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Supplemental Information

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Supporting Material

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Initiation:
$LH + R^{\bullet} \rightarrow L^{\bullet} + RH$ (1)
$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$ (2)
Propagation;
$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$ (3)
New chain reaction:
$LOOH \rightarrow LO^{\bullet} + LOO^{\bullet} + RCHO$ (4)
Termination:
$2\,LOO^{\bullet} \rightarrow LOO - OOL$ (5)

Fenton Initiation:
$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + OH^{\bullet}$ (6)
$OH^{\bullet} + LH \rightarrow H_2O + L^{\bullet}$ (7)
AAPH Initiation:
$R - N{=}N - R \rightarrow 2N + 2R^{\bullet}$ (8)
$R^{\bullet} + O_2 + LH \rightarrow ROOH + LOO^{\bullet}$ (9)

Redox cycling of Iron:
$Fe^{3+} + LOOH \rightarrow Fe^{2+} + LOO^{\bullet} + H^{+}$ (10)
$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^{\bullet} + OH^{-}$ (11)

Scheme S1: Reactions of oxidation

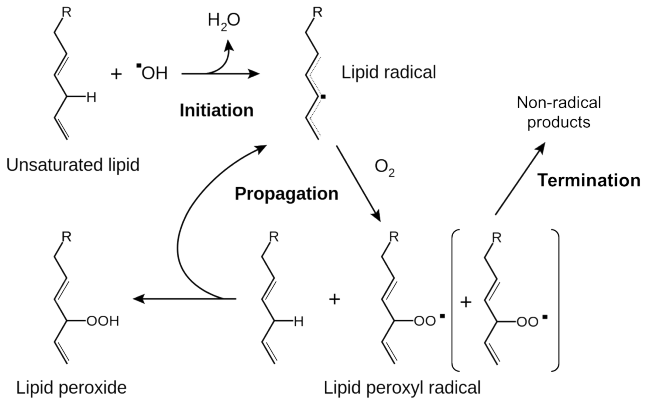

Figure S1: Mechanisms of peroxidation

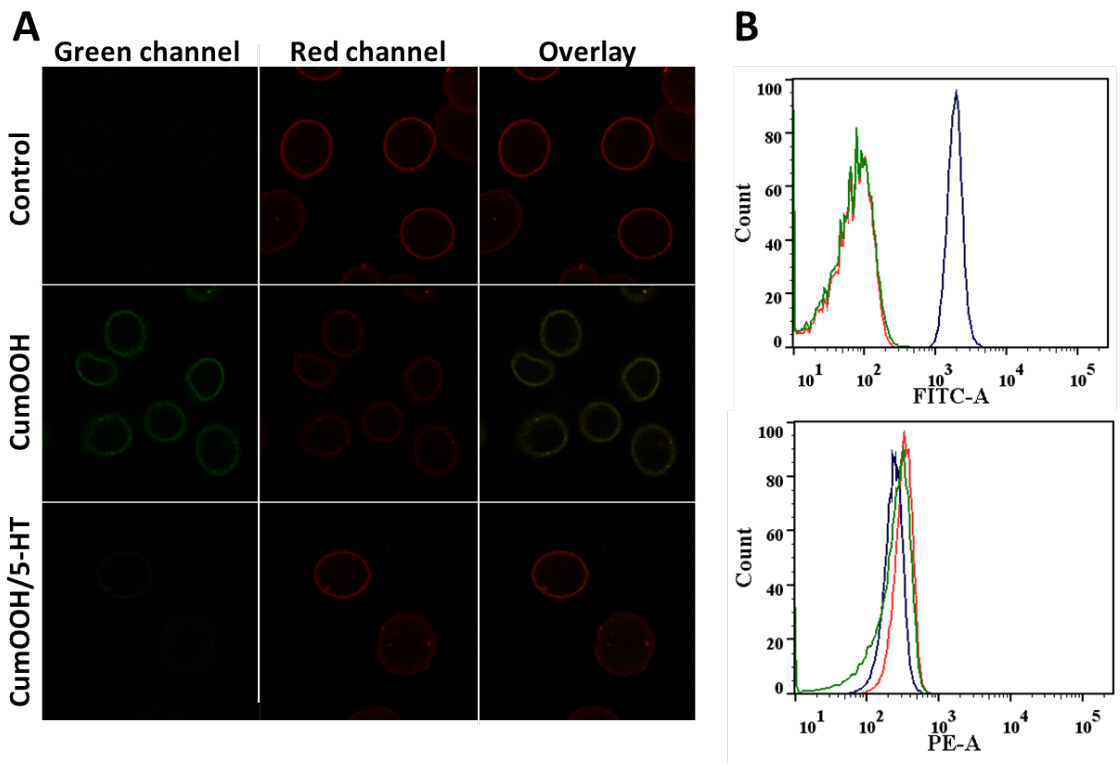

Figure S2: Detection of CumOOH-induced oxidation of BODIPY 581/591 C11 in RBCs using confocal microscopy and flow cytometry. (**A**) Typical fluorescence images obtained from the red and green channels of BODIPY 581/591 C11-labeled RBCs, incubated 2h at room temperature with 150 µM CumOOH or with 150 µM CumOOH/50 µM 5-HT. (**B**) The green and red fluorescence intensities associated with each samples were analyzed by flow cytometry. Fluorescence intensities are shown for untreated samples (red), CumOOH treatment (blue), and CumOOH/5-HT treatment (green). Upon reaction with reactive oxygen species, the red fluorescence of BODIPY 581/591 shifts to green.

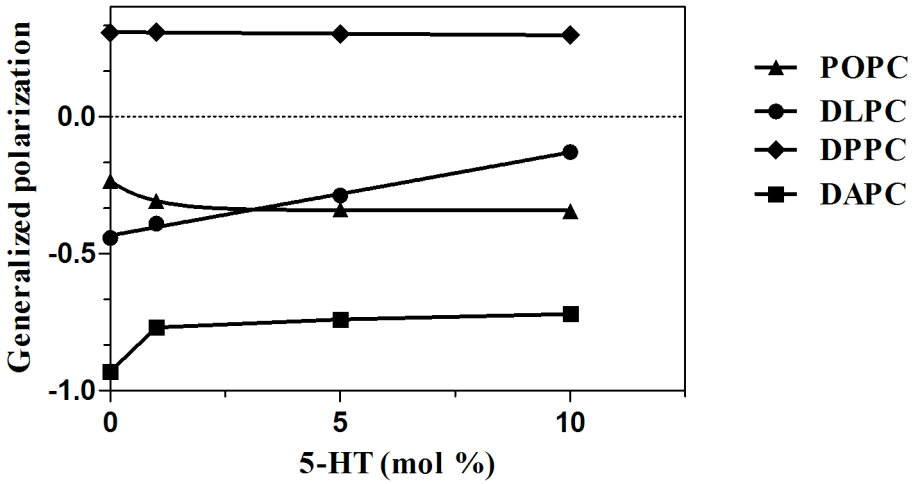

Figure S3: Generalized polarization (GP) of the Laurdan fluorescent probe was plotted as a function of 5-HT content in DAPC, DPPC, DLPC or POPC SUVs at 37°C.

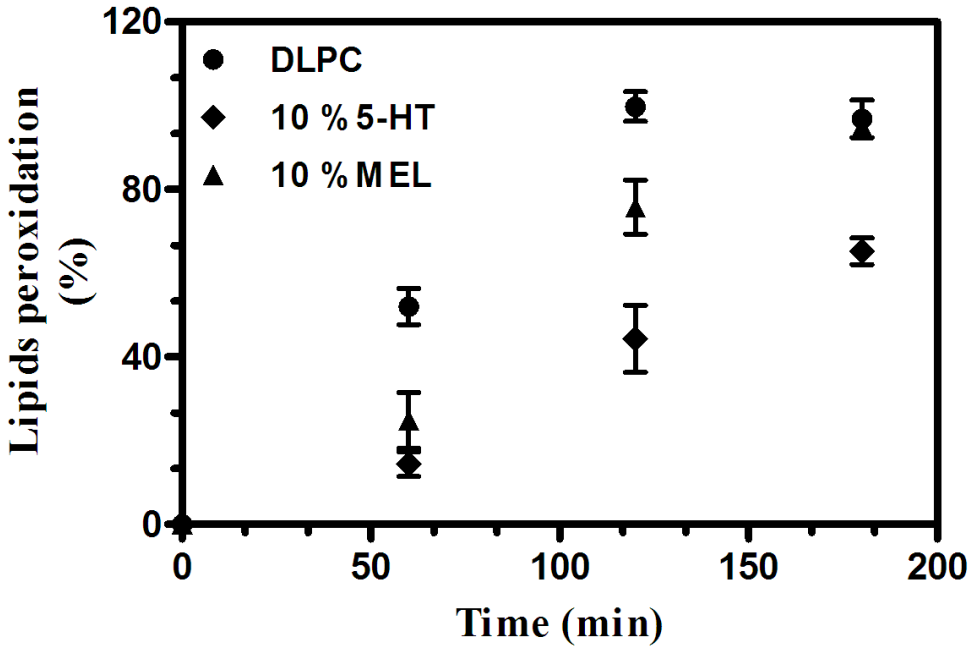

Figure S4: Kinetics of DLPC peroxidation with AAPH measured at 234 nm.

	% of 5-HT (mol)			
	0	1	5	10
DPPC	**55 ± 3 nm**	**49 ± 2 nm**	**50 ± 4 nm**	**47 ± 7 nm**
POPC	**51 ± 4 nm**	**45 ± 3 nm**	**46 ± 6 nm**	**54 ± 4 nm**
DLPC	**49 ± 3 nm**	**55 ± 1 nm**	**56 ± 4 nm**	**52 ± 8 nm**
DAPC	**67 ± 8 nm**	**62 ± 6 nm**	**68 ± 3 nm**	**57 ± 6 nm**

Table S1: The diameters of the different SUVs measured by Dynamic Light Scattering (DLS).

Systems	OH		Amine	
	Water	**Lipid**	**Water**	**Lipid**
POPC	**1.85 ± 0.04**	**0.40 ± 0.01**	**1.64 ± 0.05**	**1.30 ± 0.05**
DLPC	**1.87 ± 0.02**	**0.37 ± 0.01**	**1.73 ± 0.05**	**1.21 ± 0.05**
DAPC	**1.86 ± 0.05**	**0.39 ± 0.02**	**1.76 ± 0.20**	**1.18 ± 0.22**

Table S2: Average number of H-bonds for each system between Amine OH group of 5-HT and water or lipid molecules. The statistical errors were obtained using the block-averaging method. The values agree fairly well those reported by Wood et al (1) for the interactions with lipids (1.611 for the Amine and 0.290 for OH). For water, the values obtained are higher (1.64 comparted to 1.045 for the amine and 1.85 compared to 0.725 for the OH part). This could be explained by a longer time spent by 5-HT in water during our simulations.

	Thickness (nm)	A_L (nm²)
POPC	**3.95 ± 0.06**	**0.62 ± 0.01**
DLPC	**3.80 ± 0.07**	**0.67 ± 0.01**
DAPC	**3.69 ± 0.07**	**0.74 ± 0.02**
DLPC/Z,E-13-HPd	**3.72 ± 0.06**	**0.69 ± 0.01**
DLPC/Z,E-9-Pl$^{\bullet}$	**3.76 ± 0.06**	**0.68 ± 0.01**
DLPC/Z,E-13-Pl$^{\bullet}$	**3.79 ± 0.06**	**0.68 ± 0.01**

Table S3: Properties of pure bilayer membranes. The thickness was defined as the Phosphate-Phosphate distance. The area per lipid (A_L) was calculated by dividing the box area in the dimension parallel to the bilayer plane by the number of lipids in each leaflet.

	HeadGroup	Glycerol	Aliphatic Chains
DLPC	**41.2**	**22.4**	**36.4**
DLPC/Z,E-13-HPd	**37.3**	**21.9**	**40.8**
DLPC/Z,E-9-Pl$^{\bullet}$	**42.62**	**22.49**	**34.94**
DLPC/Z,E-13-Pl$^{\bullet}$	**40.66**	**22.25**	**37.11**

Table S4: Type of contacts (in %) between 5-HT and membrane. A Contact is defined if the distance between any atoms of the lipids and the center of mass of 5-HT lipid is inferior to 0.6 nm.

Text S1: Protocol of Potential of Mean Force

Umbrella sampling simulations were performed for serotonin crossing membrane of three different compositions, i.e pure POPC, pure DLPC and pure DAPC. Software version, forcefield parameters and simulations conditions were the same as used in the standard molecular dynamics simulations described in Material and Methods section. The starting frames for the umbrella simulations were taken from the last frame of the pure bilayers simulations. The system was composed of 128 phospholipids and around 10 000 water molecules. To construct the configurations, the center of mass (COM) of 5-HT were placing at different z-position separated by 1A with a range between -39 Å and 39 Å. To enhance sampling, 2 molecules of 5-HT were added at different z positions, keeping a distance of 39 Å between them. In each window of umbrella sampling, the simulation was carried out for 10 ns by applying a force constant of 1000 kJ/mol/nm² based on the COM of the 5-HT molecules. For the positions located within the membrane interface (between 12 Å and 26 Å and between -12 Å and -26 Å), the simulation time for sampling was lengthened up to 50 ns.

The PMFs were computed using the weighted histogram analysis method (WHAM) (2) as implemented in the g_wham software (3). Statistical errors were calculated using bootstrap analysis.

References

1. Wood, I., M.F. Martini, and M. Pickholz. 2013. Similarities and differences of serotonin and its precursors in their interactions with model membranes studied by molecular dynamics simulation. J. Mol. Struct. 1045: 124–130.
2. Kumar, S., J.M. Rosenberg, D. Bouzida, R.H. Swendsen, and P.A. Kollman. 1992. THE weighted histogram analysis method for free-energy calculations on biomolecules. I. The method. J. Comput. Chem. 13: 1011–1021.
3. Hub, J.S., B.L. de Groot, and D. van der Spoel. 2010. g_wham—A Free Weighted Histogram Analysis Implementation Including Robust Error and Autocorrelation Estimates. J. Chem. Theory Comput. 6: 3713–3720.