Supporting Information

Modulation of Membrane Fluidity to Control Interfacial Water Structure and Dynamics in Saturated and Unsaturated Phospholipid Vesicles

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Instrumentation:

Solvation Dynamics Studies: For solvation dynamics measurement, we have collected wavelength dependent emission decays of C-153. The both kinetics using up-conversion and TCSPC are taken from the blue end to red end of the emission spectra (see S4). Kinetics from up-conversion are de-convoluted (using LABVIEW) using a Gaussian-shaped function for instrument response function (IRF) and a three-exponential function given by following equation

$$I(\lambda,t) = \sum_i a_i(\lambda,t) exp\left(-t/\tau_i(\lambda)\right) \qquad (S1)$$

Where $a_i(\lambda)$ is contribution of corresponding $\tau_i(\lambda)$ decay times. Small changes to the width of this Gaussian have no significant effect on the fitted time constants. Using these parameter time-resolved emission spectra (TRES) is constructed by homebuilt MATLAB® programmes following the method described by Maroncelli et al.[28] The TRES (S(λ, t)), at a given time t, are obtained by a relative normalization of those fitted decays D(t, λ) to the steady-state spectrum, $S_0(\lambda)$:

$$S(\lambda,t) = \frac{D(\lambda,t)\, S_0(\lambda)}{\int_0^\infty D(\lambda,t)dt} \qquad (S2)$$

The time dependence of the spectral shifts and the shape of the TRES are used to determine the rates of relaxation and the nature of the relaxation process. All the TRES are fitted using a log-normal line shape function g(ν) defined by[1]

$$g(\upsilon) = g_0 \exp\left\{-ln(2)\left(\frac{ln\left[1+2b\left(\upsilon-\upsilon_p\right)/\Delta\right]}{b}\right)\right\} \qquad (S3)$$

Here g_0, b, ν_p, and Δ are the peak height, asymmetric parameter, peak frequency, and width parameter, respectively. The width parameter, Δ is related to FWHM (Γ) of this function and represented by the following equation

$$\Gamma = \Delta\frac{sinh(b)}{b} \qquad (S4)$$

The peak frequency has been obtained by fitting the spectral points to above equation. This calculated peak frequency is utilized to construct the decay of the solvent relaxation time, C(t). Correlation spectra, C(t) obtained from up-conversion measurement are then fitted using the following exponential function:

$$C(t) = \sum_{i=1}^{n} a_i exp\left({}^{t}/_{\tau_i}\right) \qquad (S5)$$

In the above-mentioned equations, τ_i denotes the solvation times and a_i represents their corresponding normalized contribution. i varies from 1 to 3 in up conversion measurement and in TCSPC measurement i varies from 1 to 2.

Fluorescence Polarization or Anisotropy Imaging (FPIM/FAIM): The same FLIM set up was used as the basis for the measurement of rotational dynamics of sulphorhaodamine at the interface of vesicle bilayer. Only change we made was at the detection path. We used a polarizing beam splitter at the detection path and signal was divided in a way that the horizontal polarization goes to the upper detector, and vertical polarization reflects down to the lower detector. But the contrast ratio is not perfect for two APD; there was difference in intensity of light in different channels for a symmetric fluorescent molecule. Therefore, we have corrected the detection efficiency of two APD placed orthogonal to each other at room temperature for 40X objective with 1.2 NA keeping same width of pinhole for parallel and perpendicular signal component. G factor calibration for anisotropy measurement was performed using Perylene. G is correction factor defined as the ratio of detection efficiency of our set up for the parallel and perpendicular signal components. The G value for our experimental set up is 0.79. The fluorescence images at parallel, perpendicular direction and their anisotropy images were generated with MATLAB software after background subtraction. The final anisotropy image was pressed to smooth by a uniform in built filter function using MATLAB. The background pixels were excluded from the 2D image matrix by threshold masking. The mean r(t) for each anisotropy image was calculated from the mean intensity-weighted pixel values. All the experimental data were collected at 298 K.

Supporting Figures:

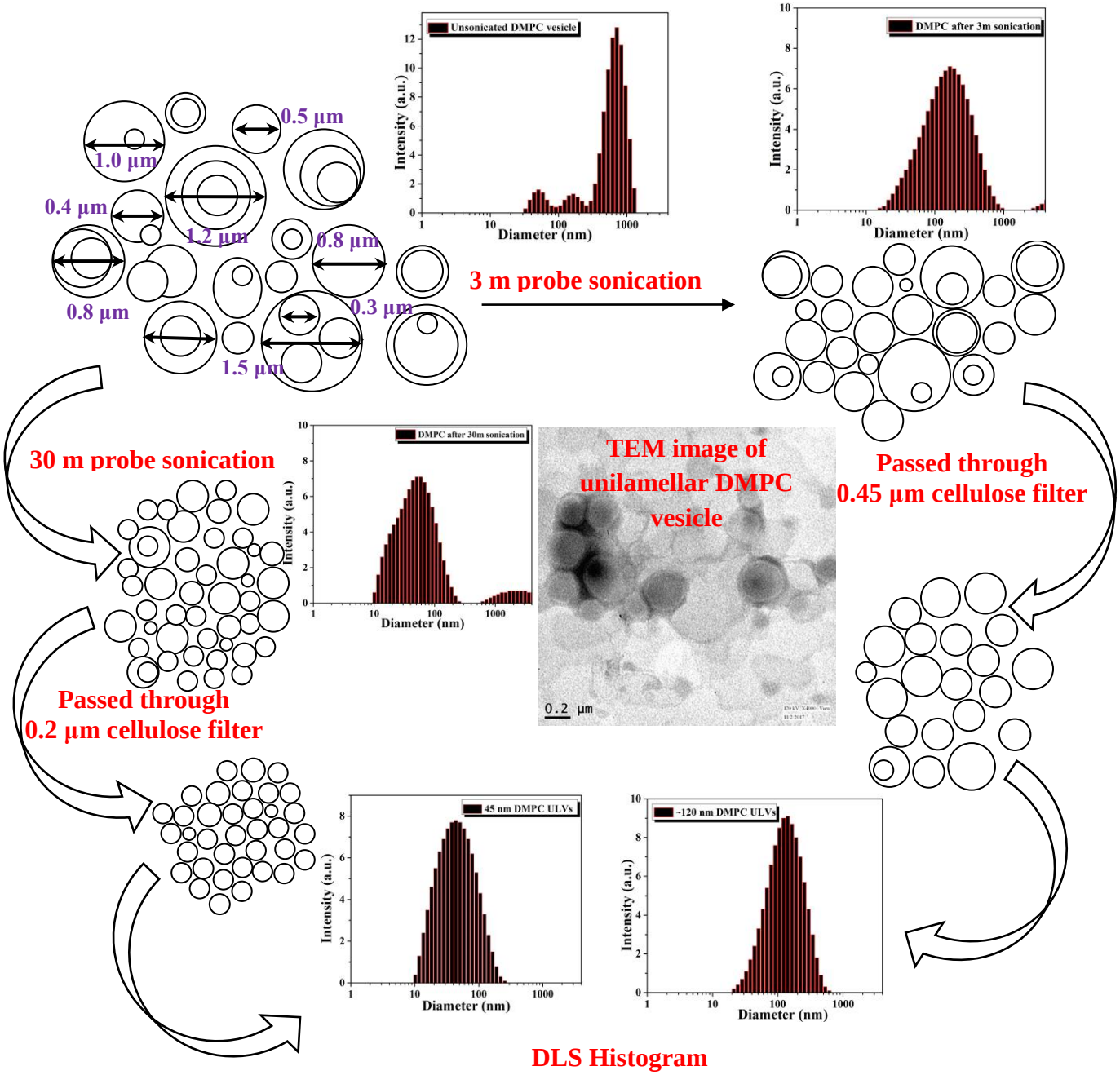

Figure S1: Sample preparation step by step

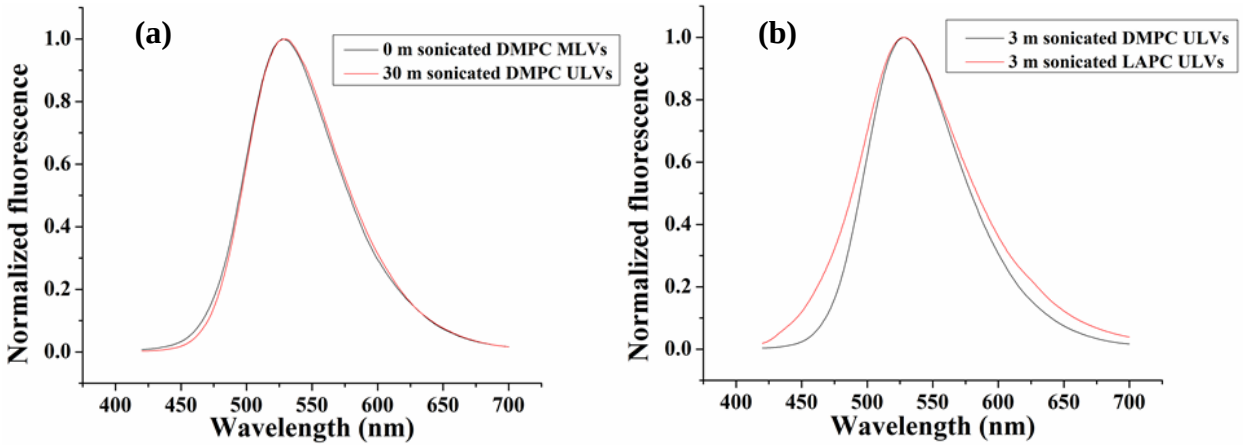

Figure S2: Normalized emission spectra of C-153 in various size of DMPC and LAPC vesicles

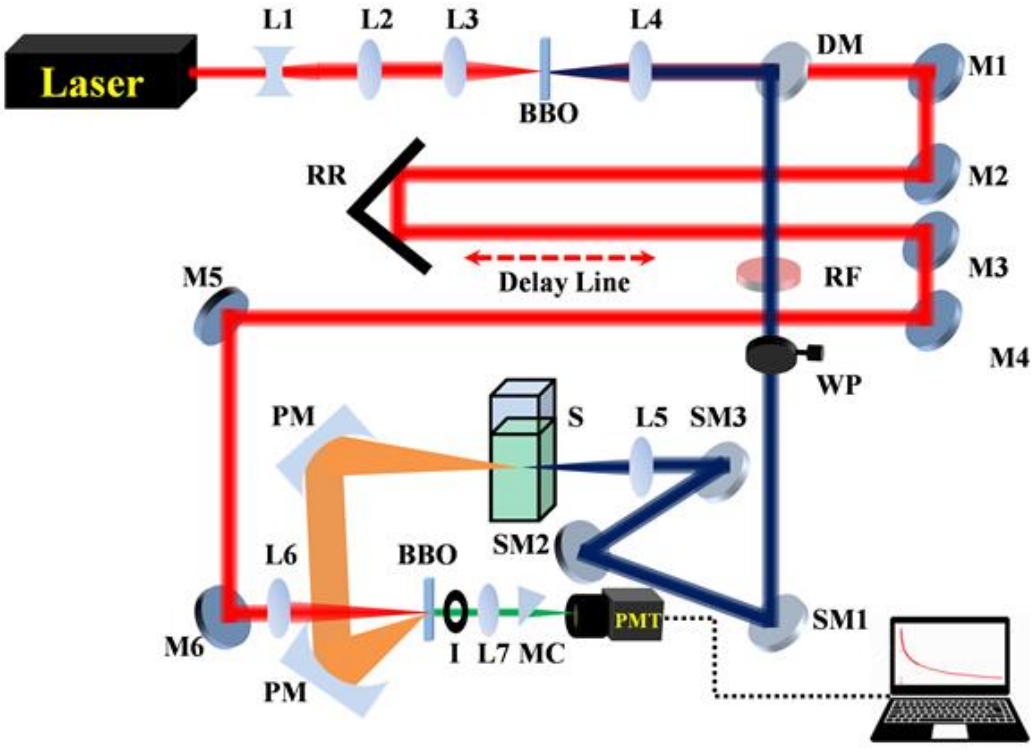

Figure S3: *Schematic diagram of the experimental femtosecond fluorescence up-conversion set-up:* L1: Concave lens; L (2-7): Concave lens; BBO: Beta barium borate; DM: Dichroic mirror; RR: Retro reflector; RF: Red filter; M (1-6): Mirror; SM (1-3): Silver mirror; PM: Parabolic mirror; I: Iris; MC: Monochromator; PMT: Photo multiplier tube; PC: Personal computer.

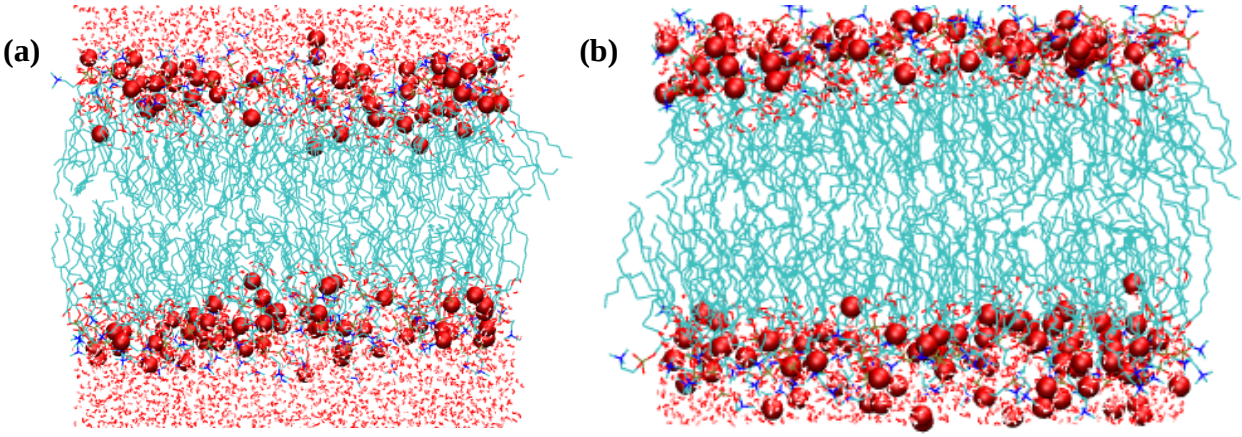

Figure S4: (a). Snapshots of DMPC Single-Bilayer with different hydration levels (a) 44 and, (b) 10. Color code with representation: Cyan line: DMPC; red line: Water; red VDW: interfacial water (IW)

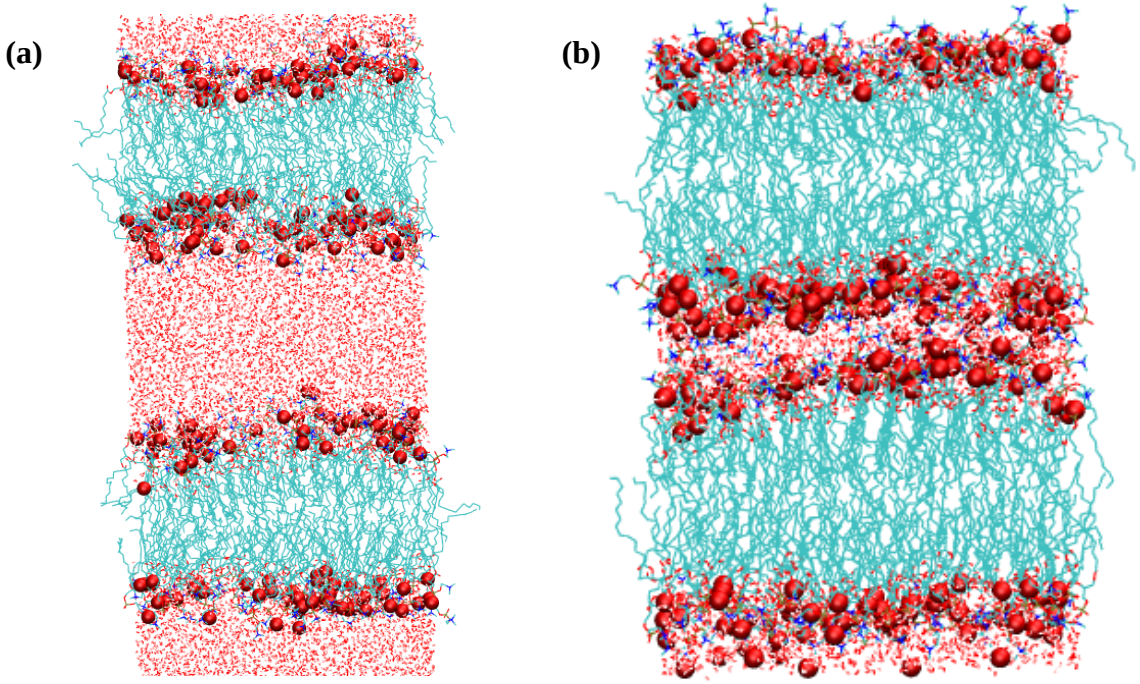

Figure S5: Snapshots of DMPC Multi-Bilayer with different hydration levels (a) 44 and, (b) 10. Color code with representation: Cyan line: DMPC; red line: Water; red VDW: interfacial water (IW)

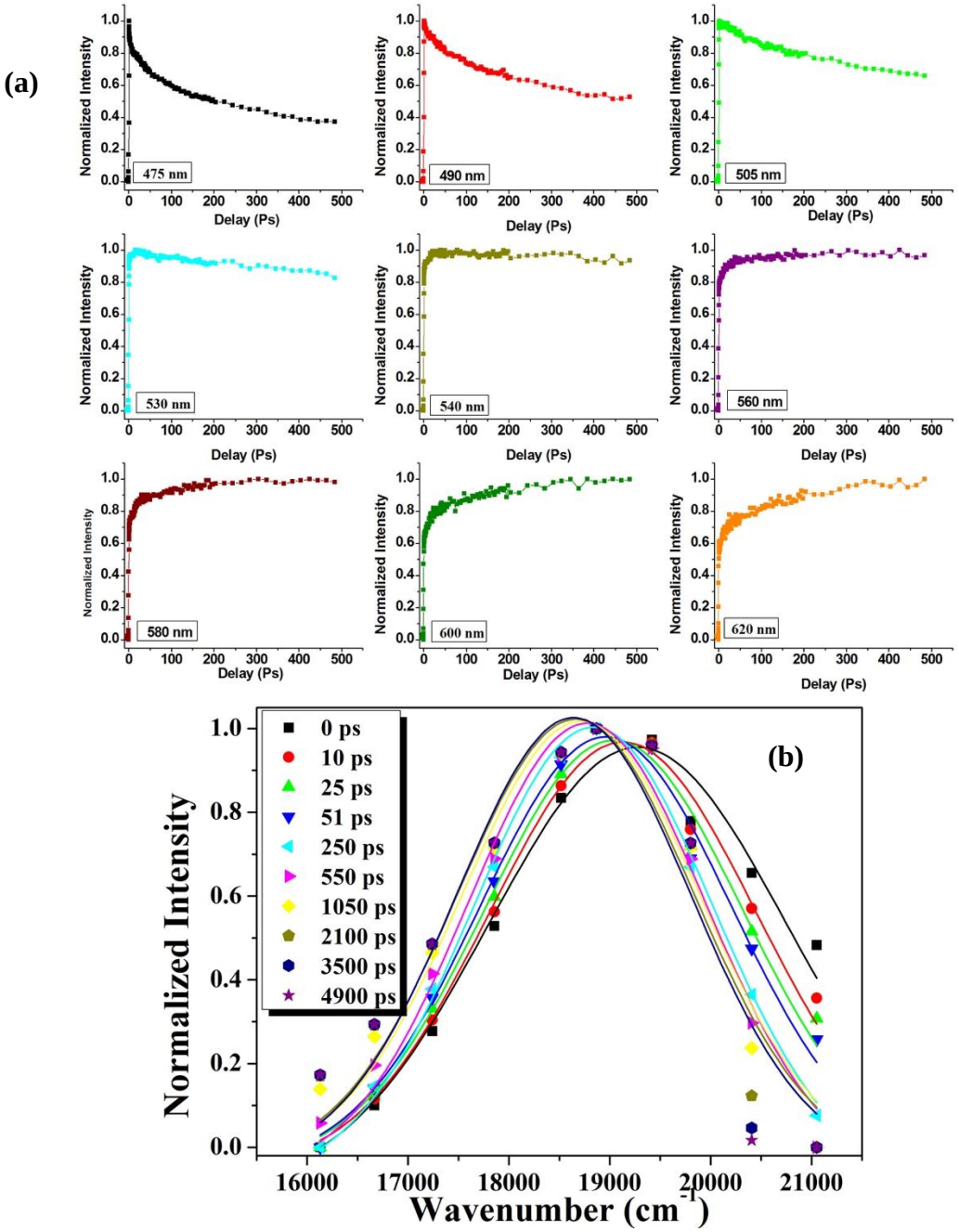

Figure S6: (a) Wavelength dependent emission decays of C-153 and (b) corresponding time resolved emission spectra (TRES) in multilamellar DMPC vesicle

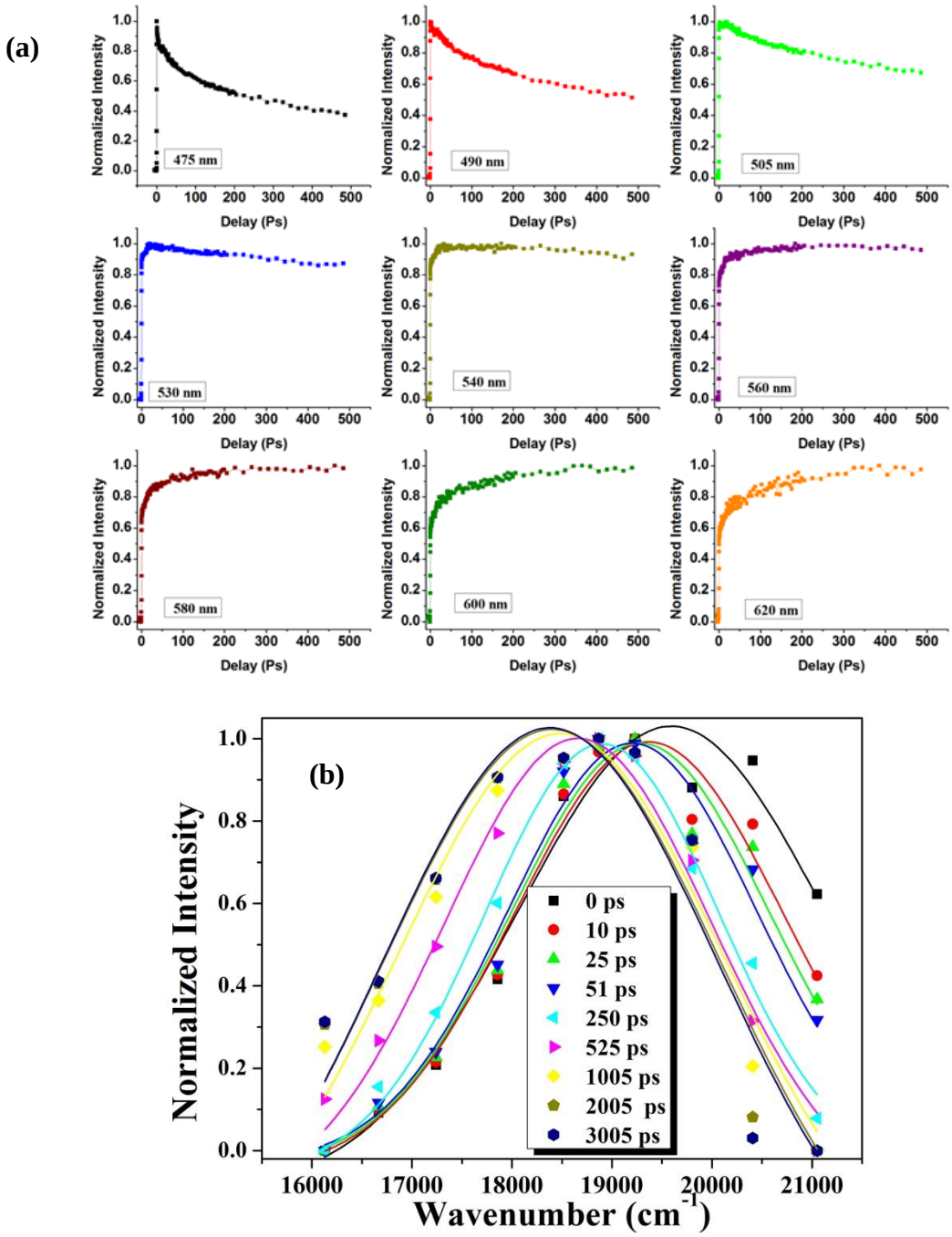

Figure S7: (a) Wavelength dependent emission decays of C-153 and (b) corresponding time resolved emission spectra (TRES) in 45 nm DMPC vesicle

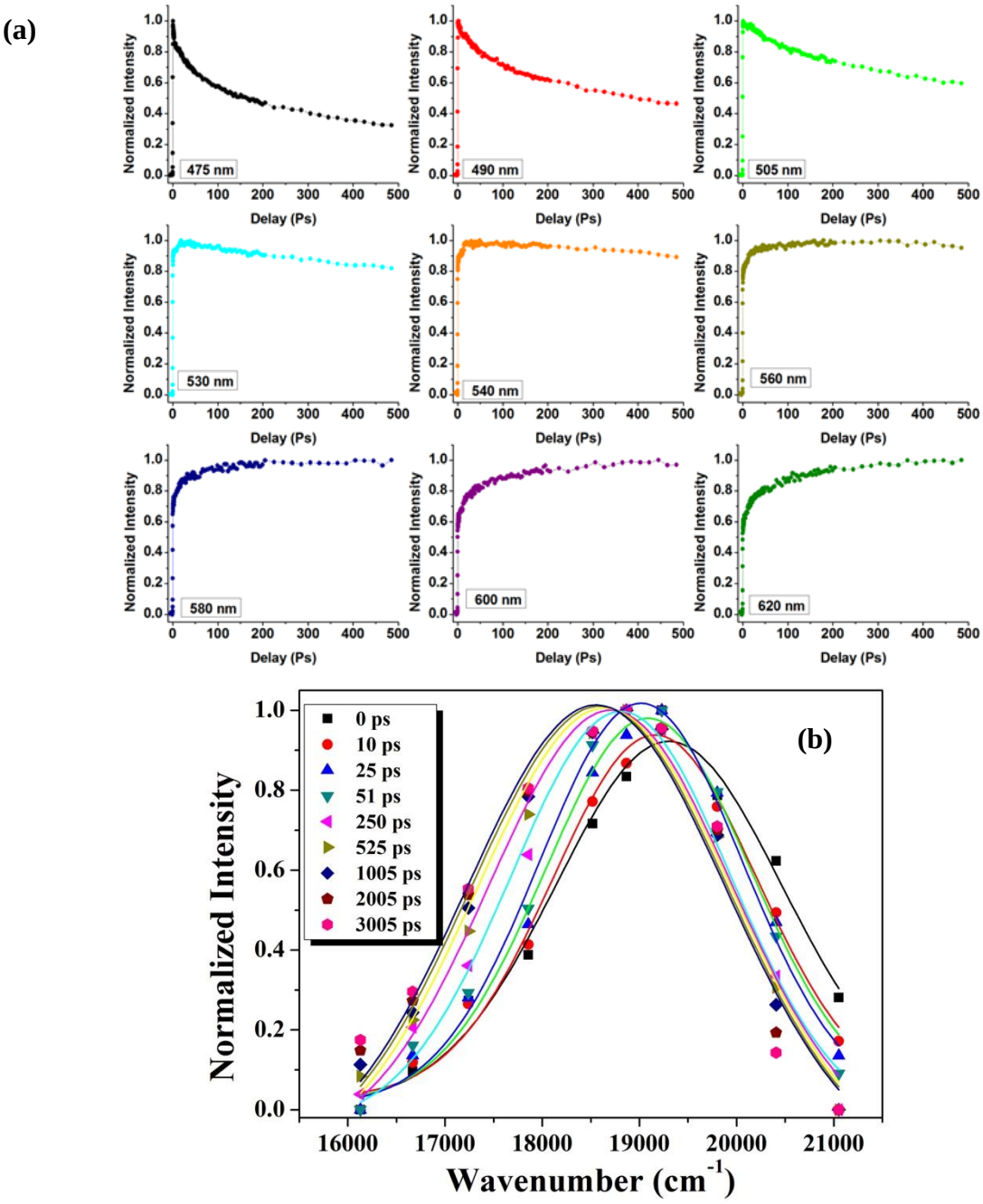

Figure S8: (a) Wavelength dependent emission decays of C-153 and (b) corresponding time resolved emission spectra (TRES) in 120 nm DMPC vesicle

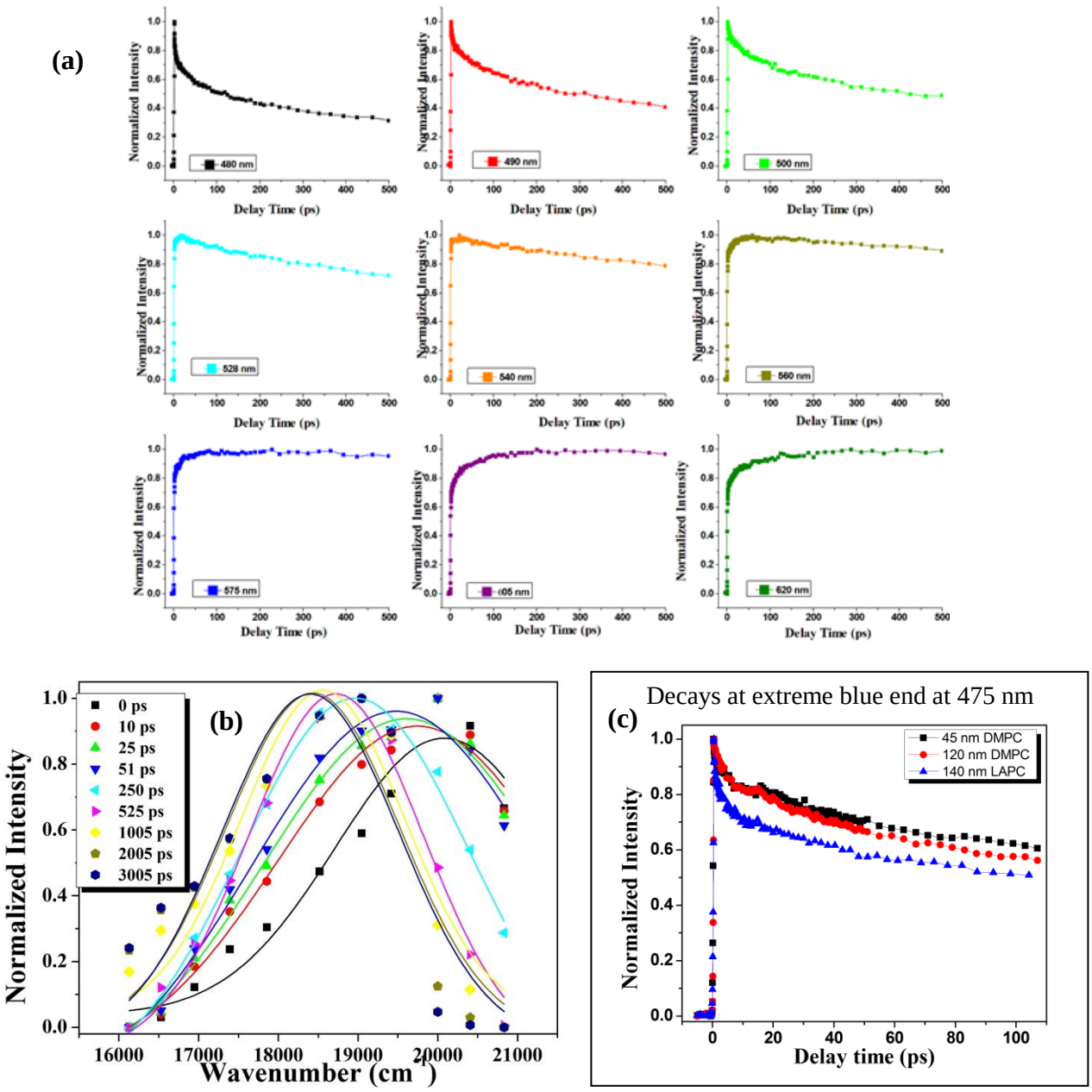

Figure S9. (a) Wavelength dependent emission decays of C-153 and (b) corresponding time resolved emission spectra (TRES) in 140 nm LAPC vesicle and (c) comparison of decay kinetics between different size vesicles

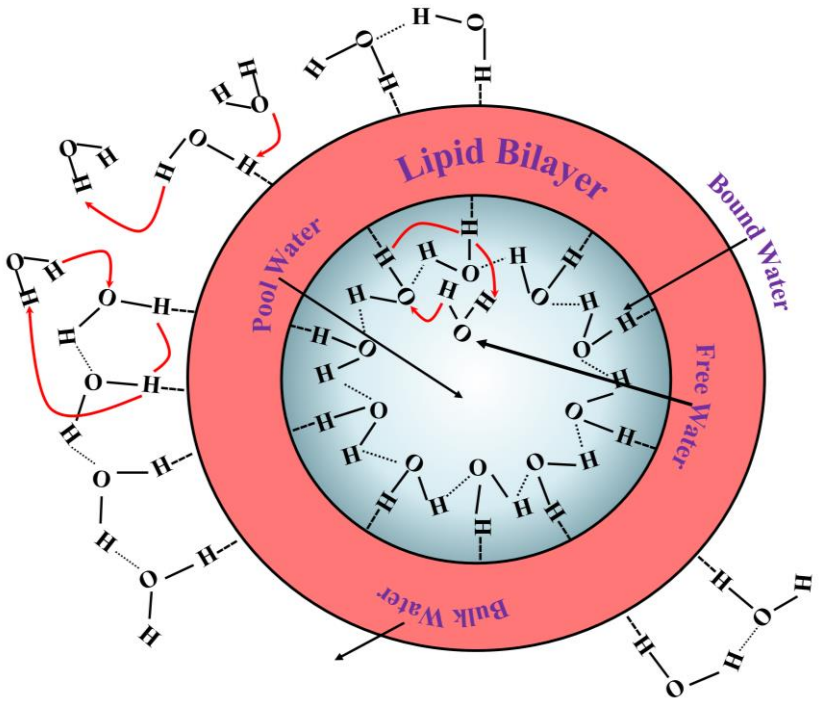

Figure S10: Various hydration layers in phospholipid vesicle

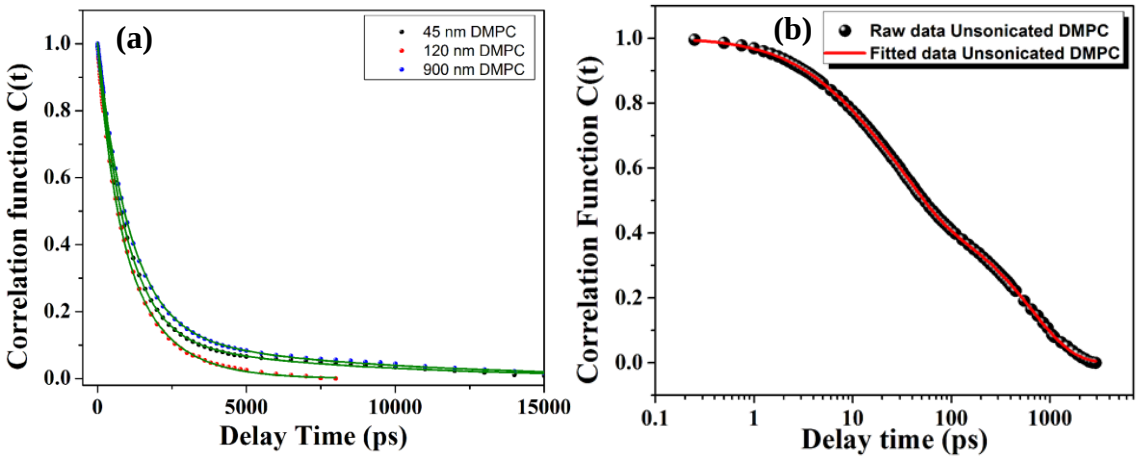

Figure S11: The decay of solvent response function C (t) of C-153 in different size DMPC and LAPC vesicle in water obtained from (a) TCSPC measurement at excitation wavelength 402 nm: 45 nm DMPC (black sphere), 120 nm DMPC (red sphere), 900 nm DMPC (blue sphere) (b) The decay of solvent response function C (t) of C-153 in unsonicated DMPC vesicle in water obtained up-conversion techniques.

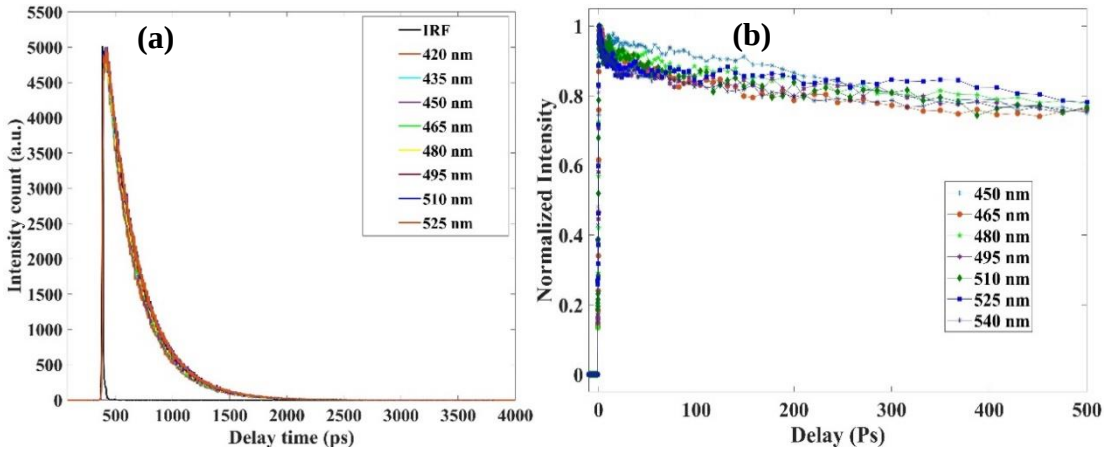

Figure S12: Fluorescent decay of C-153 in non-polar solvent n-Hexane with LAPC lipid molecules (a) in TCSPC and (b) in FFUC

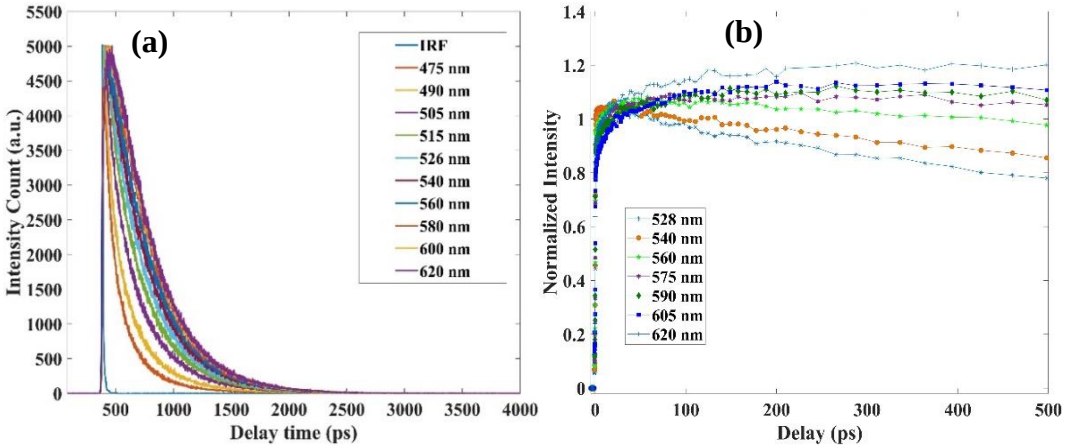

Figure S13: Fluorescent decay of C-153 in aqueous solution of LAPC vesicle (a) in TCSPC and (b) in FFUC

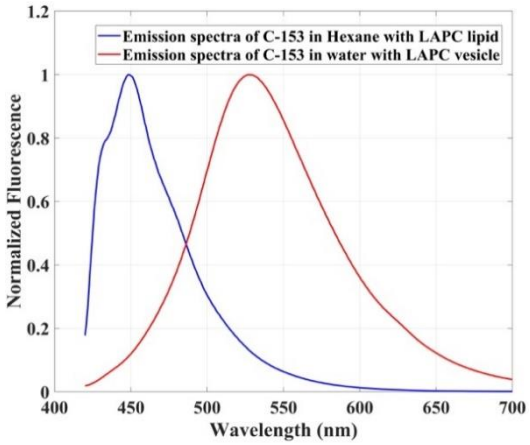

Figure S14: Normalized emission spectra of C-153 in non-polar hexane (Blue Line) with LAPC lipid and in aqueous solution (Red Line) of LAPC vesicle

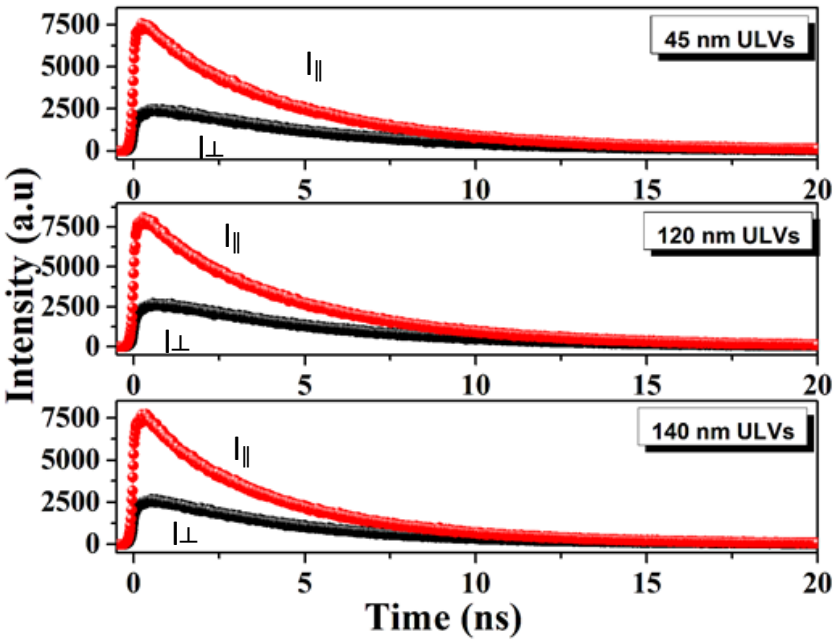

Figure S15: Polarization-resolved fluorescence decay of C-153 collected at parallel (red solid sphere) and perpendicular (black solid sphere) at corresponding emission maxima.

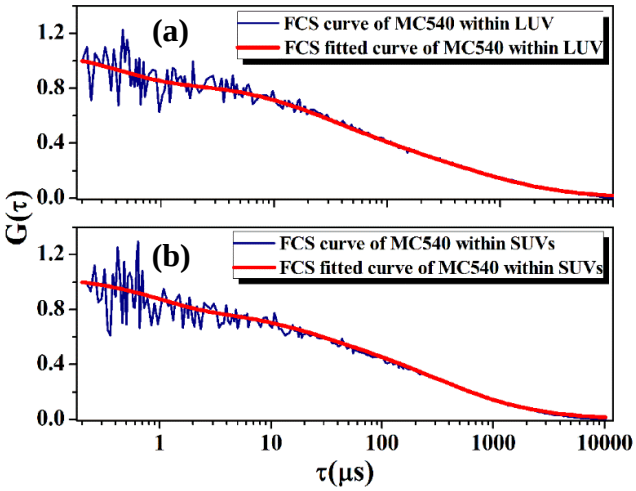

Figure S16: Fitted FCS traces of MC-540 in phospholipid vesicle bilayers made of DMPC with diameter (a) 120 nm and (b) 45 nm

Supporting Tables:

Table S1. The emission energies of the excited dipole immediately after excitation [i.e., at time zero υ (0)], and after a long time (υ (∞)) when the solvation process has come to its end.

Vesicles	υ (0)	υ (∞)
MLVs	19270	18621
45 nm DMPC	19231	18574
120 nm DMPC (ex 400 nm)	19202	18694
140 nm LAPC (ex 400 nm)	19633	18979

Table S2: Comparison of Diffusion coefficient (D), with different hydration number.

DMPC MultiBilayer	$D(10^{-5}cm^2s^{-1})$
ω = 44	0.6520±0.0137
ω = 26	0.65334±0.0989
ω = 18	0.5305±0.1159
ω = 10	0.1952±0.0054
DMPC Single Bilayer	$D(10^{-5}cm^2s^{-1})$
ω = 44	0.6740±0.0156

Table S3: Average lifetime of C-153 located at the interfacial region of a DMPC vesicle

Location at outer interface	<τ> (ps)	Location at inner interface	<τ> (ps)
1	3184	1	4197
2	3205	2	4044
3	2785	3	4221
4	3067	4	4289
5	2736	5	4159
6	3232	6	4308
7	3499	7	4002
8	3271	8	4096
Average Lifetime=	3122	Average Lifetime =	4165

References:

1. Siano; D. B.; Metzler, D. E. Band Shapes of the Electronic Spectra of Complex Molecules J. Chem. Phys. 51, 1856 (1969).