Supplemental information for:

Stability of the bi-nitroxide radical AMUPol in intact and lysed mammalian cells

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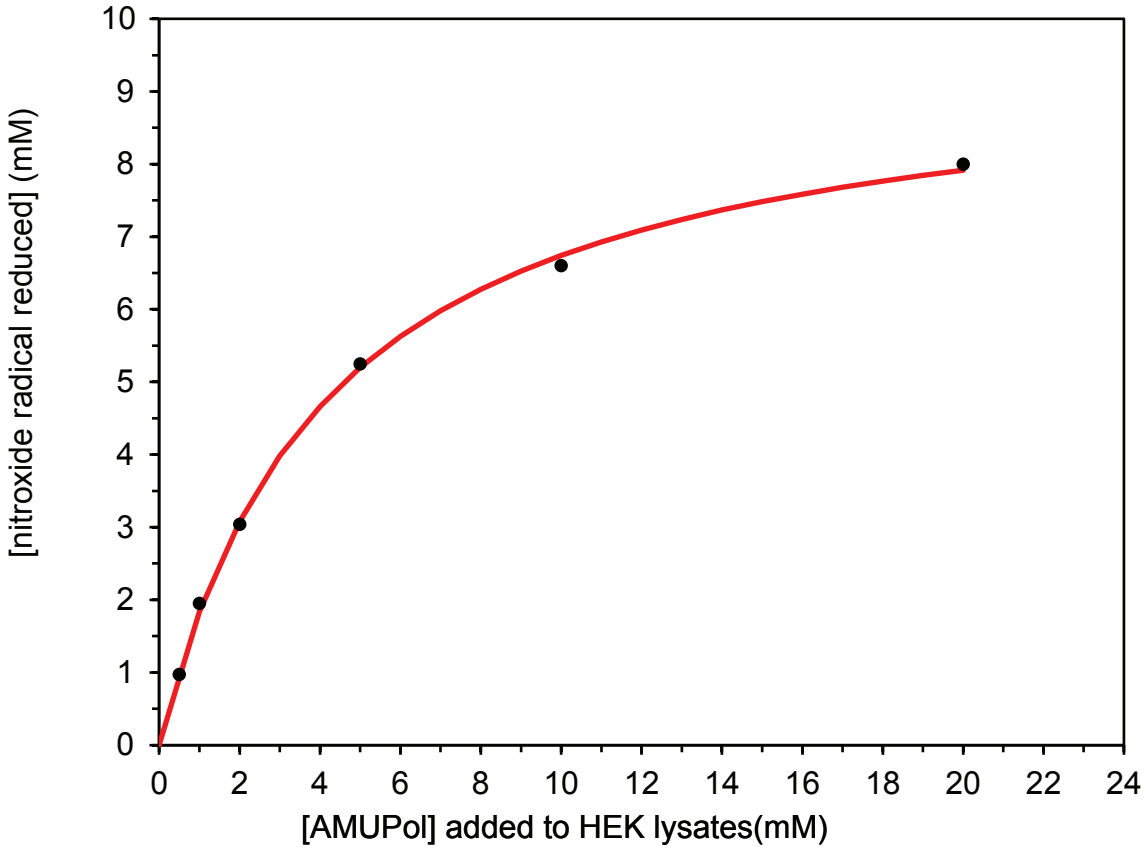

Figure S1: Concentration of nitroxide reduced (at approx. 12 h) as a function of the concentration of AMUPol added to lysate. The graph demonstrates that lysates can reduce a maximum of 9.6 mM of nitroxide radicals. The concentration of reduced nitroxide = (1- c) * conc. of AMUPol added, where c is the fitting parameter obtained from fitting the time course of nitroxide reduction in lysate to y=a*exp (-b*x) +c. The graph was fitted to y=a*x/(b+x) where a = 9.6 mM.

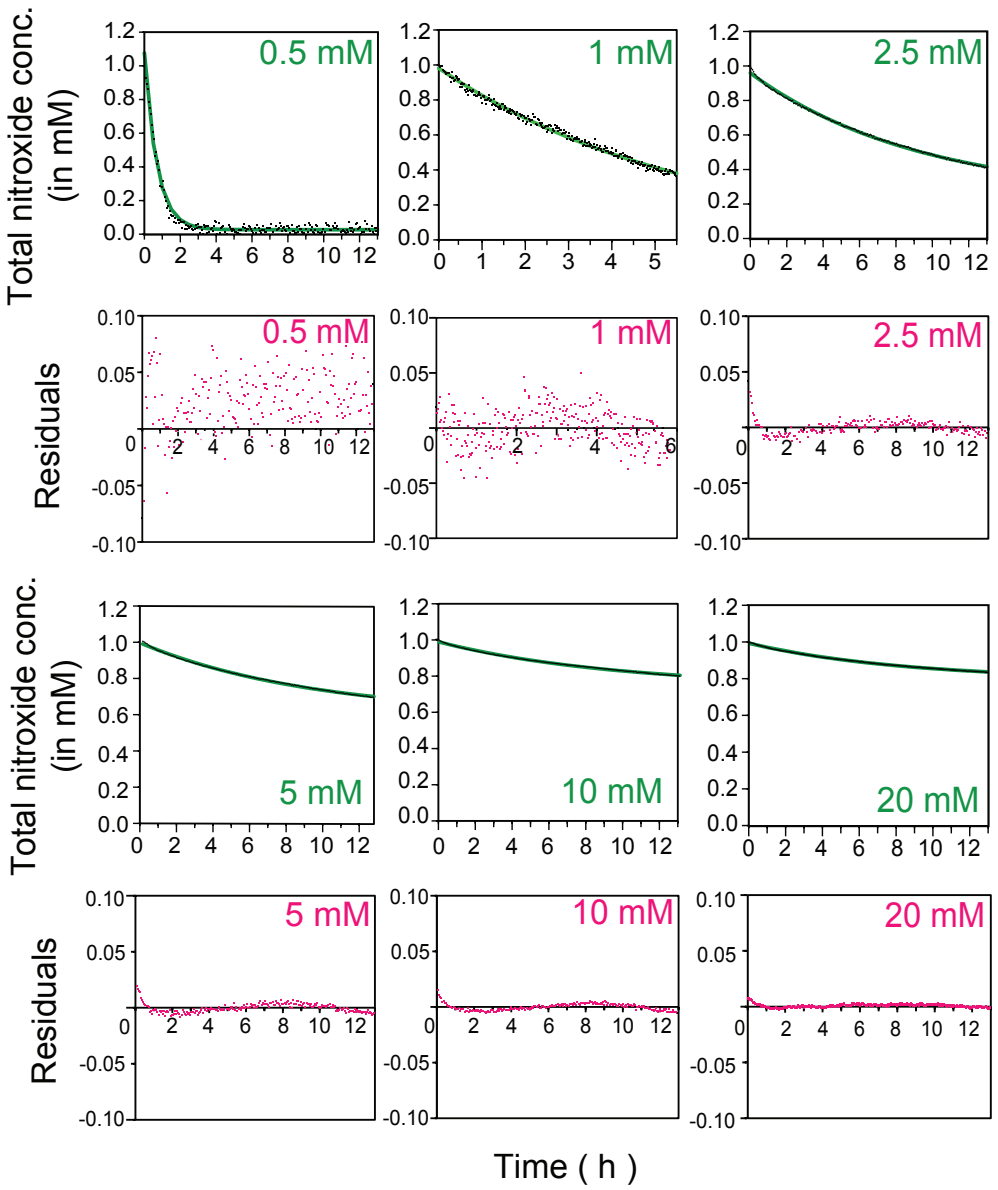

Figure S2: Deactivation kinetics of AMUPol in HEK293 cell lysates. (A) Reduction kinetics of AMUPol in lysates of HEK293 cells. AMUPol was added to lysates at the indicated concentration. Total nitroxide concentration determined by double integration of the EPR spectra at the initial timepoint was normalized to 1 for each time course. Individual data points are represented by black dots. Green lines indicate best fit to an exponential decay. The residual error was determined by subtraction of the experimental value from the fit and is plotted below in pink. The experimental value is systematically underestimated by the fit by a few percent during the first 30 minutes of the time course when the added concentration of AMUPol is 2.5 mM or greater.

Table S1: Total nitroxide reduction rates for HEK 293 lysate samples mixed with varying concentrations of AMUPol from monoexponential fits.

AMUPol Conc. (mM)	Decay Rate (% per min) from monoexponential fit	Decay Rate (mM/min) from monoexponential fit	R^2
0.5	2.4 ± 0.1	0.024 ± 0.001	0.97
1	0.22 ± 0.02	0.0044 ± 0.0004	0.99
2	0.180 ± 0.005	0.0072 ± 0.0002	0.99
5	0.172 ± 0.006	0.017 ± 0.001	0.99
10	0.177 ± 0.007	0.035 ± 0.001	0.99
20	0.200 ± 0.005	0.080 ± 0.002	0.99

Table S2: Total nitroxide reduction rates for 5 samples of HEK 293 cells electroporated with 20 mM AMUPol from monoexponential fits.

Total Nitroxide Conc. (mM) at t=0 from double integration of EPR signal	Decay Rate (% per min)	Decay Rate (mM/min)	R^2
1.62	0.26 ± 0.01	0.0043 ± 0.0001	0.97
1.93	0.36 ± 0.01	0.0069 ± 0.0001	0.99
1.61	0.26 ± 0.01	0.0042 ± 0.0001	0.99
1.73	0.22 ± 0.01	0.0038 ± 0.0001	0.99
2.40	0.24 ± 0.04	0.006 ± 0.001	0.99

AMUPol delivery	[NEM] (mM) 30 min pre-inc.	[AMU Pol] mM	protein						RNA						Lipid					
			ε	mono-exp		stretch-exp			ε	mono-exp		stretch-exp			ε	mono-exp		stretch-exp		
				$T_{B,on}$	reg.	$T_{B,on}$	**β**	reg.		$T_{B,on}$	reg.	$T_{B,on}$	**β**	reg.		$T_{B,on}$	reg.	$T_{B,on}$	**β**	reg.
EP	0	20	32.2	6.3	1.3%	6.7	0.92	0.5%	22.6	6.4	1.5%	6.7	0.93	1.1%	26.5	7.1	1.1%	7.4	0.94	0.6%
EP	2.5	20	8.1	3.4	1.5%	3.6	0.91	0.9%	9.6	3.8	2.9%	3.9	0.92	2.7%	6.5	3.7	1.3%	3.8	0.92	0.6%

Table S3: DNP parameters for HEK293 cells that were pre-incubated in either PBS or in PBS with 2.5 mM NEM.

Mono-exp reports values determined from a fit to a mono-exponential function. Stretch-exp reports values determined from a fit to a stretched exponential function.

$T_{B,on}$ is reported in units of seconds and was determined from 13 time points that ranged from 0.5~60 sec. (0.1, 0.5, 1.0, 1.5, 2.5, 3.0, 3.5, 5.0, 7.5, 10, 15, 30, and 60 sec, respectively).

reg. is the regression error, determined as reported in the methods section.