

Supplementary material

Detection of superoxide production in stimulated and non stimulated living cells using new cyclic nitron spin traps

Kahina Abbas, Micael Hardy, Florent Poulhès, Hakim Karoui, Paul Tordo, Olivier Ouari, and Fabienne Peyrot

Table of content

Table S1.....page 1
Figure S1.....page 2
Figure S2.....page 3
Figure S3.....page 4

Table S1: Parameters used for the simulation of the spectra in Fig. S1.

Spin trap	Spin adduct	Conformer	Exchange time	A_P (mT)	A_N (mT)	$A_{H\beta}$ (mT)	$A_{H\gamma}$ (mT)
BMPO	BMPO-OH (100%)	-	-	-	1.41	1.27	-
DEPMPO	DEPMPO-OOH <i>trans</i> (20%)	T1 (53%)	32 ns	5.19	1.31	1.19	0.08
		T2 (47%)		4.85	1.32	1.01	0.09
	DEPMPO-OH (80%)	-	-	4.71	1.40	1.32	-
DIPPMPO	DIPPMPO-OH (100%)	-	-	4.68	1.40	1.33	-
Mito-DIPPMPO	Mito-DIPPMPO-OOH <i>trans</i> (75%)	T1 (52%)	41 ns	5.33	1.28	1.48	-
		T2 (48%)		5.29	1.29	0.87	-
	Mito-DIPPMPO-OH (25%)	-	-	5.29	1.36	1.05	-
CD-DIPPMPO	CD-DIPPMPO-OOH <i>trans</i> (100%)	T1 (54%)	64 μ s	5.34	1.26	1.25	-
		T2 (46%)		5.26	1.30	1.12	-

Figure S1: Superimposition of the representative ESR spectra obtained with various cyclic nitrone spin traps in the presence of PMA-stimulated RAW macrophages (black lines) and their computer simulations (red lines). See legend of Fig. 2 for experimental conditions and Table S1 for simulation parameters.

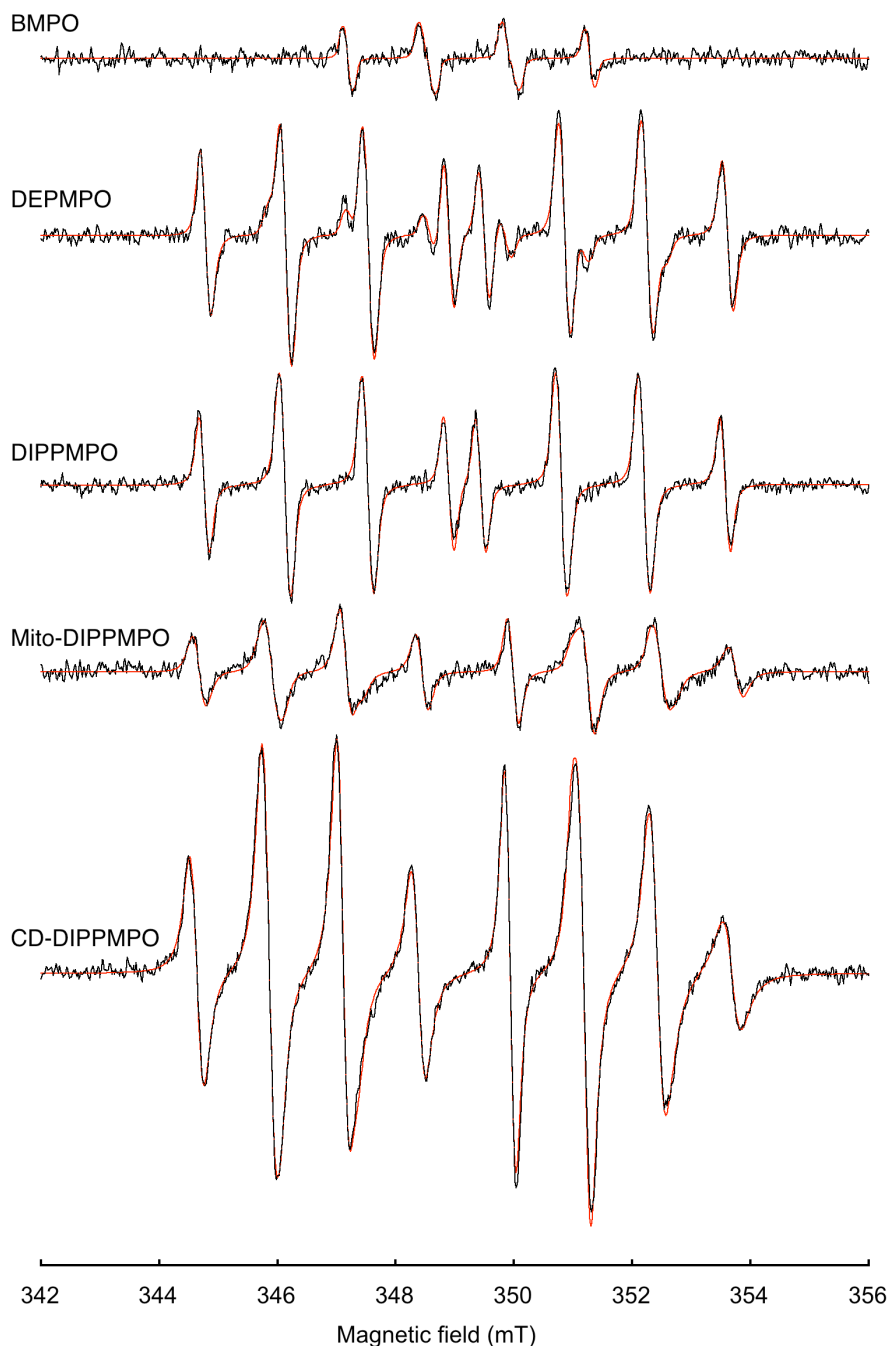


Figure S2: Comparison of the representative ESR spectra obtained with DEPMPO or CD-DIPPMPO in the presence of unstimulated macrophages. DEPMPO (10 mM) or CD-DIPPMPO (5 mM) was incubated with unstimulated RAW macrophages ($3.6 \pm 0.3 \times 10^6$ cells/100 μ L) in PBS (pH 7.1-7.4) and 1 mM DTPA. ESR settings are described under the Experimental procedures. The data are the same as those presented in Figures 2 and 5 but the sum of 30 scans without application of the SVD noise filtration is shown here.

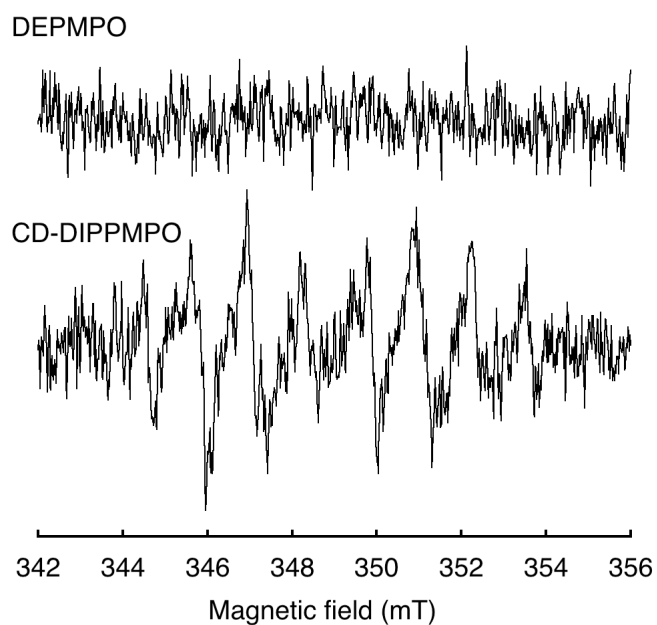


Figure S3: Use of TEMPOL and Ni(en)₃²⁺ to distinguish between intra- and extracellular compartments in RAW macrophage suspensions. (a) The ESR spectrum of a suspension of RAW macrophages ($3.6 \pm 0.3 \times 10^6$ cells in 100 μ L PBS containing 1 mM DTPA) was recorded immediately after addition of the aminoxyl radical TEMPOL (500 μ M), which passively equilibrates across the cell membrane, and of the membrane-impermeable spin probe Ni(en)₃²⁺ (100 mM), which broadens the extracellular radical signal; (b) simulation of the broad component in (a); (c) simulation of the intracellular TEMPOL signal in (a), using a coupling constant A_N of 1.7 mT.

