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Supporting Information for

A Unique Self assembly driven sensing of lipid bilayer: ratiometric probing of Vesicle to Micelle Transition

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Materials and methods

All reagents, starting materials, and silica gel for TLC and column chromatography were obtained from the best known commercial sources. Solvents were distilled and dried prior to use. The lipids molecules (DMPC, DOPC, and DOPG) were bought from Avanti Polar Lipids. The starting materials and surfactants (CTAB, Tween20) were purchased from Sigma-Aldrich and were used without further purification. FTIR spectra were recorded on a Perkin-Elmer FT-IR Spectrum BX system and were reported in wave numbers (cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker Advance DRX 400 spectrometer operating at 400 and 100 MHz for ^{1}H and ^{13}C NMR spectroscopy, respectively. Chemical shifts were reported in ppm downfield from the internal standard, tetramethylsilane (TMS). Mass spectra were recorded on Micro mass Q-TOF Micro TM spectrometer.

UV-visible and fluorescence Experiment

The UV−vis and fluorescence spectra were recorded on a Shimadzu model 2100 UV-vis spectrometer and Cary Eclipse spectrofluorimeter respectively. In the emission experiments, the slit widths used for recording various emission and excitation spectra were 10 nm (excitation) and 5 or 10 nm (emission).

Studies in micelles and phospholipid bilayer

Pytpy fluorescence in micelles was measured in the following way. The micelle forming surfactant was taken a glass vial in appropriate quantity and Tris. HCl buffer (pH 7.5) was added to the surfactant. The micellar 'solution' was obtained after mixing for ~5 minutes followed by brief sonication (~ 2 minutes). The solutions were equilibrated for about 30 minutes before adding highly concentrated THF stock solution of the probe Pytpy. The co-solvent THF was present in very small amounts (< 0.5 %) and did not affect the fluorescence behavior.

The unilamellar vesicles or related membranous aggregates doped with the fluorescence probe were prepared as reported previously. Chloroform or chloroform/methanol (in a few cases, depending on the solubility) solution of the lipid(s) was taken in the required amount and a THF stock solution of the probe, Pytpy was added to it to get the desired probe/lipid ratio. The concentration of the probe was 1.2×10^{-6} while the lipid concentration was 10^{-3} M giving a probe to lipid ratio ~ 1/1000. 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 Wheaton glass vial by evaporating the chloroform 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 the adding required amount of 50 mM Tris.HCl buffer (pH 7.5). Lipid suspensions were then freeze-thawed between 0 °C and 60 °C with

intermittent vortexing. This on further sonication in a bath type sonicator for 30~min at 50-60°C afforded unilamellar vesicular suspensions as evidenced from electron microscopy.

Fluorescence Decay Experiment

Fluorescence lifetime values were measured by using a time-correlated single photon counting fluorimeter (Horiba Jobin Yvon). The system was excited with nano LED of Horiba - Jobin Yvon with pulse duration of 1.2 ns. Average fluorescence lifetimes (τ_{av}) for the exponential iterative fitting were calculated from the decay times (τ_i) and the relative amplitudes (a_i) using the following relation

$\tau_{av}=(a_1\tau_1^2+a_2\tau_2^2+a_3\tau_3^2)/(a_1\tau_1+a_2\tau_2+a_3\tau_3)$

Where a1, a2 and a3 are the relative amplitudes and τ1, τ2, and τ3 are the lifetime values, respectively. For data fitting, a DAS6 analysis software version 6.2 was used.

Transmission Electron Microscopy

Samples for TEM were prepared under dust-free conditions on a Formvar-coated, 400 mesh copper grid, which was allowed to stand for 15 min. The grid was then air-dried and the last traces of solvent were removed under high vacuum. Changes in morphologies were observed on a transmission electron microscope (TECNAI T20) operating at an acceleration voltage (direct-current (DC) voltage) of 100 keV. Micrographs were recorded at a magnification of 10000–80000V.

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.

Fluorescence anisotropy measurements

$$r = \frac{I_{II}-GI_{\perp}}{I_{II}+2GI_{\perp}}$$

Where, I_7 and $I_{\perp}$ are the polarized components parallel and perpendicular to the direction of polarization of the incident light. G or the 'grating' factor was calculated as;

$G = I_{HV}/I_{HH}$

'G factor' is the ratio of the vertical (V) and horizontal (H) emission components for horizontally polarized excitation light.

Methods	Working principle	Advantages/disadvantages of the methods
Turbidity measurement	Increase in turbidity during micelle to vesicle transition	clear, dust-free sample is needed to avoid false positive result. This procedure is less sensitive, difficult to determine small amount of vesicle in micelle-vesicle mixture
Conductivity measurement	Increase in conductivity during micelle to vesicle transition	Costly instrumental set up required. Also conductivity depends on the concentration of free ion present in the solution.
Cryogenic TEM	Increase in particle size (also presence of bilayer can be visualized) during micelle to vesicle transition	Costly instrumental set up required, high maintenance cost, need trained personnel. Moreover, size of the particles will also depend on sample preparation procedure and nature of the surfactant systems
Dynamic Light Scattering	Increase in hydrodynamic diameter during micelle to vesicle transition	Costly instrumental set up required, clear, dust-free sample preparation is important for analysis. Confusion may arise if the sample contains vesicles or micelles of different size
Rheological Investigations	Change in viscoelastic property of the system	Costly instrumental set up required, viscoelasticity of the system also depends on the nature of the surfactants involved.
NMR self-diffusion and relaxation	NMR relaxation provides information about dynamics of probe molecule on bilayer surface, while self-diffusion reveals presence of enclosed water pocket	Need costly instrumental set up and ^{2}H-labelled surfactant system, dynamics of the probe might also be affected by its position and microenvironment. On the other hand, self-diffusion technique can't distinguish between vesicles and reversed micelles
Fluorescence probing	Use of micropolarity, hydration or microviscosity sensitive probes	The response of the probe depends on the change in the microenvironment, which can also happen due to many reasons other than micelle to vesicle transition. These are not direct-detection methods.
Present method*	Ratiometric change in emission color (blue to cyan during micelle to vesicle transition)	Exclusive nanoassembly formation on the bilayer surface, thus this method can directly report presence of bilayer membrane in mixture (micelle to vesicle transition). No indirect assumption by monitoring changes in microenvironment.

Table S1. A Comparison table summarizing the advantages and disadvantages of different methods known to study the phase transition process

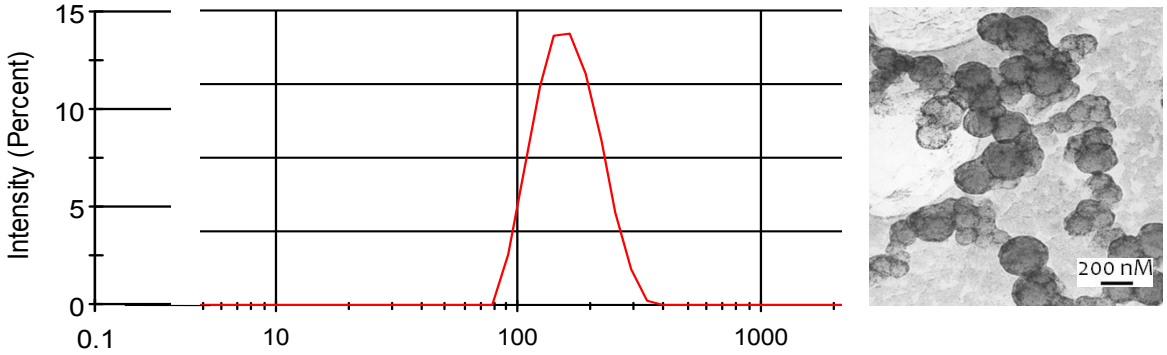


Figure S1. Hydrodynamic diameter (D_h) of Pytpy (10 μM) in water estimated using dynamic light scattering experiment. Right hand side figure shows TEM images of Pytpy (10 μM) in water.

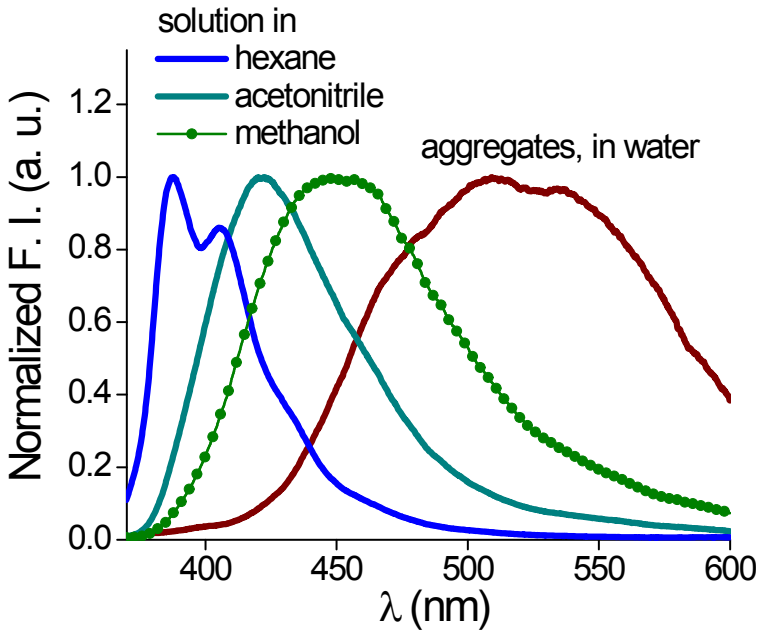


Figure S2. Normalized emission spectra of Pytpy (10 μM, λ_{ex}= 350 nm) in water and its comparison with emission spectra of Pytpy in organic solvents.

	Wavelength (nm)					
	410	430	467	480	500	535
DOPC	0.173	0.147	0.134	0.134	0.128	0.131
Tris.HCl	-	-	0.016	0.015	0.02	0.022

Figure S3. Anisotropy (r) values for Pytpy in PC lipid bilayers and in buffer at various wavelengths.

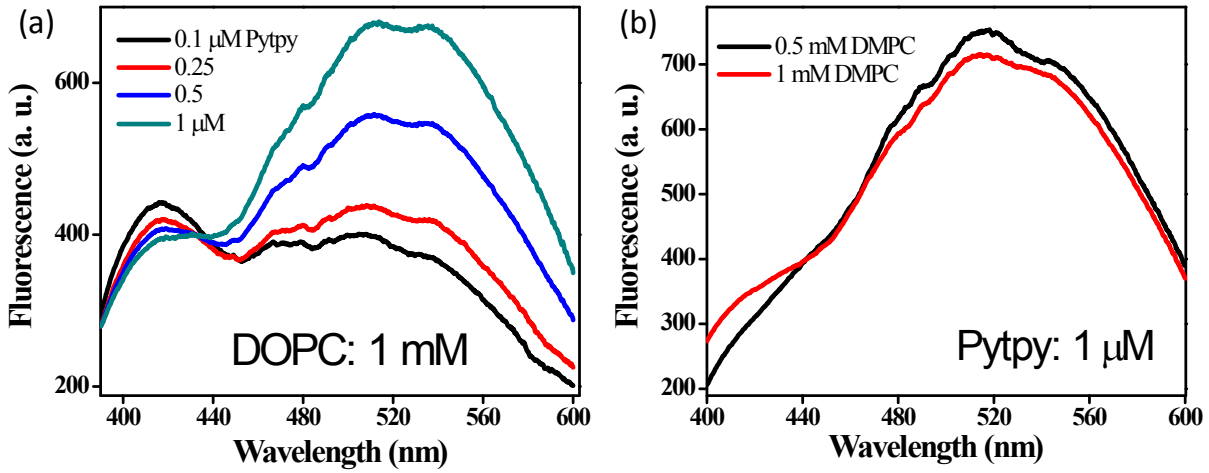


Figure S4. (a) Emission spectra of Pybpa (λex = 350 nm) at different concentrations in DMPC bilayer (1 mM DMPC). (b) Effect of DMPC concentration (1 and 2 mM) on the emission spectra of Pybpa (1 μM, λex = 350 nm) in PC bilayer.

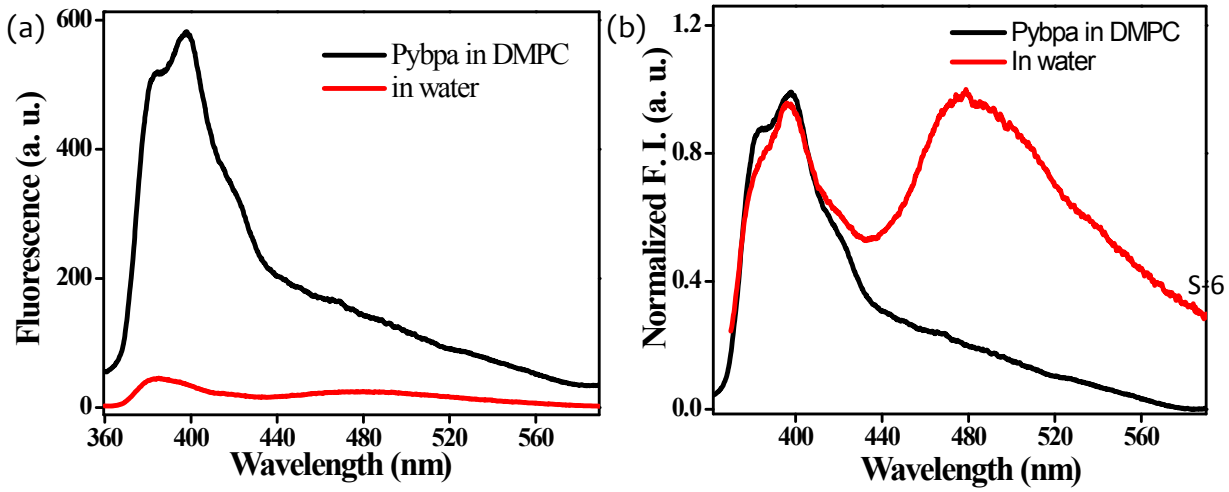


Figure S5. (a) Emission spectra of Pybpa (λex = 350 nm, 10 μM) in water and in DMPC bilayer (1 μM in 1 mM DMPC). (b) Normalized emission spectra of Pybpa (λex = 350 nm, 10 μM) in water and in DMPC bilayer (1 μM in 1 mM DMPC).

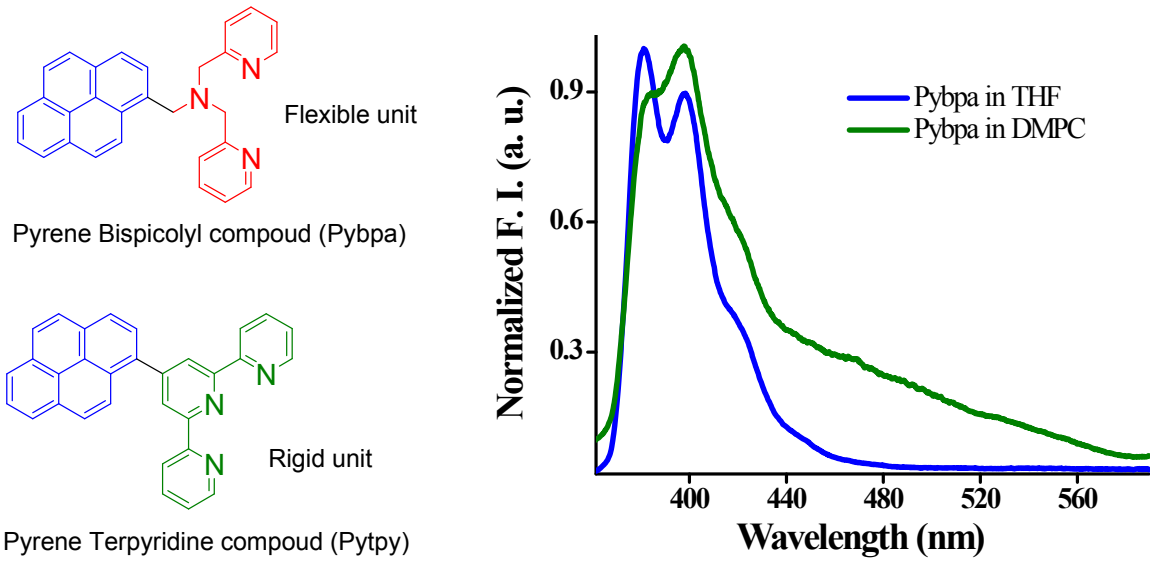


Figure S6. Normalized emission spectra of Pybpa (λex = 350 nm, 10 μM) in THF and in DMPC bilayer (1 μM in 1 mM DMPC) [structures of both Pybpa and Pytpy have been shown on left side].

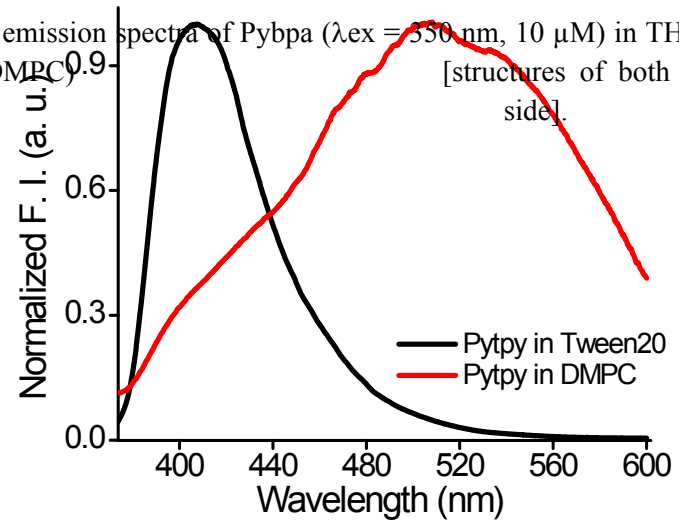


Figure S7. Normalized emission spectra of Pytpy (λex = 350 nm) in DMPC bilayer (1 μM in 1 mM DMPC) and Tween20 (1 μM in 0.06 mM Tween20) micelle medium

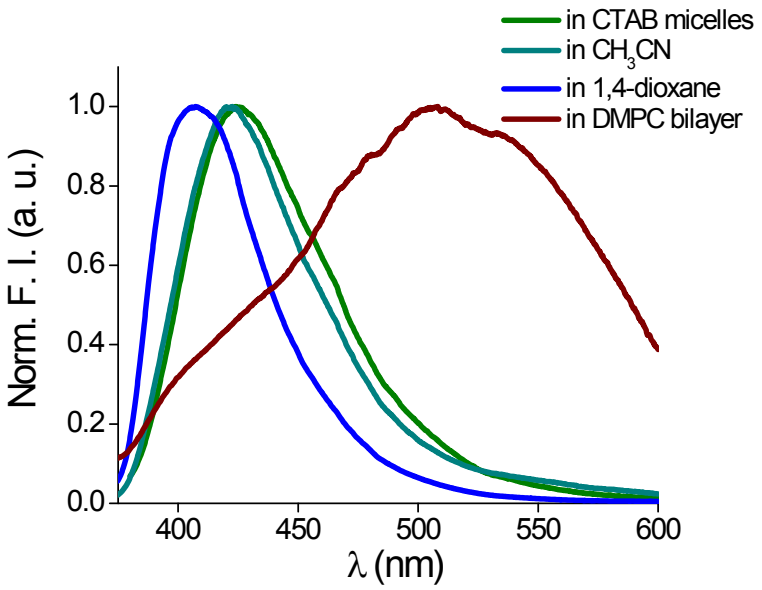


Figure S8. Normalized emission spectra of Pytpy (λex = 350 nm) in CH_3CN, 1,4-Dioxane (10 μM) and in DMPC bilayer (1 μM in 1 mM DMPC) and CTAB micelle (1 μM in 10 mM CTAB).

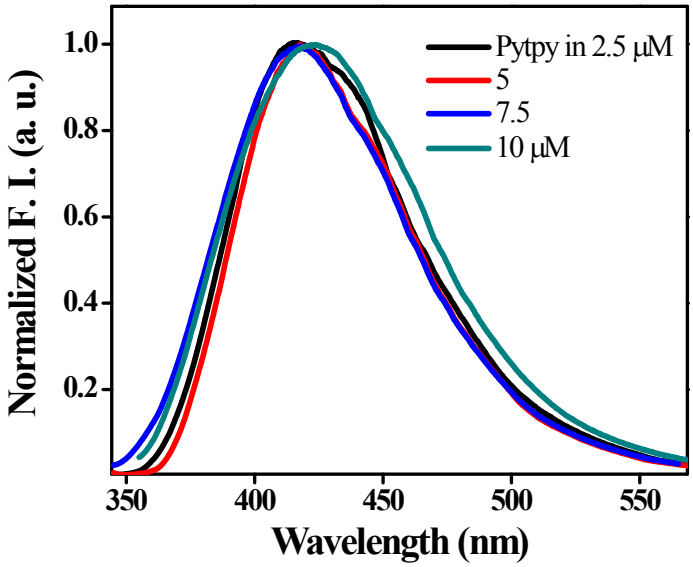
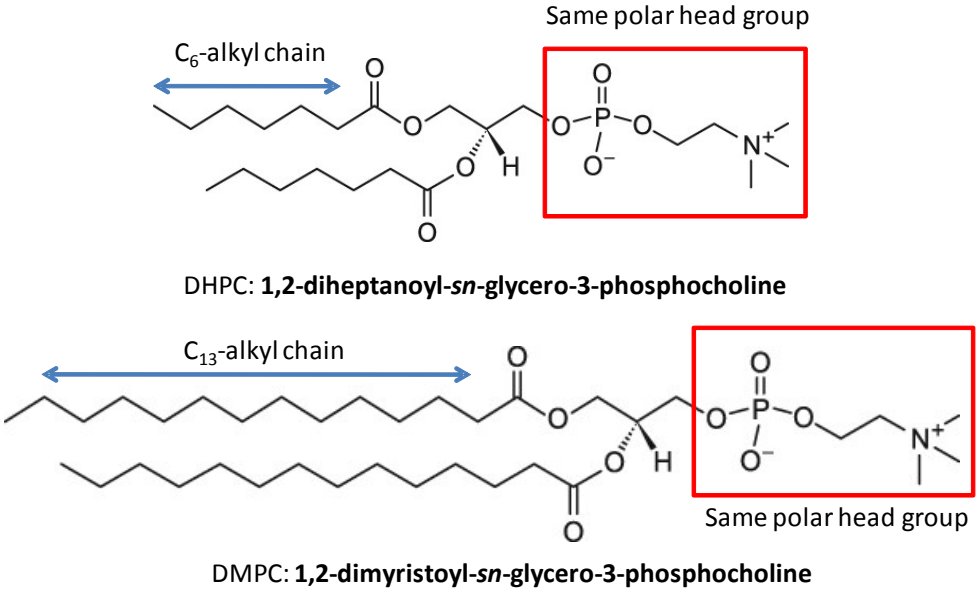


Figure S9. Normalized emission spectra of Pytpy (λex = 350 nm) at different concentrations in CTAB (10 mM) micelle medium.



Scheme S1. Structures of DHPC (micelle-forming surfactant) and DMPC (vesicle-forming surfactant).

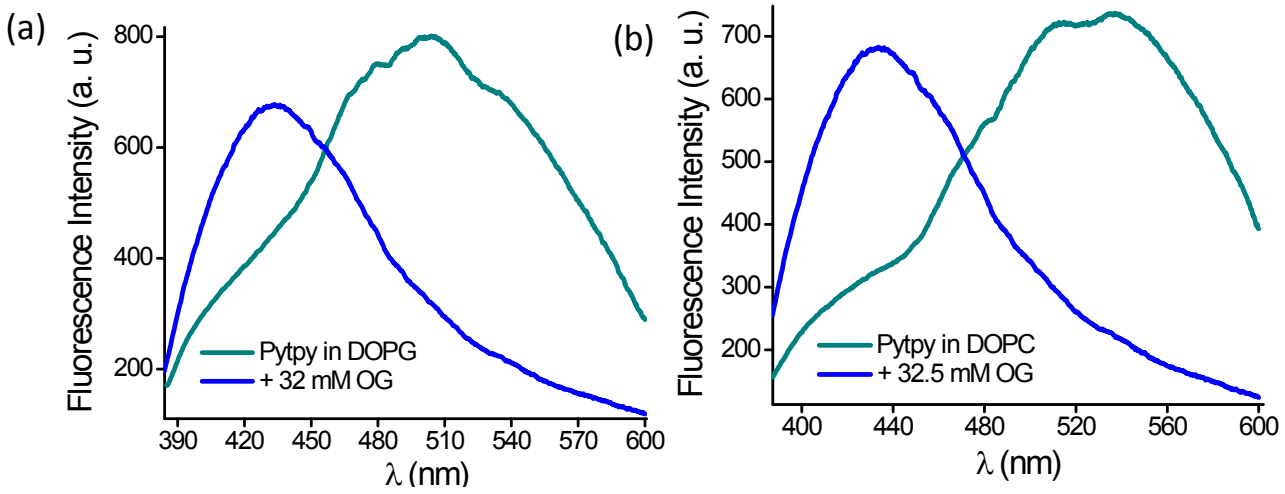


Figure S10. (a) Change in emission behavior of Pytpy (1 μM) doped in DOPG (1 mM) on the addition of OG (32 mM) at 30 °C. (b) Change in emission behavior of Pytpy (1 μM) doped in DOPC (1 mM) on the addition of OG (32 mM) at 30 °C.

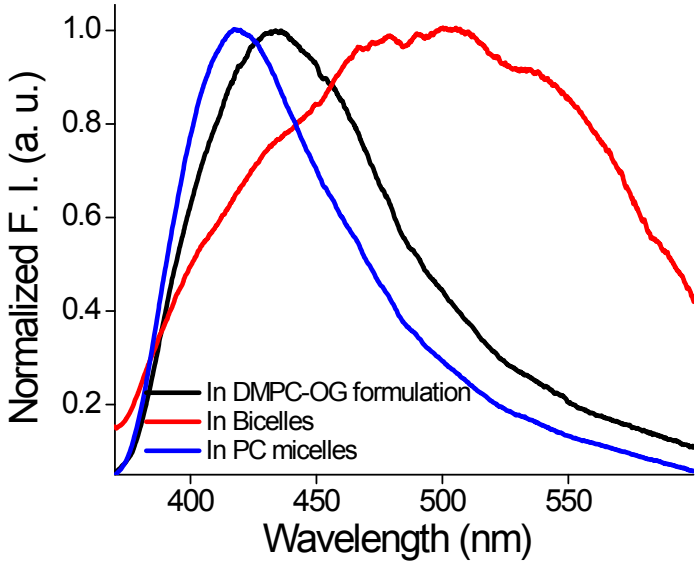


Figure S11. Normalized emission spectra of Pytpy in CTAB micelles and its comparison with Pytpy in bicelles (di-C_6 PC: 20 mM and DMPC: 10 mM) at different emission wavelengths (410 and 430 nm).

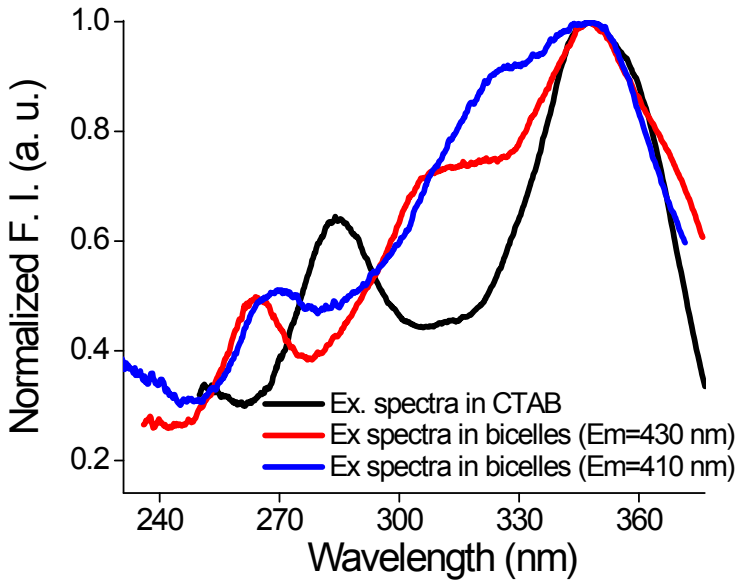


Figure S12. Excitation spectra of Pytpy in CTAB micelles and its comparison with Pytpy in bicelles (di-C_6 PC: 20 mM and DMPC: 10 mM) at different emission wavelengths (410 and 430 nm).

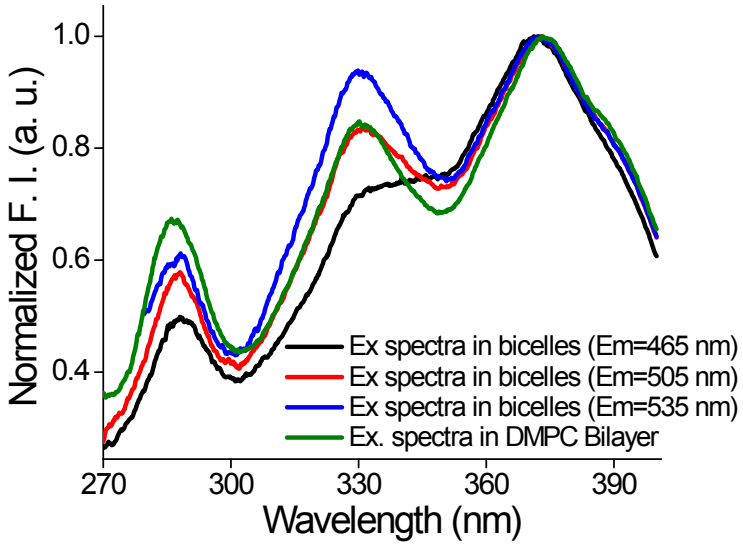


Figure S13. Excitation spectra of Pytpy in DMPC bilayer and its comparison with Pytpy in bicelles (di-C_6 PC: 20 mM and DMPC: 10 mM) at different emission wavelengths (465, 505 and 535 nm).