Supplementary Information (SI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2026

Supporting Information

Impact of Phospholipase A2 Hydrolysis on Triplet-Triplet Annihilation Upconversion Liposomes

Amrutha Prabhakaran,[a] Nirod Kumar Sarangi,[a] Colm Smith[a], Ruben Arturo Arellano Reyes,[a] and Tia E. Keyes *[a]

[a]School of Chemical Sciences and National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland.

E-mail: tia.keyes@dcu.ie

Fabrication of gold and PDMS microcavity array

Polystyrene (PS) microsphere lithography was used to fabricate both gold and PDMS microcavity array electrodes, as reported previously.[1–4] In brief, gold coated silicon wafer electrodes were initially cut into ~1 cm ×1.5 cm and cleaned by washing with tetrahydrofuran (THF) then ethanol before drying under mild stream of high purity N_2. The electrodes were then air plasma treated for 5 minutes which both cleans and renders the gold surface hydrophilic. The gold substrate was placed over chamber in a slanted manner and calculated amount of 1% (v/v) of PS spheres (1 µm diameter) aqueous solution were drop cast over the gold chip. A coverslip was placed over the chip so that the PS sphere uniformly covers across the whole gold surface area and sandwiched between gold and coverslip. The substrate was left overnight to evaporate the aqueous solvent in a sealed box with dry silica gel. Next, controlled potential (-0.6 V vs. Ag/AgCl (1 M KCl)) electrochemical deposition of gold through the hexagonally close packed self-assembled 1 µm sized PS microsphere array until it reached precisely 0.5 µm thickness. The thickness was controlled using the shape of a well characterised amperometric I-t curve as reported previously. After gold deposition, the substrate was rinsed with Milli-Q water and subsequently electrochemically cleaned by sweeping the potential from -0.2 to 1.6 V (3 cycles) in 0.05 M H_2SO_4 using cyclic voltammetry. This step results removal of the top oxide layers and helps self-assembled monolayer formation. The substrate was then rinsed with milli-Q water, dried under N_2, and soaked for at least 48 hrs in an ethanolic solution of 1 mM 6-Mercapto-1-hexanol (MH) to form self-assembled monolayer (SAM). During these steps, the PS spheres template remained in place hexagonally ordered throughout and allowed MH SAM formation exclusively to the interstitial planar top gold regions. The chemical modification of the substrate renders top gold surface hydrophilic due to the terminal -OH group of MH. After SAM formation, the substrates were washed with copious amount of ethanol to remove any unbound thiols and further washed multiple times with THF. The THF wash step results in the complete removal of PS spheres leaving periodic micropore arrays with pore diameters of 1 µm and 0.5 µm depth with SAM at the interstitial planar regions. Before and after PS removal steps were confirmed using cyclic voltammetry. The cavities were then PBS buffer filled and used for bilayer formation for electrochemical impedance study.

For fluorescence lifetime imaging (FLIM) and fluorescence lifetime correlation spectroscopy (FLCS) studies, the arrays were prepared using optically transparent PDMS substrate. Freshly cleaved mica sheets of a few micrometres in thicknesses (height, h) were cut to ~1 cm (length, l)×1 cm (breadth, b) dimension and glued to glass cover slides. 20 µL of ethanolic 4.61 µm PS solution (0.1% v/v) was drop cast onto the mica surface. The hexagonally packed array was formed spontaneously after ethanol evaporation

and was then used as master mould where PDMS was cast over the array performed and cured at 90 °C for 1 hour. After curing was completed, the PDMS was gently peeled off. The thin chamber created at the PDMS has identical dimensions to the mica sheet thickness, making it ideal for confocal imaging and spectroscopy. The microcavity array was then formed by dissolving the PS sphere template from the PDMS substrate in tetrahydrofuran (THF) for 15 min via sonication. During the THF treatment, PDMS substrate was swollen and upon drying overnight in vacuum, it regained its usual size with the resulting micropore cavity hexagonal array with a pore diameter of ~2 µm and pore depth of ~0.5 µm confirmed by AFM. The PDMS substrates were air plasma cleaned for 5 min to make the surface hydrophilic and the microcavities were buffer filled by sonication and stored in buffer until use.

Fabrication of microcavity suspended lipid bilayers

The assembly of lipid bilayer spanned across the aqueous filled microcavity array, both gold and PDMS array substrates were completed as described previously[3,4] and explained in ESI by using a combination of Langmuir-Blodgett transfer followed by vesicle fusion (LB-VF) method. Briefly, in order for a Langmuir-Blodgett transfer, 50 µL of 1 mg/mL of DOPC dissolved in chloroform was added dropwise to the Langmuir trough (KN2006, KSV-NIMA technology). A lag time of 10 minutes was set before compressing and decompressing the lipid monolayers (two times each) below the collapse surface pressure. Next, the monolayer is compressed until the surface pressure is reached 33 mN/m and allowed to equilibrate for at least 5 minutes at the designated surface pressure. Then, the buffer filled gold and/or PDMS which was submerged in the LB trough using a clip holder was vertically pulled from the water surface at a speed of 5 mm/min resulting a transfer of a single layer of lipid as proximal leaflet. For distal leaflet, vesicle fusion (VF) method was carried out. The fusion process on gold was carried out immediately by submerging the LB transferred monolayer spanned array into the LUV solution (0.2 mg/mL) and allowing it to fuse for at least one hour. For PDMS substrate, the monolayer spanning PDMS substrate was sealed within a microfluidic chamber and subjected for vesicle fusion. For fluorescence-based imaging and spectroscopy, the liposomes are doped with 0.01 mol% of DOPE-ATTO655. After the completion of VF process, the substrates were washed with Tris-HCl buffer gently to remove any unfused vesicles and at no scenario, the membranes are exposed to air.

Structure and Abs/Em spectra of sensitizer

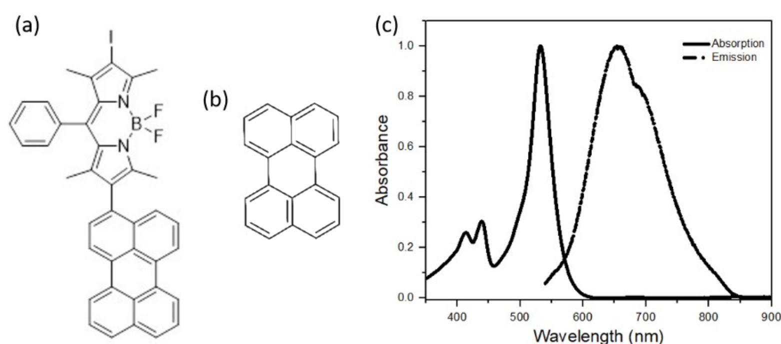

Fig. S1 Structure of (a) Ph-Bodipy-2-perylene-Iodine (B2PI) and (b) perylene. (c) Normalized absorption and emission spectra of 10 µM B2PI in chloroform.

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

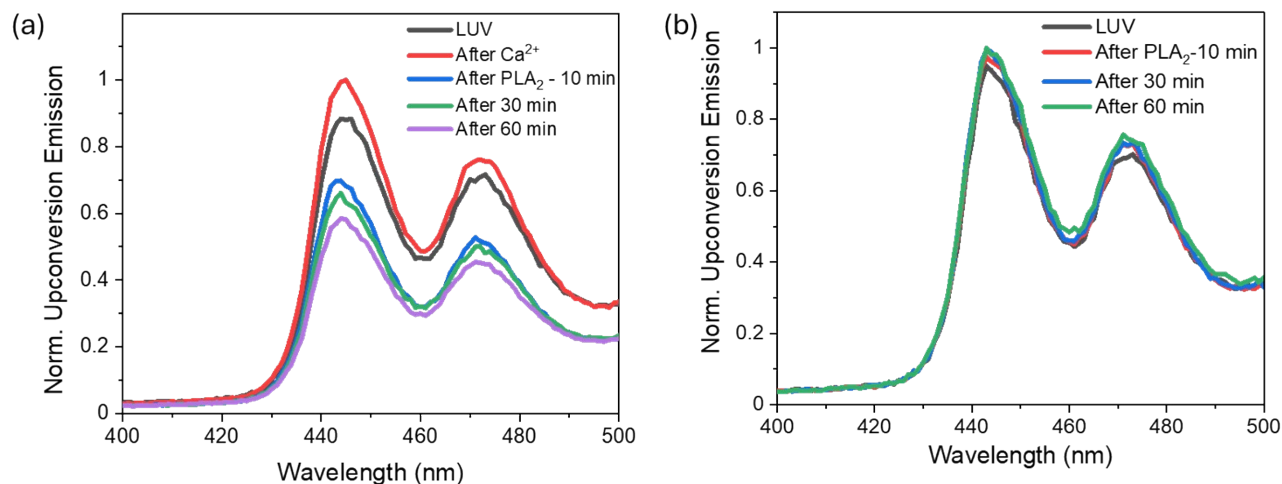

Fig. S2 a) Representative normalized TTA-UC emission spectra from DOPC liposomes incorporated with 0.5 µM B2PI and 5 µM perylene before (black) and after (red) the addition of 5 mM Ca^{2+} and after the subsequent addition of 5 µM PLA2 measured at 10, 30 and 60 minutes. b) Normalized TTA-UC emission spectra from DOPC liposomes incorporated with 0.5 µM B2PI and 5 µM perylene in presence of 5 µM PLA2 in the absence of Ca^{2+}. All the measurements are carried out in deaerated Tris-HCl buffer of pH 7.4 and were excited with a 532 nm CW laser.

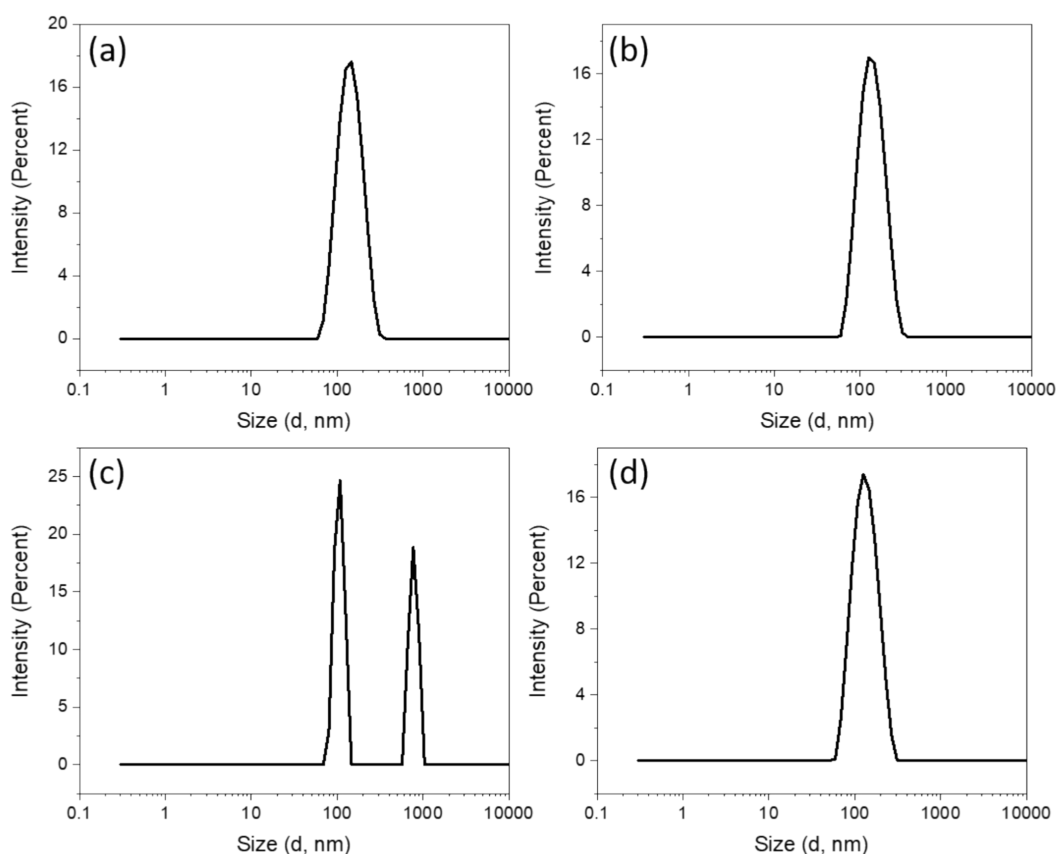

Fig. S3 Dynamic light scattering (DLS) spectra showing the hydrodynamic radius of DOPC liposomes incorporated with 0.5 µM B2PI and 5 µM perylene a) without and b) with the presence of 5 µM PLA_2 in the absence of Ca^{2+}, (c) in presence of 5 mM Ca^{2+} after incubation with PLA_2 and (d) after incubation of imipramine treated PLA_2 in presence of Ca^{2+}. All liposomes are in Tris-HCl buffer of pH 7.4.

Please do not adjust margins

Please do not adjust margins

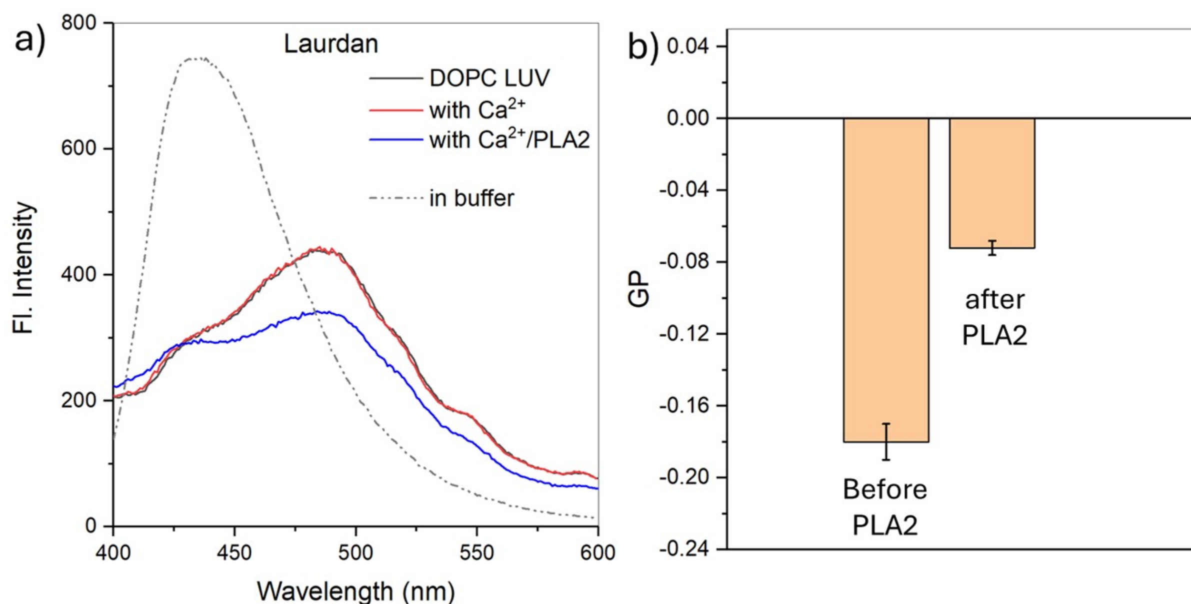

Fig. S4 a) Fluorescence spectra of 10 mM Laurdan in Tris buffer (dotted line), in DOPC LUV in the absence (solid black line) and in the presence (solid black line) of 5 mM Ca^{2+}. Blue solid line in the panel a represents the Laurdan Fluorescence in DOPC LUV in presence of both Ca^{2+} and 5 mM PLA2. b) Calculated GP values for DOPC before and after PLA2 in presence of Ca^{2+}.

Experimental condition of Laurdan fluorescence spectroscopy

Fluorescence measurements were performed with a Cary fluorimeter (Varian). The excitation and emission band-pass were set at 10 nm. Spectra were recorded 30 min after addition of Ca^{2+} and PLA2 to Laurdan containing DOPC LUVs using a 1 cm path length quartz cuvette, at 22±1 °C. Laurdan emission spectra were recorded from 400 to 600 nm using a 365 nm excitation wavelength in 10 mM Tris buffer (pH 7.4). The excitation generalized polarization (GP) was calculated as;

$$GP = \frac{I_{430} - I_{490}}{I_{430} + I_{490}} \qquad \text{(S1)}$$

where I_{430} and I_{490} are the fluorescence intensities at the maximum emission wavelength in the ordered (430 nm) and disordered (490 nm) phases.

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

Please do not adjust margins

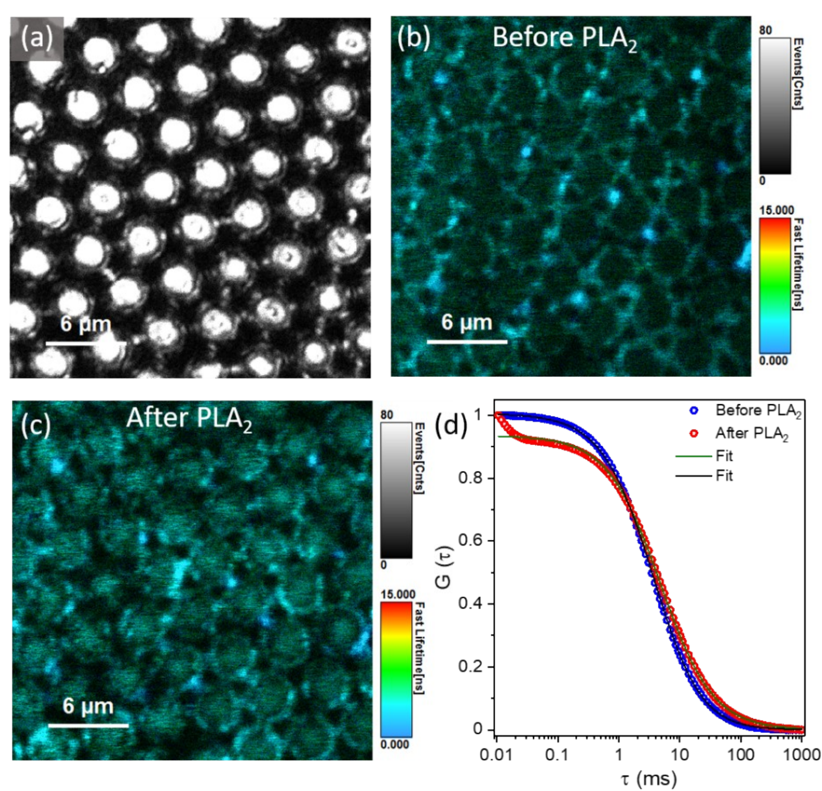

Fig. S5 Representative reflectance image of DOPC MSLBs with scale bar 6 µm and fluorescence lifetime images (FLIM) of the same DOPC MSLB labelled with 0.01 mol% DOPE-ATTO655 (upper leaflet) (b) before, (c) after the addition of 5 µM PLA_2 in the absence of Ca^{2+} with 9 µm scale bar. (d) Representative FLCS autocorrelation functions of DOPC MSLB labelled with 0.01 mol% DOPE-ATTO655 (upper leaflet) before (open blue), and after the addition of 5 µM PLA_2 (open red) in the absence of Ca^{2+}. FLCS was measured over 40-50 cavities and the average is shown. The solid lines are the 2D diffusion fit using equation 1. All measurements were carried out under Tris-HCl buffer of pH 7.4.

Please do not adjust margins

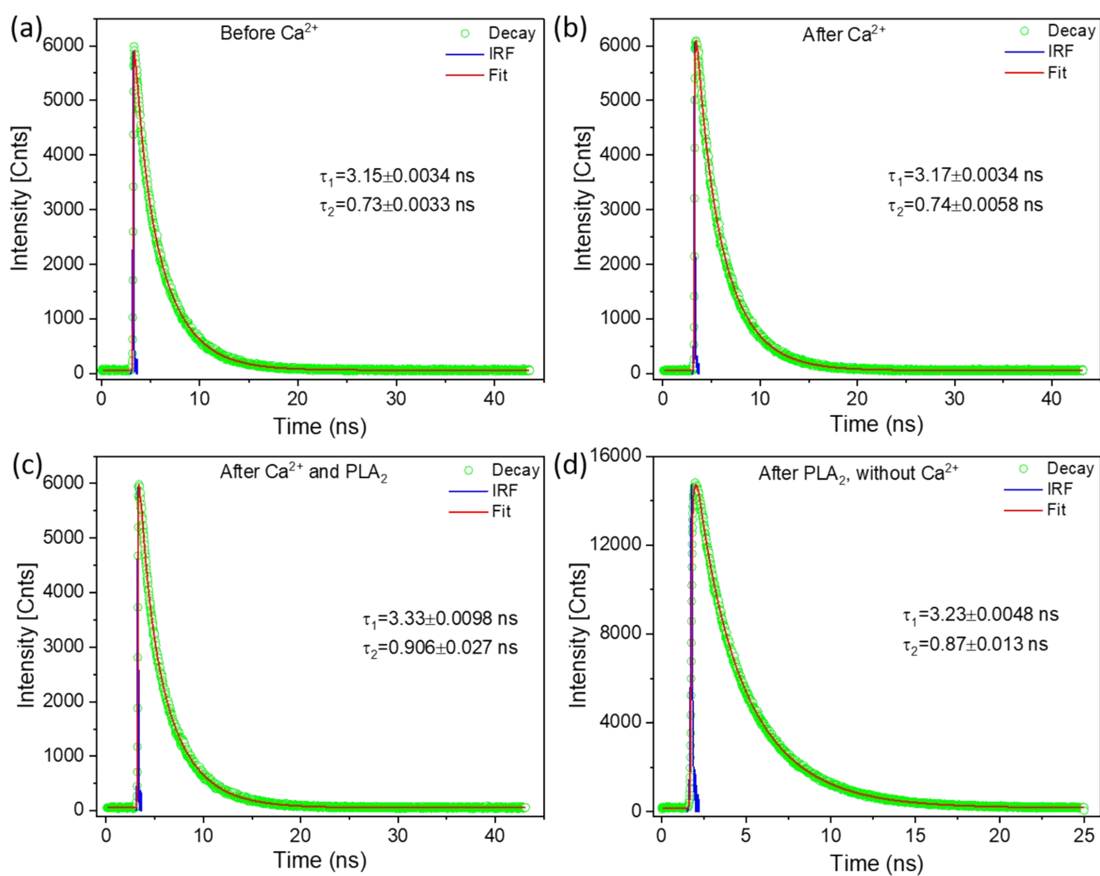

Fig. S6 Emission decays (green) extracted from FLIM of DOPC MSLBs labelled with 0.01 mol% DOPE-ATTO655 (upper leaflet) (a) before, (b) after the addition of 5 mM Ca^{2+}, (c) after the addition of 5 µM PLA_2 in presence of Ca^{2+}, and (d) after the addition of 5 µM PLA_2 in the absence of Ca^{2+}. All measurements were carried out in Tris-HCl buffer at pH 7.4. The instrument response function (IRF) is shown in blue and the bi-exponential fit is given in red. The lifetimes are given inside each figure.

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

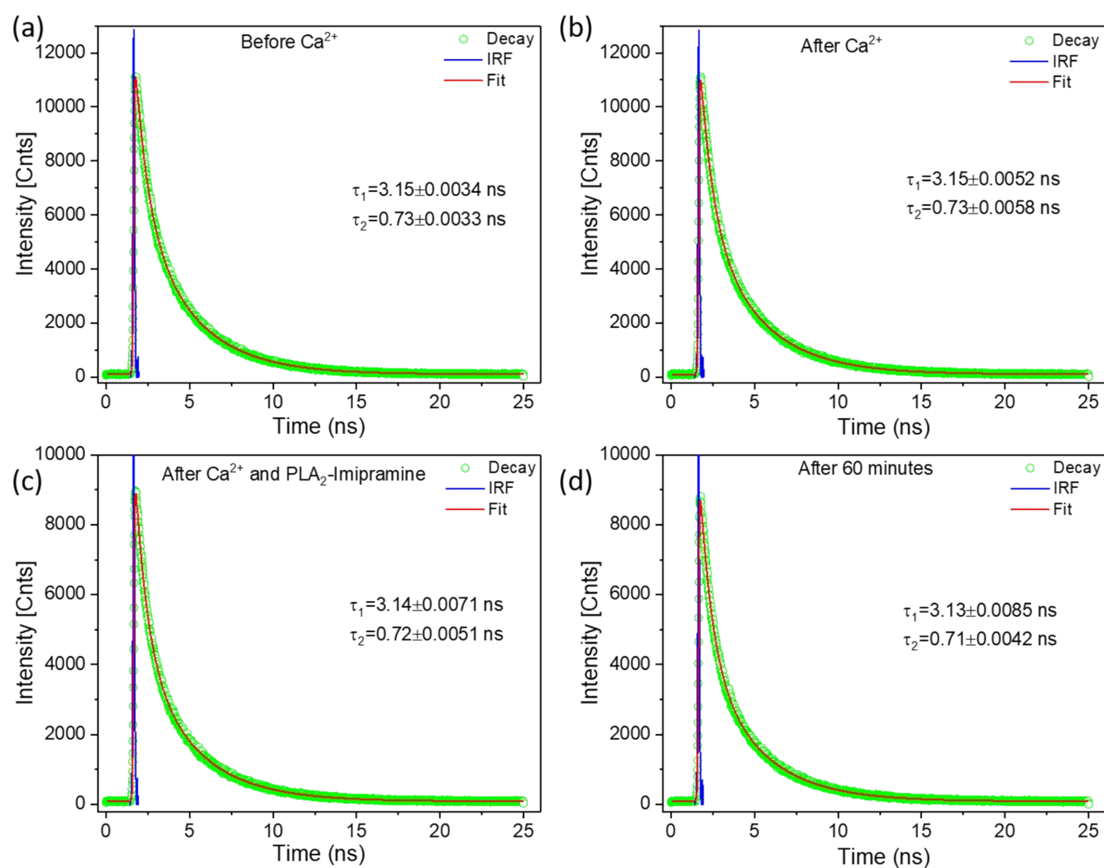

Fig. S7 Emission decays (green) extracted from FLIM of DOPC MSLBs labelled with 0.01 mol% DOPE-ATTO655 (upper leaflet) (a) before, (b) after the addition of 5 mM Ca^{2+}, (c) 10 minutes after the addition of 5 µM PLA_2 pre-incubated with 20 µM imipramine in presence of Ca^{2+}, and (d) after 60 minutes. All measurements were carried out in Tris-HCl buffer at pH 7.4. The instrument response function (IRF) is shown in blue, and the bi-exponential fit is given in red. The lifetimes are given inside each figure.

Please do not adjust margins

Please do not adjust margins

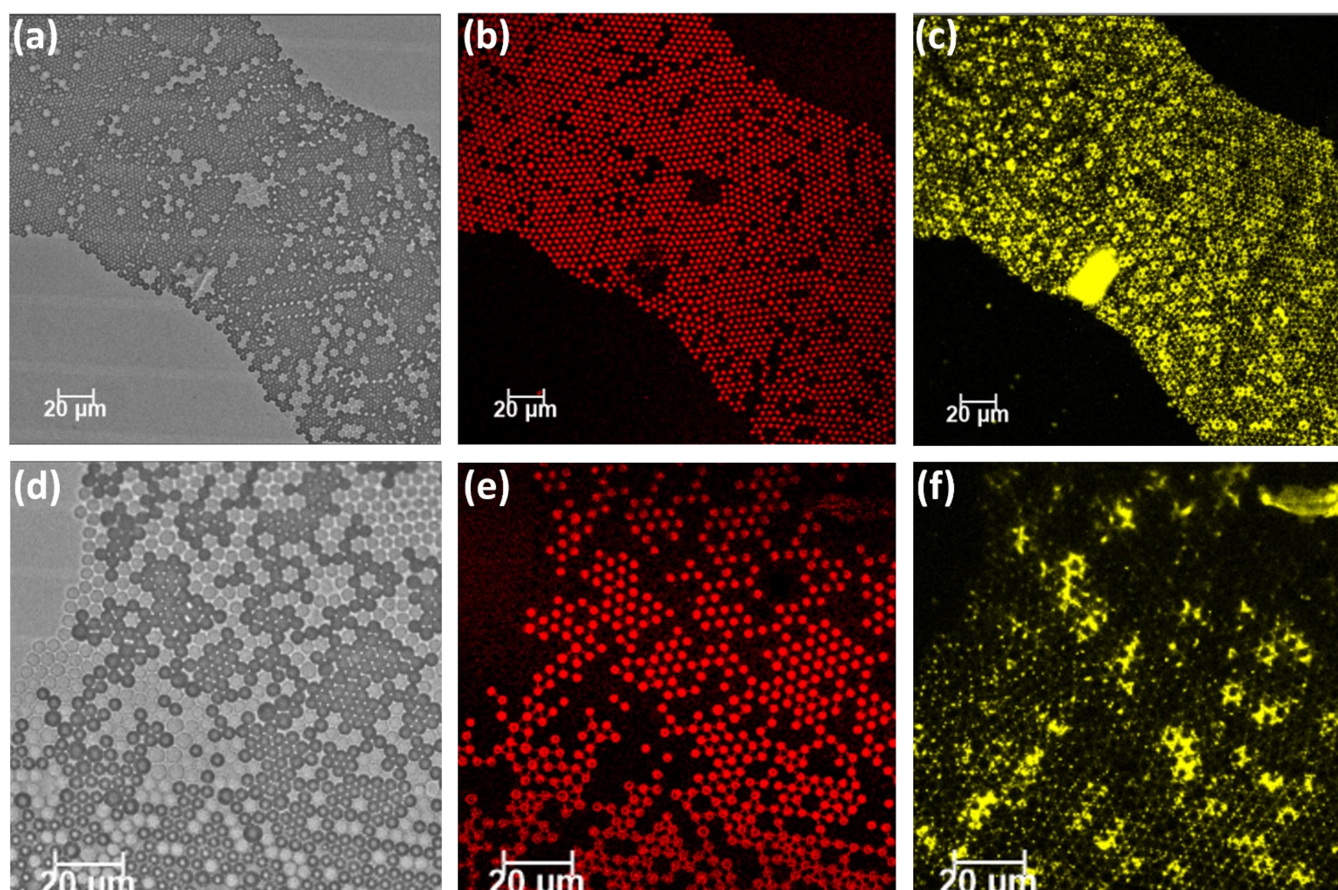

Fig. S8 (a and d) Reflectance images of DOPC MSLB spanned over pyranine containing buffer filled cavities on PDMS substrate. The dark grey circular features are the aqueous filled cavities, and the occasional light grey circles are unfilled cavities. Confocal fluorescence images of DOPE-ATTO655 labelled DOPC MSLB (red) across the same region as the reflectance images, showing the bilayer spanned over pyranine/buffer filled cavities (b) before and (e) after the addition of 5 µM PLA_2 in the presence of 5 mM Ca^{2+}. Confocal fluorescence images of the pyranine containing buffer filled (yellow) cavities below DOPC MSLB (c) before and (f) after the addition of 5 µM PLA_2 in presence of 5 mM Ca^{2+}. For DOPE-ATTO655 $\lambda_{ex}/\lambda_{em}$= 633/640-680 nm and for pyranine $\lambda_{ex}/\lambda_{em}$= 405/440-560 nm. In each panel, the scale bar is 20 µm.

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

Please do not adjust margins

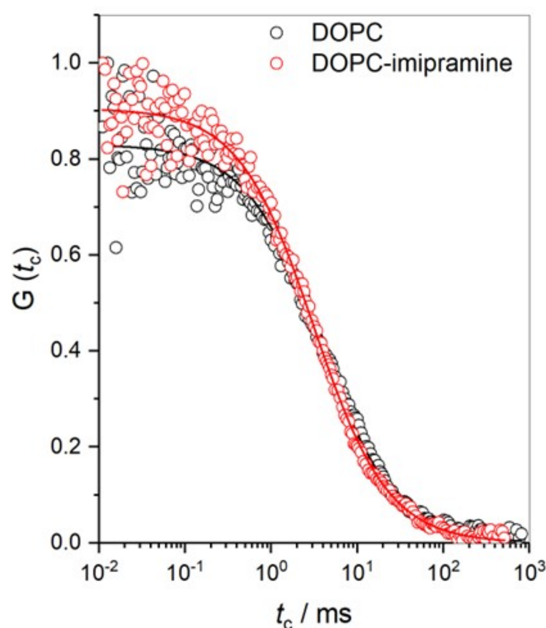

Figure S9. FLCS Traces for DOPC MSLB in the presence and absence of 20 µM imipramine inhibitor. Impipramine causes a modest but significant increase in membrane diffusivity.

References:

1 H. Basit, V. Gaul, S. Maher, R. J. Forster and T. E. Keyes, *Analyst*, 2015, **140**, 3012–3018.

2 S. Maher, H. Basit, R. J. Forster and T. E. Keyes, *Bioelectrochemistry*, 2016, **112**, 16–23.

3 S. Ramadurai, N. K. Sarangi, S. Maher, N. MacConnell, A. M. Bond, D. McDaid, D. Flynn and T. E. Keyes, *Langmuir*, 2019, **35**, 8095–8109.

4 G. B. Berselli, N. K. Sarangi, S. Ramadurai, P. V. Murphy and T. E. Keyes, *ACS Appl. Bio Mater.*, 2019, **2**, 3404–3417.

Please do not adjust margins