Photochemical &

Photobiological Sciences

Cytochrome c-promoted cardiolipin oxidation generates singlet molecular oxygen

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Eletronic Supplementary Information

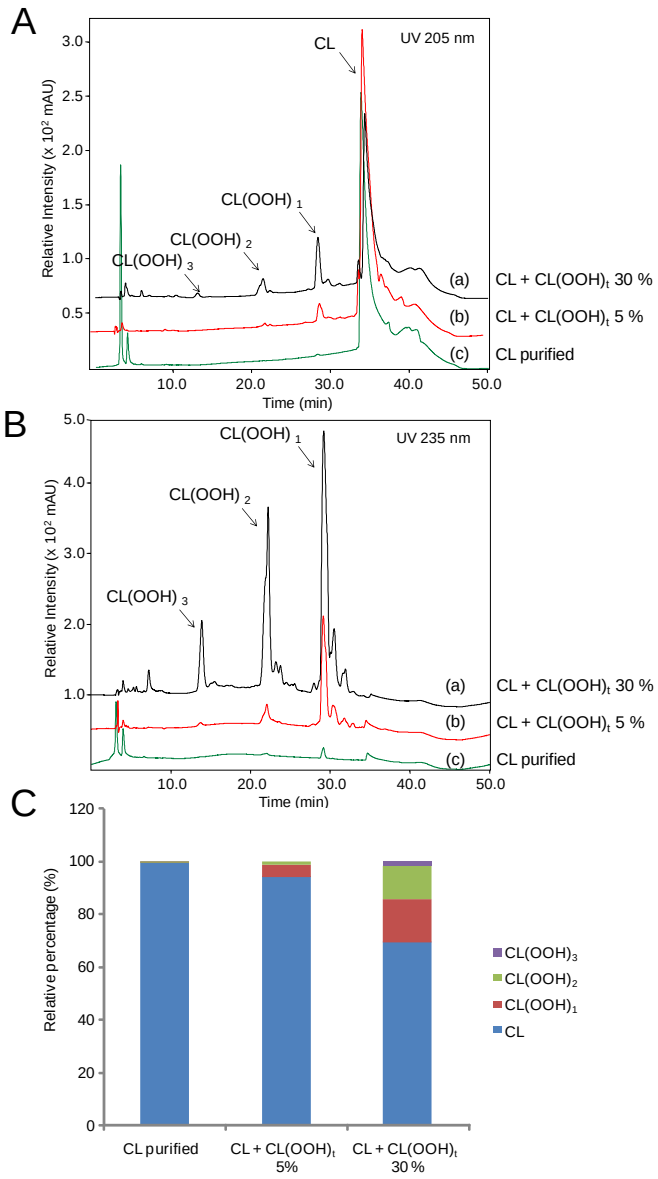


Figure S1. Analysis of CL hydroperoxide content in CL stock solutions. An aliquot of 5 µl of CL stock solutions [(a) containing 30 % CLOOH; (b) containing 5 % CLOOH; and (c) purified CL] was injected into a HPLC system. The eluent was monitored at 205 nm for CL analysis (A) and at 235 nm for $CL(OOH)_1$, $CL(OOH)_2$ and $CL(OOH)_3$ analysis (B). The peaks corresponding to each compound was integrated and concentrations (mM) were calculated using the following standard curves: CL ($y=7\times10^6x+525102$, $R^2=0,9961$), $CL(OOH)_1$ ($y=5\times10^6x+5809$, $R^2=0,9996$), $CL(OOH)_2$ ($y=4\times10^6x-23605$, $R^2=0,9911$) and $CL(OOH)_3$ ($y=1\times10^7x+12704$, $R^2=0,9726$). Pure CL hydroperoxide standards were prepared by photooxidation using methylene blue as a photosensitizer and purified by HPLC using the same condition described for CLOOH analysis.

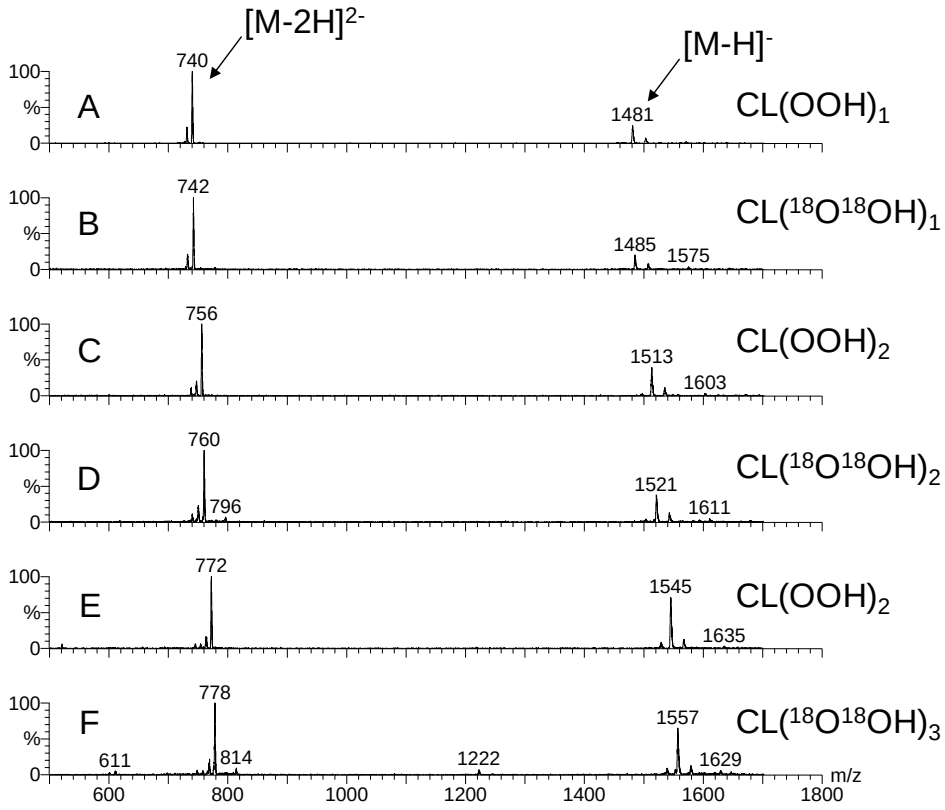


Figure S2. Analysis of unlabeled and ^{18}O-labeled cardiolipin hydroperoxides by HPLC-MS using electrospray ionization in the negative ion mode. The source temperature was set to 150 °C and the cone voltage was set to 35 V. Aliquots (10 µl) of purified cardiolipin hydroperoxides (150 – 300 µM) were injected to the HPLC-MS. $CL(OOH)_1$ (A), $CL(^{18}O^{18}OH)_1$ (B), $CL(OOH)_2$ (C), $CL(^{18}O^{18}OH)_2$ (D), $CL(OOH)_3$ (E) and $CL(^{18}O^{18}OH)_3$ (F).

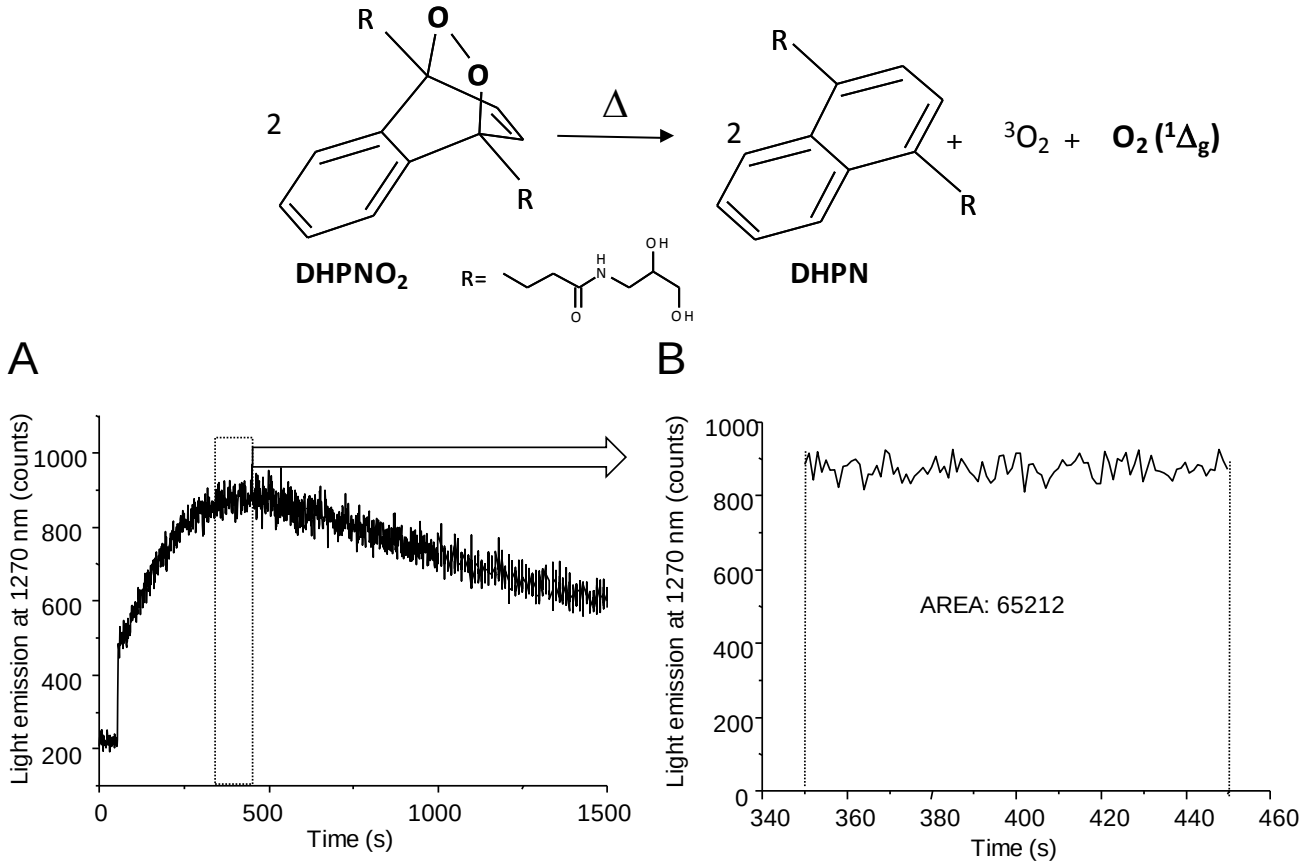
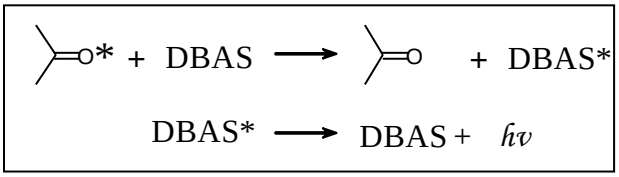


Figure S3. Singlet oxygen monomolecular light emission at 1270 nm observed for $DHPNO_2$. A) Light emission recorded for $DHPNO_2$ (6.3 mM) thermolysis in D_2O at 37°C. B) Expanded view of the emission signal obtained in the period between 350 – 450 s. The area under the emission signal was integrated and correlated to the amount of singlet oxygen generated. Thermolysis of this endoperoxide yields 59% of singlet oxygen at 5.02×10^{-4} s^{-1}. Considering that thermolysis of a 6.3 mM solution of $DHPNO_2$ at 37 °C yields 1.9 μM s^{-1} of singlet oxygen, the integrated area (65212) corresponds to 190 μM of singlet oxygen. This correlation was used to convert the emission signal to singlet oxygen concentrations.



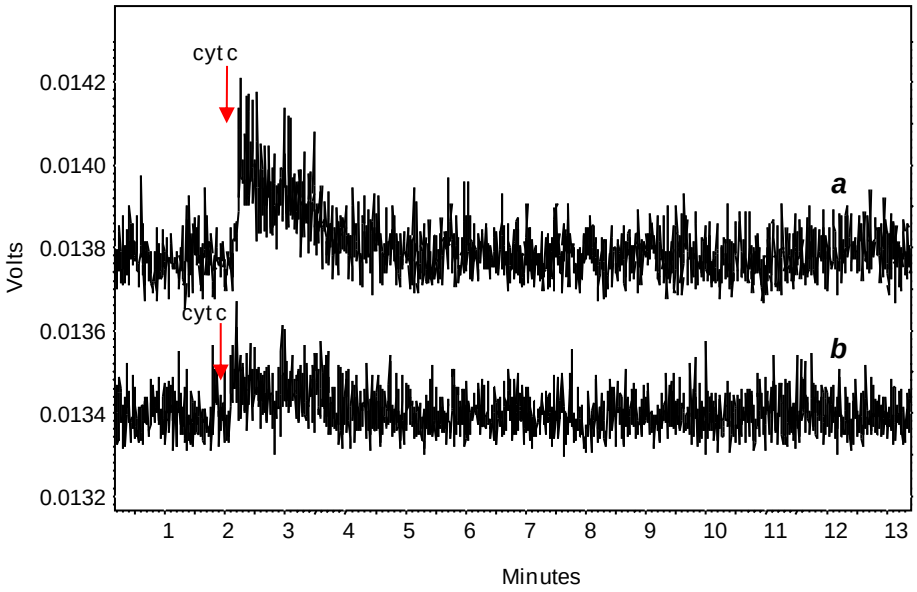


Figure S4. Visible light emission in the reaction of cyt c (50 μM) with liposomes containing DMPC:CL (1.7 mM:1.7 mM in 10 mM deuterated phosphate buffer, pH 7.4) in the presence (a) and absence (b) of 9,10-dibromoanthracene sulphonate, DBAS (14 μM).