Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Electronic Supplementary Information for

DEPMPO: an Efficient Tool for the Coupled ESR-Spin Trapping of Alkylperoxyl radicals in Water

Hakim Karoui,[*] Florence Chalier, Jean-Pierre Finet[†] and Paul Tordo[*]

Universités d'Aix-Marseille I, II et III - CNRS UMR 6264. Laboratoire Chimie Provence, Equipe SREP, 13397 Marseille Cedex 20, France. Tel: + 33 4 91 28 86 10; Fax: + 33 4 91 28 87 58; E-mails: hakim.karoui@univ-provence.fr; paul.tordo@univ-provence.fr

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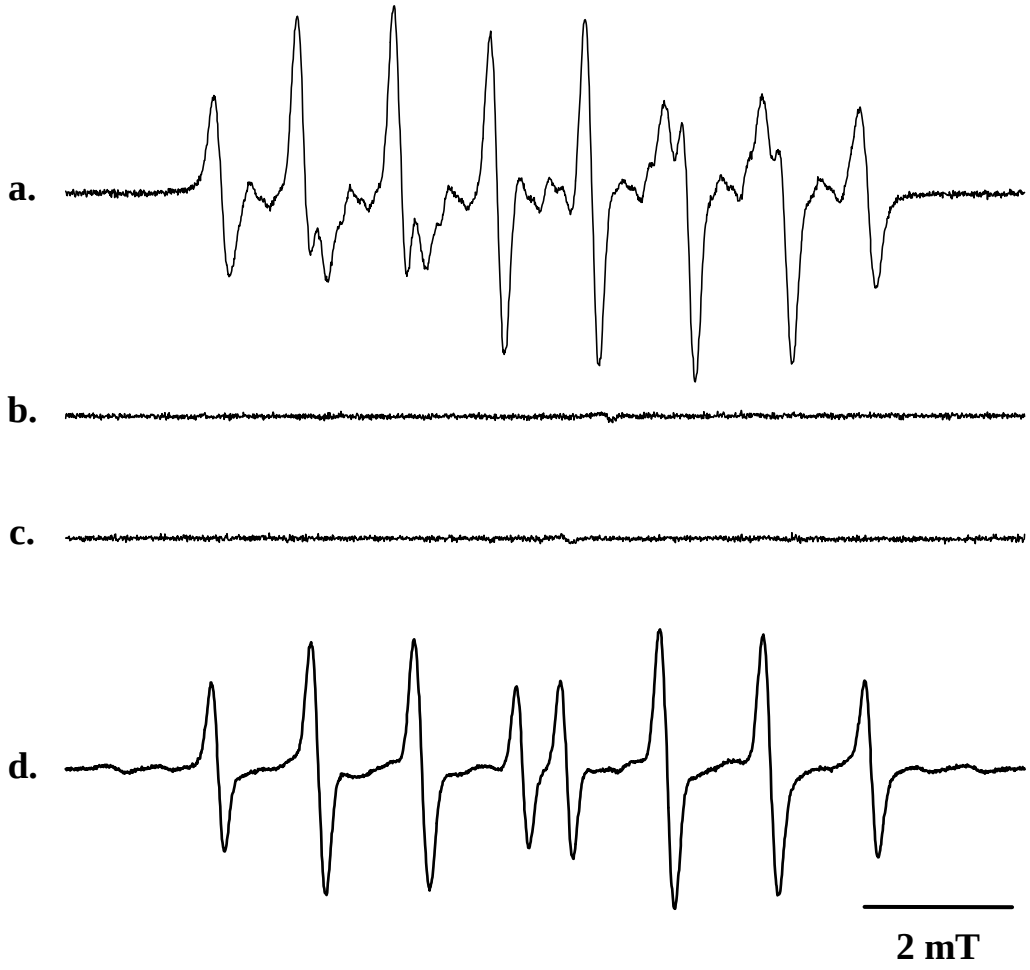

Figure S1: ESR signals during superoxide and hydroxyl radicals formation. a, signal obtained upon incubating xanthine (0.4 mM), XOD (0.04 U/ml), DTPA (0.3 mM) and DEPMPO (50 mM) in phosphate buffer (0.1 M, pH 7.4), **b**., as (**a**) but in the presence of SOD (0.1 mg.mL^{-1}), **c**, as (**b**) but in the presence of irradiated SOD (0.1 mg.mL^{-1}), **d**, UV-photolysis of a solution containing DEPMPO (5 mM), H_2O_2 (1%). Spectrometer settings: microwave power, 10 mW; modulation amplitude, 0.1 mT; time constant, 0.128 s; gain, 5x10^4; scan range, 14 mT and scan time, 43 s (4 scans for each signal).

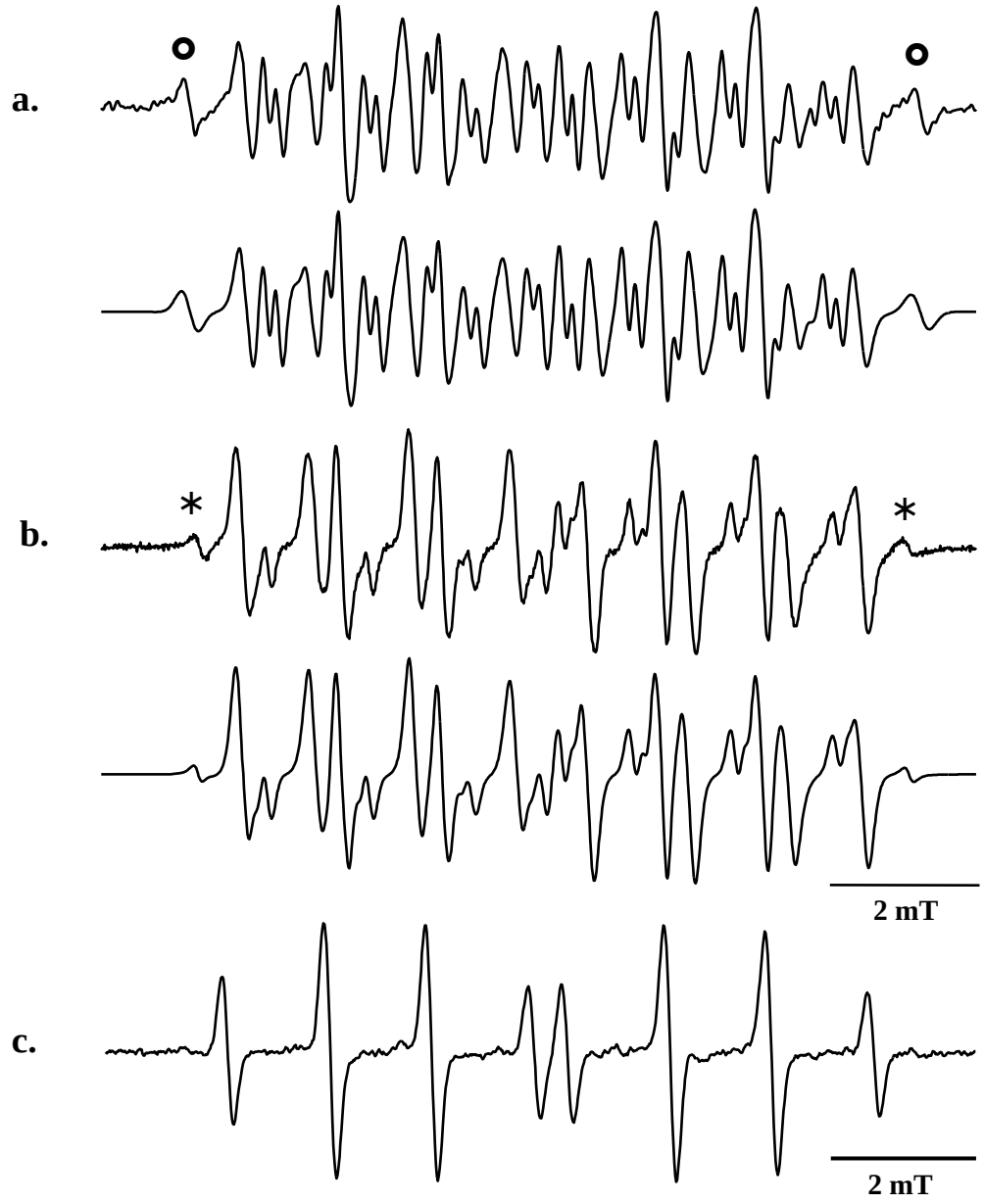

Figure S2: Authentic ESR spectra of DEPMPO-OR spin adducts in water. DEPMPO-OR spin adducts were obtained by addition of $FeCl_3$ (2 mM) to the respective ROH solution of DEPMPO (0.5 M) for 2 min; then 30 µL of this solution were added to 270 µL of a phosphate buffer solution (0.1 M, pH 7.4) containing DTPA (20 mM). **a,** DEPMPO-O*n*Bu. The spectrum below represents the simulation. **b,** DEPMPO-O*i*Pr. The spectrum below represents the simulation. **c**, DEPMPO-O*tert*-Bu: a solution of DEPMPO (50 mM) and *tert*-BuOO*tert*-Bu (0.5 M) in *tert*-butanol was irradiated during 30 s and 30 µL of this solution were added to 270 µL of phosphate buffer (0.1 M, pH 7.4); Spectrometer settings: microwave power, 10 mW; modulation amplitude, 0.05 mT; time constant, 0.128 s; gain, 5 $x10^4$; scan range, 12 mT and scan time, 42 s (4 scans for **a.** and **b.**; 84 s for **c.**).

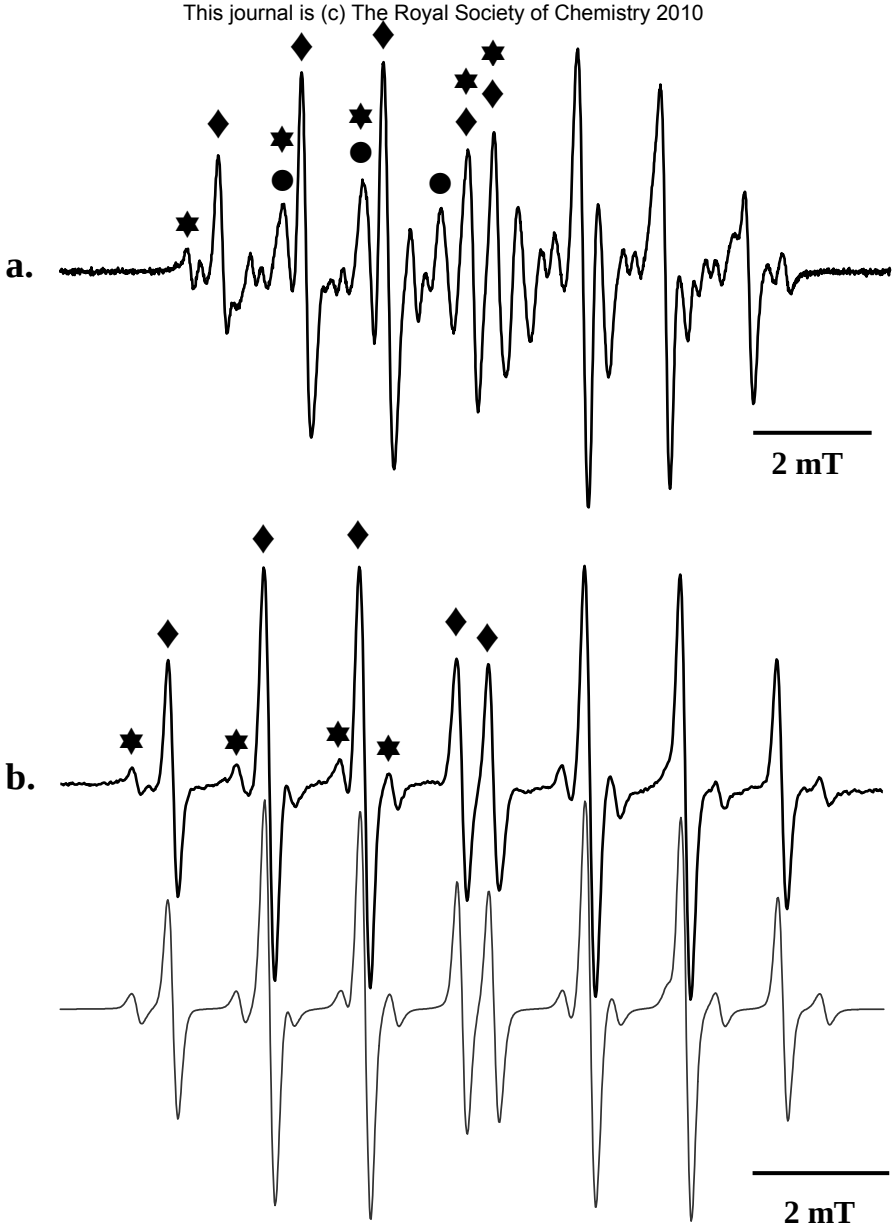

Figure S3: Free radicals trapped during the reaction between cytochrome c and *t*-BuOOH in the presence of different DEPMPO concentrations. a, incubation mixture containing cytochrome c (20 μM), *t*-BuOOH (15 mM), DTPA (0.3 mM) and DEPMPO (100 mM) in oxygen-saturated phosphate buffer (0.1 M, pH 7.4). The ESR spectrum observed corresponds to a mixture of DEPMPO-O*tert*-Bu (◆), DEPMPO-$OOCH_3$ (●) and DEPMPO-CH_3 (✸) spin adducts. **b**, as (**a**) but in the presence of DEPMPO (400 mM). The ESR spectrum observed corresponds to a mixture of DEPMPO-O*tert*-Bu (◆) and DEPMPO-CH_3 (✸) spin adducts The grey spectrum below represents the computer simulation. Spectrometer settings: microwave power, 10 mW; modulation amplitude, 0.1 mT; time constant, 0.128 s; gain, $1x10^5$; scan range, 14 mT for **a.** and 12 mT for **b.** and scan time, 43 s.

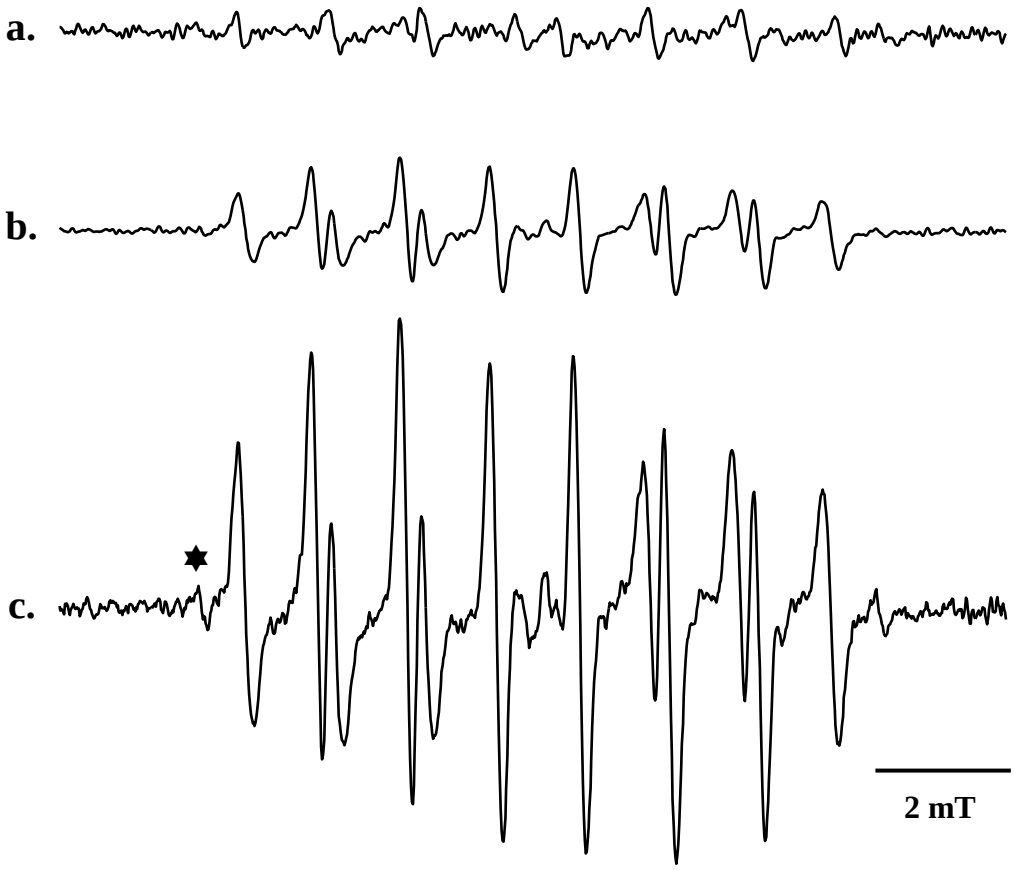

Figure S4: DEPMPO-$OOCH_3$ spin adduct generation during the reaction between cytochrome c and cumylhydroperoxide. **a**, EPR spectrum obtained from a solution containing DEPMPO (8 mM) and cumylhydroperoxide (CumOOH) (12.5 mM) in phosphate buffer (0.1 M, pH 7.4). **b**. EPR signal obtained upon adding Cytochome c (20 μM) to the previous incubation mixture **c**, as (**b**) after 4 scans. Spectrometer settings: microwave power, 10 mW; modulation amplitude, 0.1 mT; time constant, 0.128 s; gain, $5x10^4$; scan range, 14 mT and scan time, 43 s.

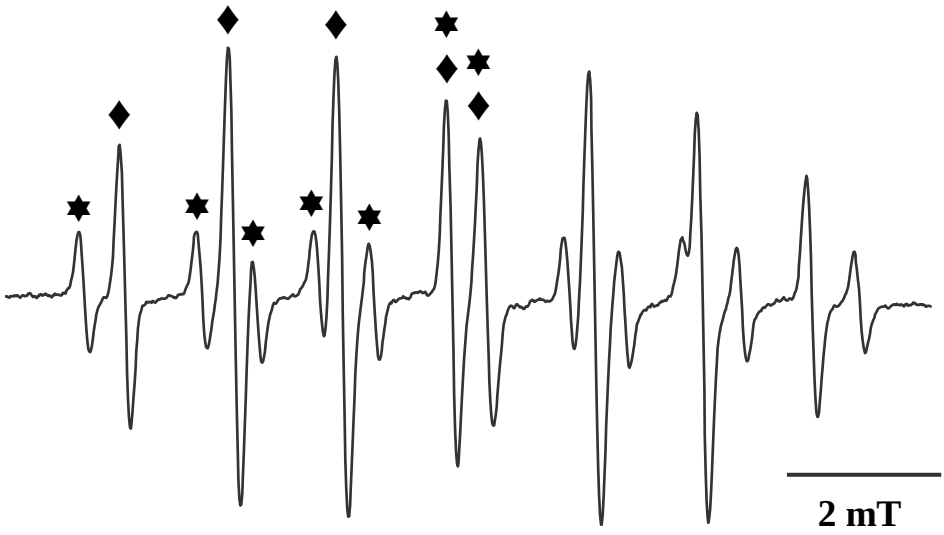

Figure S5: DEPMPO spin adducts obtained during the reaction between *t*-BuOOH and Fe^{2+} in the presence of DEPMPO. Addition of Fe^{2+} (0.5 mM) to a deoxygenated phosphate buffer solution (0.1 M, pH 7.4) containing *tert*-BuOOH (4 mM) and DEPMPO (0.05 M) led to a mixture of DEPMPO-O*tert*-Bu (◆, a_N = 1.39; $a_{H\beta}$ = 1.45 and a_P = 4.66 mT; 73 %) and DEPMPO-CH_3 (✸, a_N = 1.52; $a_{H\beta}$ = 2.23 and aP = 4.76 mT; 27 %). Spectrometer settings: microwave power, 10 mW; modulation amplitude, 0.1 mT; time constant, 0.128 s; gain, $5x10^4$; scan range, 12 mT and scan time, 83 s.