Supporting Information for

Engineering Anthraimidazoledione Charge-Transfer Fluorophores for Phospholipid Detection through Self-Assembly-Induced Emission

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Experimental Section

Materials and Methods

All the chemicals (reagents, solvents, etc) were purchased from the best-known local suppliers. The lipid molecules, such as DOPC (1,2-dioleoyl-*sn*-glycero-4-phosphocholine), TOCL (1,1′,2,2′-tetraoleoyl cardiolipin), DOPS (1,2-dioleoyl-*sn*-glycero-3-phospho-l-serine (sodium salt)), DPPE (1,2-dipalmitoleoyl-*sn*-glycero-3-phosphoethanolamine), and soy PI (l-α-phosphatidylinositol (soy) (sodium salt)), were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). SM (*N*-hexanoyl-d-sphingomyelin) and Acridine orange 10-nonyl bromide (nonyl acridine orange) were purchased from Sigma-Aldrich

Preparation of vesicles

Chloroform or chloroform/methanol solutions of the lipid(s) were taken in the required amount, and a THF stock solution of the probe, compound 1, was added to it to get the desired probe/lipid ratio. In case of mixed lipid aggregates, individual lipid solutions were mixed to get the desired lipid composition. Lipid films doped with the probe were made in a Wheaton glass vial by evaporating the chloroform (or chloroform/methanol) solution of the lipid under a steady stream of dry N_2. Traces of organic solvents were removed by keeping these films under vacuum overnight. Lipid films were then hydrated at 4°C for ~12 hrs by adding the required amount of 50 mM PBS buffer (pH 7.0). Lipid suspensions were then freeze-thawed with intermittent vortexing. This, on further sonication in a bath-type sonicator for 30~min at 50- 60°C afforded unilamellar vesicular suspensions.

Dynamic Light Scattering Studies

DLS measurements were performed by using a Malvern Zetasizer NanoZS particle sizer (Malvern Instruments Inc., MA) instrument. Samples were prepared and examined under dust-free conditions. Mean hydrodynamic diameters reported were obtained from Gaussian analysis of the intensity-weighted particle size distributions.

Fluorometric analysis (Steady state/ Time-dependent)

The fluorescence experiments were performed in FluoroLog-TM (Horiba Scientific). The slit width for the fluorescence experiment was kept at 5 nm (excitation) and 5 nm (emission) and the excitation wavelength was set at 505 nm.

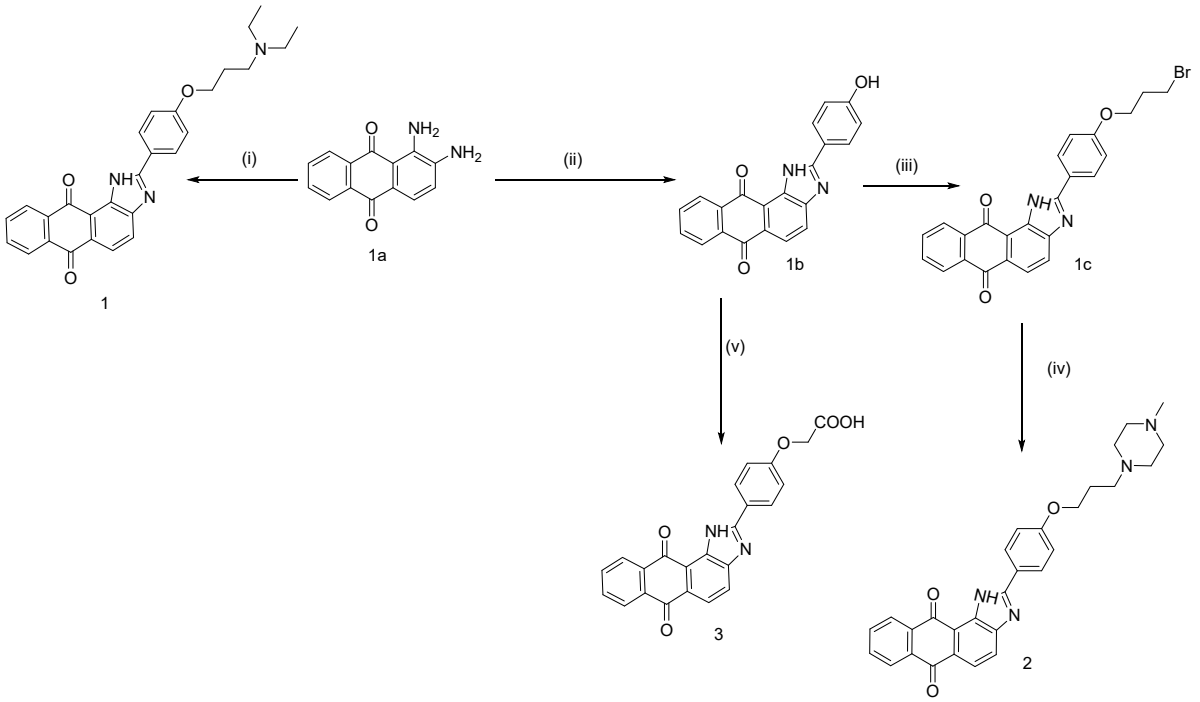
Fluorescence lifetime measurements were performed using Horiba Delta flex Modular fluorescence lifetime system with following instrumental parameters: 510 nm NanoLED excitation source with an instrument response function of about 165 ps, and peak preset 1000 counts.

Scanning Electron Microscopy (SEM)

Samples for SEM were drop casted on a silicon wafer with the required concentrations, and the solvent was allowed to evaporate overnight. The silicon wafer was then sputter-coated using a Leica Ultra Microtome EM UC7, and the stubs were loaded into an FEI Apreo LoVac to obtain images at 1 μm magnification.

Synthesis of Probe Molecules

Probes 1, 2, and 3 were synthesized following the reported procedure.[1] General synthesis scheme and conditions are given below.



Reagents, conditions: (i) 4-($Et_2N(CH_2)_3O$)-C_6H_5CHO, dry $PhNO_2$, 130 °C, 16 h. (ii) 4-OH-C_6H_5CHO, dry $PhNO_2$, 120 °C,20 h. (iii) $Br(CH_2)_3Br$, K_2CO_3, dry acetone, reflux, 12 h. (iv) N-methyl Piperazine, K_2CO_3, dry acetone, reflux, 12-15 h. (v) $Cl(CH_2COOCH_2CH_3)$, K_2CO_3, KI, DMF, 75 °C, 12 h (vi) 7.2 % KOH, EtOH, rt, 1.5 h

Reference:

1. Chaudhuri, P., Majumder, H. K., & Bhattacharya, S. *J. Med. Chem.* **2007**, 50, 2536-2540.

Additional Spectroscopic Data

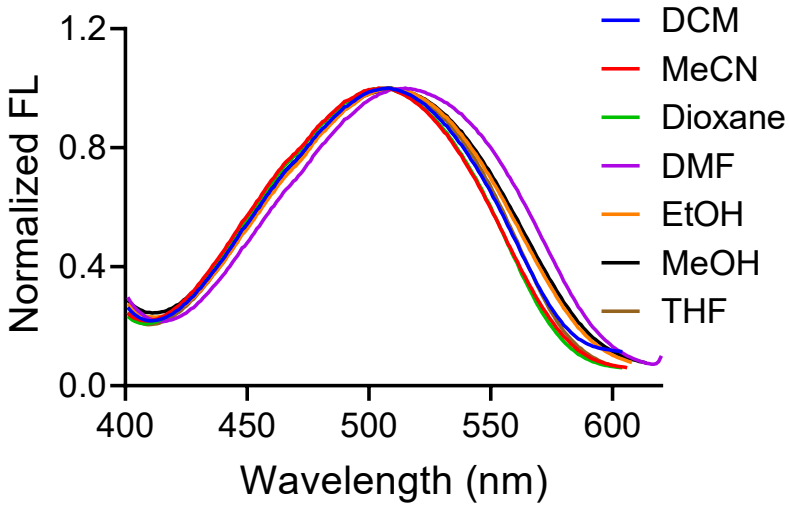


Figure S1. Fluorescence excitation spectra of 1 (10 μM) in different organic solvents.

Solvents	Average Lifetime (ns)	Exponentiality	τ_1 (ns)	τ_2 (ns)
in MeCN	0.54	Bi-exponential	0.156529	0.609415
in DCM	0.63	Bi-exponential	0.200664	0.678323
in Dioxane	0.56	Bi-exponential	0.104793	0.635863
in DMF	0.43	Mono-exponential	0.434784	
in EtOH	0.41	Mono-exponential	0.410587	
in MeOH	0.37	Mono-exponential	0.374382	
in THF	0.569	Bi-exponential	0.166627	0.622289
in Water	0.217	Mono-exponential	0.217968	

Table S1. Average fluorescence lifetime of compound 1 calculated in different organic solvents and water medium.

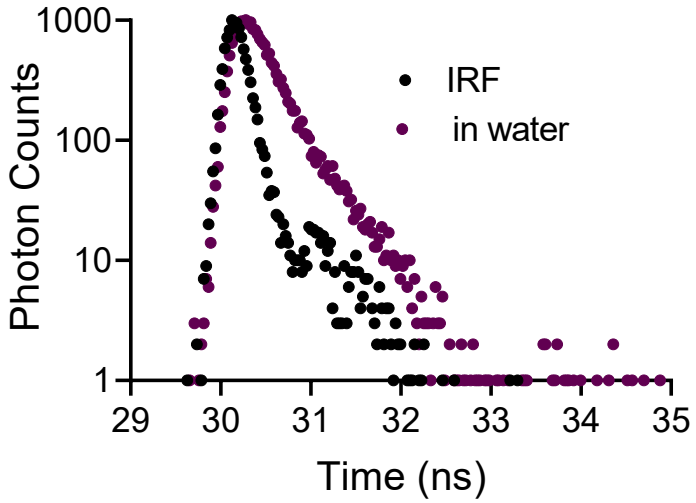


Figure S2. Fluorescence decay profile of 1 (10 μM, λex = 505 nm) at 654 nm in PBS buffer at pH 7.0.

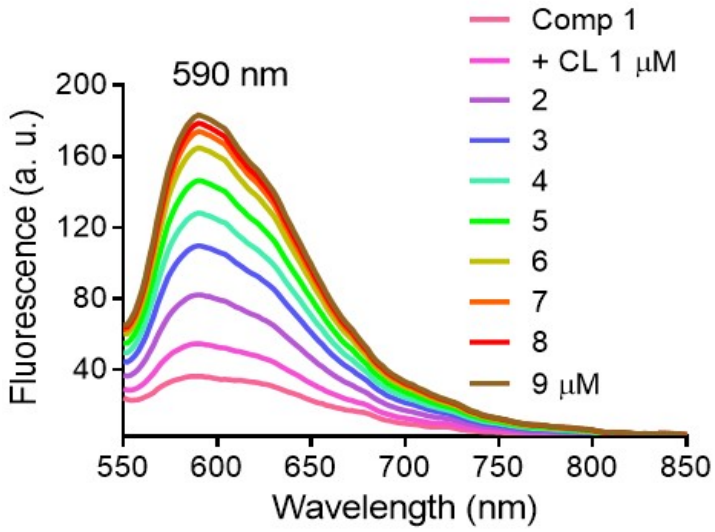


Figure S3. Fluorescence titration of 1 (10 μM, λex = 505 nm) with CL (0 – 9 μM)-doped DPPC (1 mM) vesicles in PBS buffer (pH 7.0).

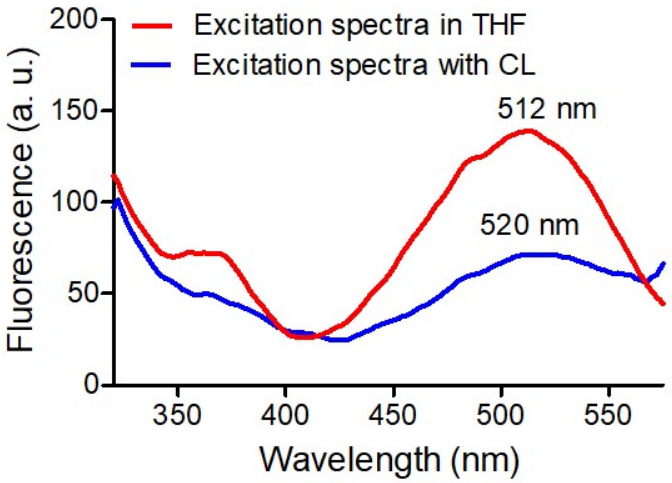


Figure S4. Fluorescence excitation spectra of 1 (10 μM, λex = 505 nm) with CL (10 μM)-doped DPPC (1 mM) vesicles in PBS buffer (pH 7.0) and in THF medium.

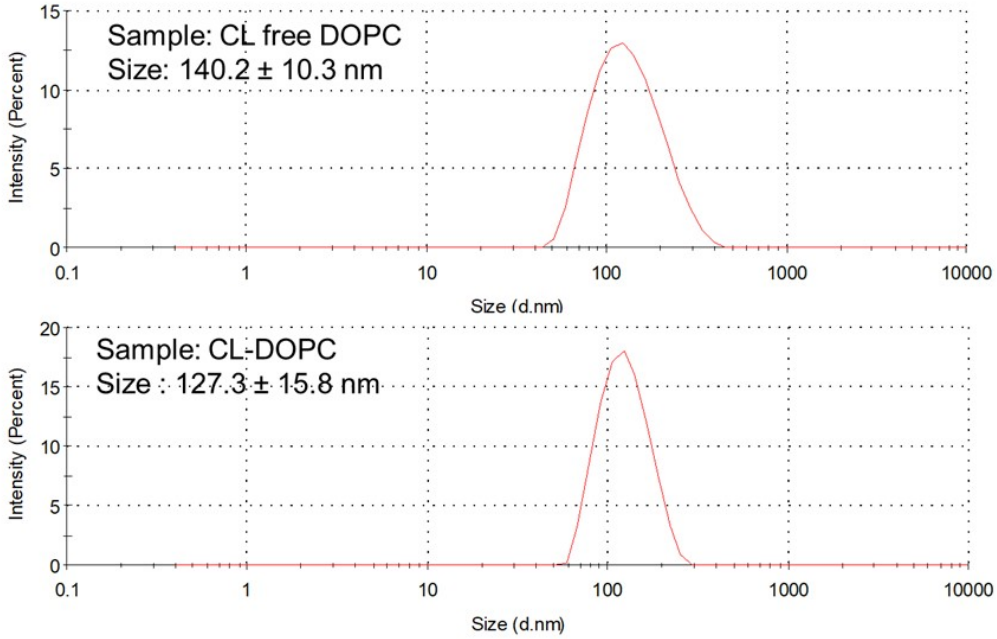


Figure S5. Size analysis of CL-containing (down) and (B) CL-free (top) DOPC vesicles in presence comp 1 in PBS buffer at pH 7.0.