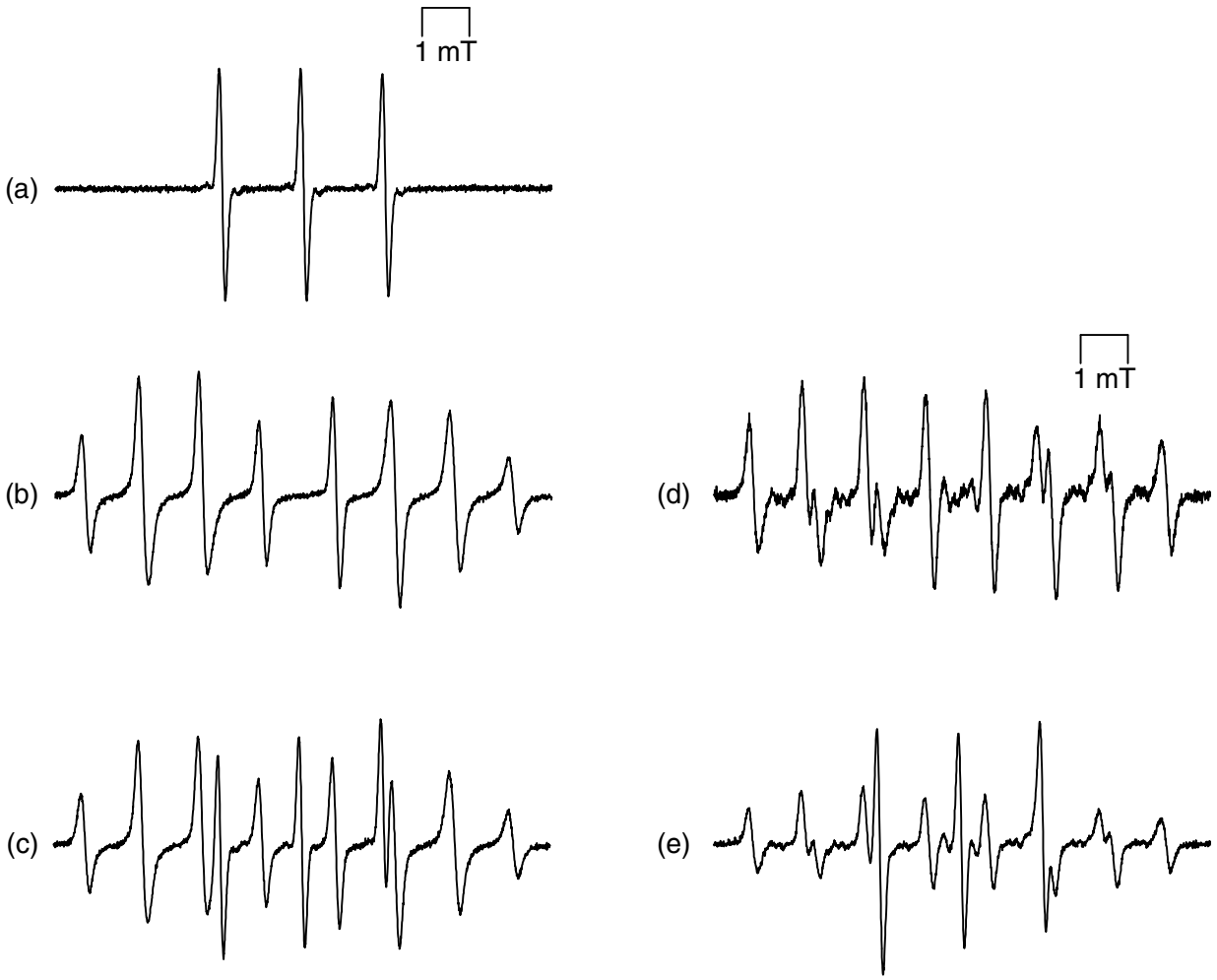

Figure 5S. Absolute concentration of the superoxide spin adducts DEPMPO and Mito-DEPMPO by TEMPO calibration. (a) EPR spectrum obtained after incubation of a mixture containing TEMPO (0.01 mM) in a phosphate buffer (0.1M, pH 7.3); (b) EPR spectrum obtained after incubation of a mixture containing HX (0.4 mM), XO (0.04 U mL^{-1}), DTPA (1 mM) and Mito-DEPMPO (20 mM) in a phosphate buffer (0.1 M, pH 7.3). The formation of superoxide anion radicals is terminated by addition of a large amount of SOD (1200 U mL^{-1}) after 9 min; (c) as in (b) but with TEMPO (0.01 mM); (d) as in (b) but with DEPMPO (20 mM) and (e) as in (d) but with TEMPO (0.01 mM). Spectrometer settings: microwave power 10 mW; modulation amplitude, 0.63 G; time constant, 0.128 s; gain 10^5; sweep time, 0.163 s; conversion time, 0.8192 s.

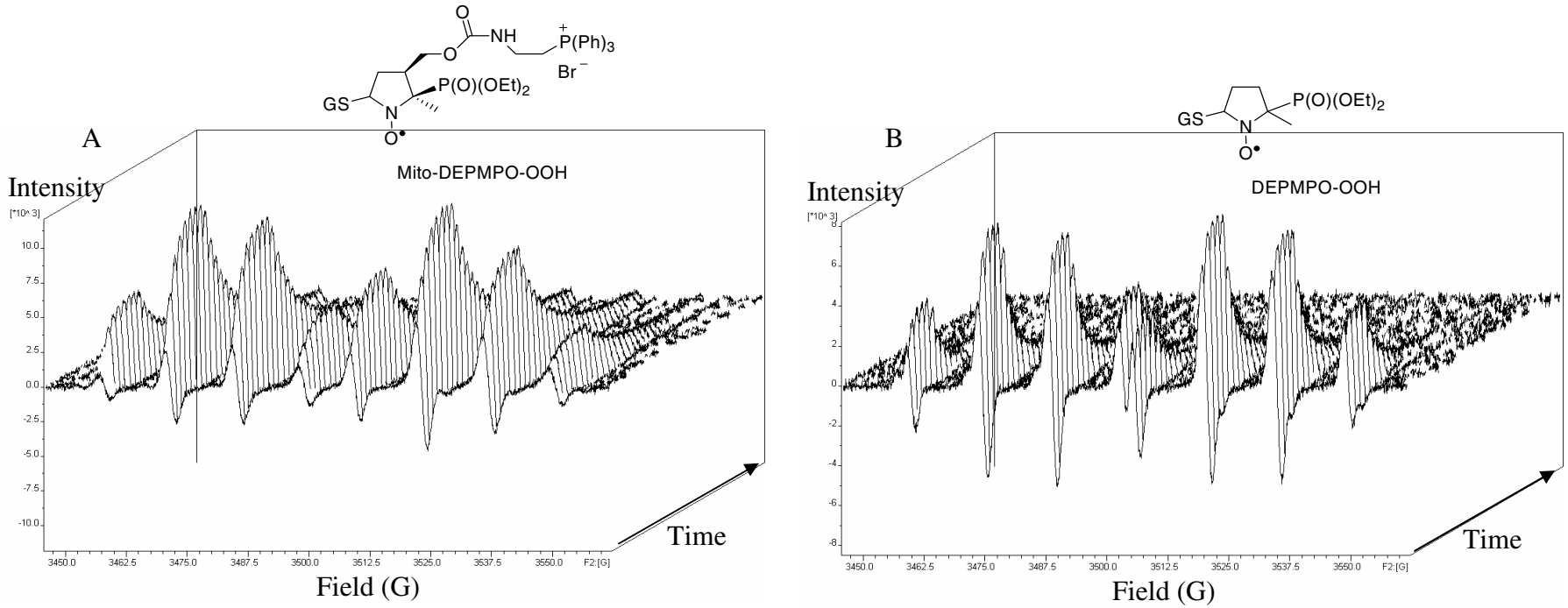

Figure 6S. Kinetics of the decay of the Mito-DEPMPO-SG and the DEPMPO-SG. (A) EPR spectra obtained after photolysis (170 W) of a mixture containing GSNO (1 mM), DTPA (1 mM) and Mito-DEPMPO (20 mM) in a phosphate buffer (0.1 M, pH 7.3); (B) As in (A) but in the presence of DEPMPO. The photolysis was terminated once the concentration of the adduct reached a maximal value. Spectrometer settings: microwave power 10 mW; modulation amplitude, 0.63 G; time constant, 0.128 s; gain 10^5; sweep time, 0.8192 s.

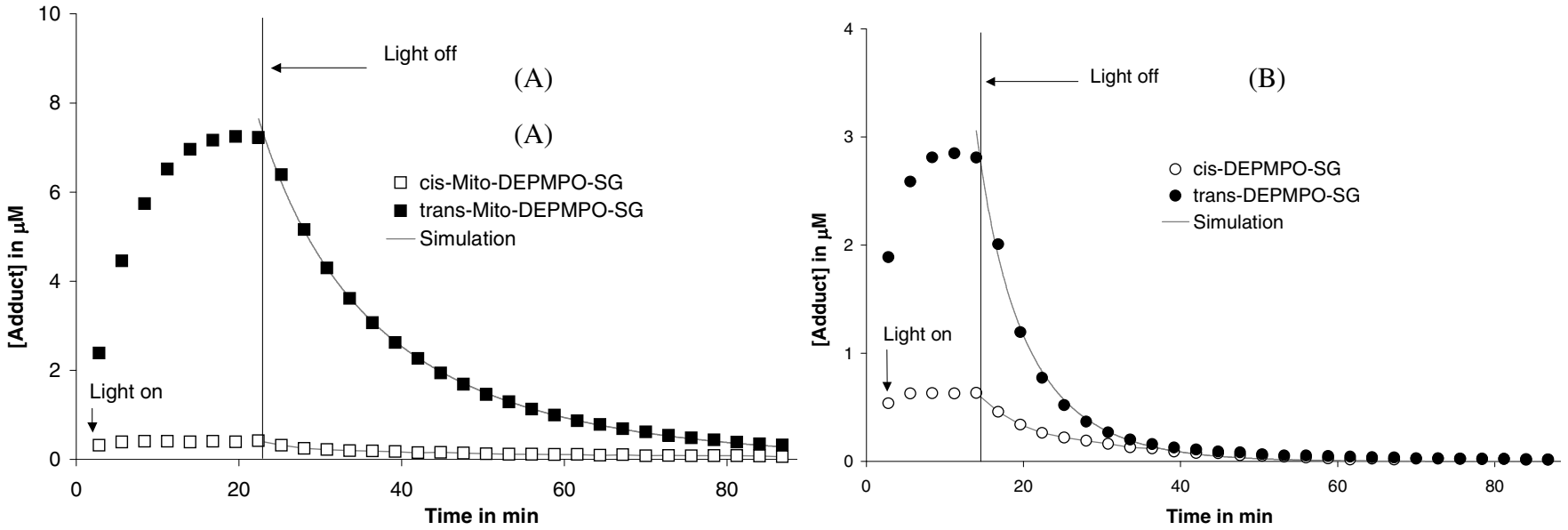

Figure 7S. Kinetics of the decay of the Mito-DEPMPO-SG and the DEPMPO-SG. (A) Decay curves for the Mito-DEPMPO-SG adduct at room temperature; (B) Decay curves for the DEPMPO-SG adduct at room temperature. The gray line was computed by a combination of first and second order kinetics: $-dR/dt = k_1 \times R + k_2 \times R^2$. The symbols (■ and ●) represent the 32 experimental spectra of Mito-DEPMPO-SG and DEPMPO-SG respectively.

	nmoles/mg/min	Percent control
Isolated mitochondria (200 μg)	-7.3×10^{-2}	100%
Isolated mitochondria (200 μg) DEPMPO (50 mM)	-5.6×10^{-2}	78%
Isolated mitochondria (200 μg) Mito-DEPMPO (50 mM)	-5.8×10^{-2}	80%

Table 1S. Mitochondrial Oxygen Consumption measured in the presence of Mito-DEPMPO or DEPMPO.