How does Bile Salt Penetration Affect the Self Assembled Architecture of Pluronic P123 Micelle? - Light Scattering and Spectroscopic Investigation

Arpita Roy, Niloy Kundu, Debasis Banik, Jagannath Kuchlyan, Nilmoni Sarkar*

Department of Chemistry, Indian Institute of Technology, Kharagpur 721302, WB, India

E-mail: nilmoni@chem.iitkgp.ernet.in

Fax: 91-3222-255303

1. Instrumentations:

1.1. Malvern Nano ZS instrument:

In Malvern Nano ZS instrument, a 4 mW He−Ne laser (λ = 632 nm) is used with detector angle poisoned at 173º. In DLS experiment, the hydrodynamic diameter (d_h) of particles was calculated by analyzing the collected scattering intensity using the following equation:

$$d_{\mathrm{h}} = \frac{k_B T}{3\pi\eta D} \qquad (1)$$

Where, k_B, T, D and η denotes Boltzmann constant, Temperature, diffusion coefficient and viscosity respectively.

1.2. Cryogenic Transmission Electron Microscope (Cryo-TEM) Measurements:

In Cryo TEM measurements, the samples were examined under an acceleration voltage of 200 kV in the conventionally operated TEM mode. The temperature was kept at -173^{0}C. Images were recorded by using a Gatan CCD Orius Camera. The measurements were executed by blotting the appropriately diluted sample on a holey carbon grid of 200 mesh. The grid was then subsequently plunged in a VITROBOT (FEI) instrument at room temperature.

1.3. Fluorescence Correlation Spectroscopy (FCS) Measurement:

3D diffusion model have been used in order to fit the correlation curves. For K fraction of dyes which are diffused within the system with distinct diffusion coefficients, the correlation function G(τ) is defined as,[61]

$$G(\tau) = \frac{1}{N}\sum_{i=1}^{K} \varphi_i \left(1+\frac{\tau}{\tau_i}\right)^{-1} \left(1+\frac{\tau}{\tau_i S^2}\right)^{-0.5} \quad (2)$$

In the above equation, N denote the number of fluorescent species with the focal volume, φ_i is the fractional weighting factor for the i-th contribution to the autocorrelation curve and τi is the diffusion time of the fluorescent species within the observation volume and τ is the delay or lag time. S denotes the structure parameter of the excitation volume and it is defined as (l/r), where l is the longitudinal radii and r is the transverse radii. Transverse radii (r) can be determined through the fitting of an autocorrelation curve of a fluorescent species with known diffusion constant. We have used R6G in water for this purpose and the diffusion coefficient (D_t) is (426 $\mu m^2\ S^{-1}$).[62]The curves are fitted with the following equation in order to determine the global parameter r and S.

$$G(\tau) = \frac{1}{N}\left(1+\frac{4D_t\tau}{r^2}\right)^{-1}\left(1+\frac{4D_t\tau}{S^2 r^2}\right)^{-0.5} \quad (3)$$

Where D_t the diffusion coefficient of the fluorescent species and τ is the diffusion time and in the fitting analysis r and S are kept as linked global parameter. S= 5 is obtained after fitting the correlation curves of R6G in water and observation volume (V_{eff}) is obtained from the following equation,

$$V_{eff} = \pi^{3/2} r^3 S \quad (4)$$

The final value of r is obtained as 365 nm and V_{eff} is 1.35 fl. All the FCS experiments were performed at 20^0C and the diffusion coefficient can be obtained from the following equation,

$$D_t = \frac{r^2}{4\tau} \quad (5)$$

Some useful Equations to calculate the Analytical parameters for Rotational Motions:

The experimentally measured τ_{slow} and τ_{fast} are related to τ_D, τ_e,τ_M and D_W by the following equations,

$$\frac{1}{\tau_{slow}} = \frac{1}{\tau_D} + \frac{1}{\tau_M} \quad (6)$$

$$\frac{1}{\tau_{fast}} = \frac{1}{\tau_e} + \frac{1}{\tau_S} \quad (7)$$

The cone angle, θ_0 and wobbling diffusion coefficient, D_W has been estimated using the following equations,

$$\theta_0 = cos^{-1}[0.5\{(1+8S)^{0.5} - 1\}] \quad (8)$$

$$D_W = \frac{7\theta^2}{24\tau_e} \quad (9)$$

We have used the following Stokes-Einstein-Debye equation to calculate the microviscosity of the mixed micelles:

$$<\tau_r> = \frac{\eta_{mic} V}{kT} \quad (10)$$

Where V, $<\tau_r>$ and T represent the volume of probe molecule, average rotational time and absolute temperature respectively. The volume of C153 and C480 were taken as 246 Å^3.

Table S1. Time-Resolved Fluorescence Decay Parameters of C153 and C480 in the molar ratio of NaTC and P123 1:3 and 3:1 respectively at λ_{exc} = 408 nm[a]

	Systems	α_{1a}	α_{2a}	τ_1 (ns)[a]	τ_2 (ns)[a]	$<\tau_{avg}>$ (ns)
	NaTC:P123 = 1:3	0.14±0.01	0.86±0.02	1.93±0.01	5.92±0.01	5.36
	NaTC:P123 = 3:1	0.19±0.02	0.81±0.01	1.93±0.01	5.78±0.02	5.04
C-153	NaDC:P123 = 1:3	0.15±0.01	0.85±0.02	1.93±0.02	5.88±0.02	5.12

	NaDC:P123 = 3:1	0.21±0.03	0.79±0.01	1.93±0.01	5.58±0.02	4.81
C-480	NaTC:P123 = 1:3	0.10±0.01	0.90±0.01	2.78±0.01	5.76±0.02	5.46
	NaTC:P123 = 3:1	0.12±0.04	0.88±0.01	3.38±0.02	5.92±0.01	5.61
	NaDC:P123 = 1:3	0.10±0.02	0.90±0.01	2.75±0.01	5.79±0.01	5.47
	NaDC:P123 = 3:1	0.14±0.06	0.86±0.02	3.01±0.03	6.04±0.01	5.61

[a]*The statistical errors were obtained from the fit of the experimental data*

Table S2: Decay Parameters of Rotational Relaxation of C153 and C480 in 5 wt % P123 and in mixed micelle of bile salts and P123 at 5:1 molar ratio ($\lambda_{ex} = 408\ nm$) at different temperatures.

System	Temperature (K)	a_{1a}	a_{2a}	τ_1(ns.)[a]	τ_2(ns.)[a]	$<\tau_r>$ (ns.)	η_{mic} (cP)
C153 in P123	293	0.13±0.01	0.87±0.02	0.62±0.01	4.03±0.01	3.58	58.80
	303	0.18±0.01	0.82±0.01	0.53±0.01	3.58±0.01	3.03	51.50
	313	0.24±0.02	0.76±0.01	0.44±0.03	3.17±0.02	2.51	44.10
	323	0.28±0.02	0.72±0.02	0.38±0.02	2.78±0.02	2.10	38.00
C153 in NaTC:P123= 5:1	293	0.23±0.01	0.77±0.01	0.57±0.02	3.38±0.03	2.73	44.80
	303	0.40±0.02	0.60±0.02	0.45±0.01	2.92±0.03	1.93	32.80
	313	0.47±0.02	0.53±0.01	0.38±0.03	2.64±0.01	1.57	27.56
	323	0.49±0.01	0.51±0.02	0.35±0.02	2.50±0.01	1.44	26.09
C153 in NaDC:P123= 5:1	293	0.29±0.01	0.71±0.02	0.53±0.02	3.04±0.01	2.31	37.96
	303	0.42±0.02	0.58±0.02	0.40±0.01	2.66±0.01	1.71	29.06
	313	0.46±0.03	0.54±0.01	0.34±0.02	2.45±0.02	1.47	25.81
	323	0.49±0.01	0.51±0.02	0.32±0.02	2.39±0.01	1.37	24.82
C480 in P123	293	0.31±0.01	0.69±0.01	0.59±0.01	2.40±0.01	1.83	30.07
	303	0.39±0.02	0.61±0.01	0.48±0.01	2.00±0.01	1.40	23.79
	313	0.45±0.01	0.55±0.01	0.40±0.01	1.81±0.01	1.17	20.54
	323	0.47±0.02	0.53±0.02	0.35±0.01	1.69±0.02	1.06	19.20
C480in NaTC:P123= 5:1	293	0.48±0.01	0.52±0.01	0.52±0.01	2.10±0.01	1.34	22.02
	303	0.51±0.01	0.49±0.01	0.42±0.01	1.86±0.01	1.12	19.03
	313	0.53±0.02	0.47±0.03	0.36±0.01	1.72±0.02	1.00	17.55

C480 in NaDC:P123= 5:1	323	0.57±0.01	0.43±0.01	0.32±0.01	1.64±0.01	0.88	15.94
	293	0.49±0.01	0.51±0.01	0.49±0.01	1.97±0.01	1.24	20.38
	303	0.52±0.02	0.48±0.01	0.38±0.02	1.76±0.02	1.04	17.67
	313	0.53±0.03	0.47±0.01	0.33±0.01	1.65±0.01	0.95	16.68
	323	0.59±0.02	0.41±0.02	0.30±0.01	1.60±0.01	0.83	15.03

[a]*The errors correspond to the average errors or propagation of errors*

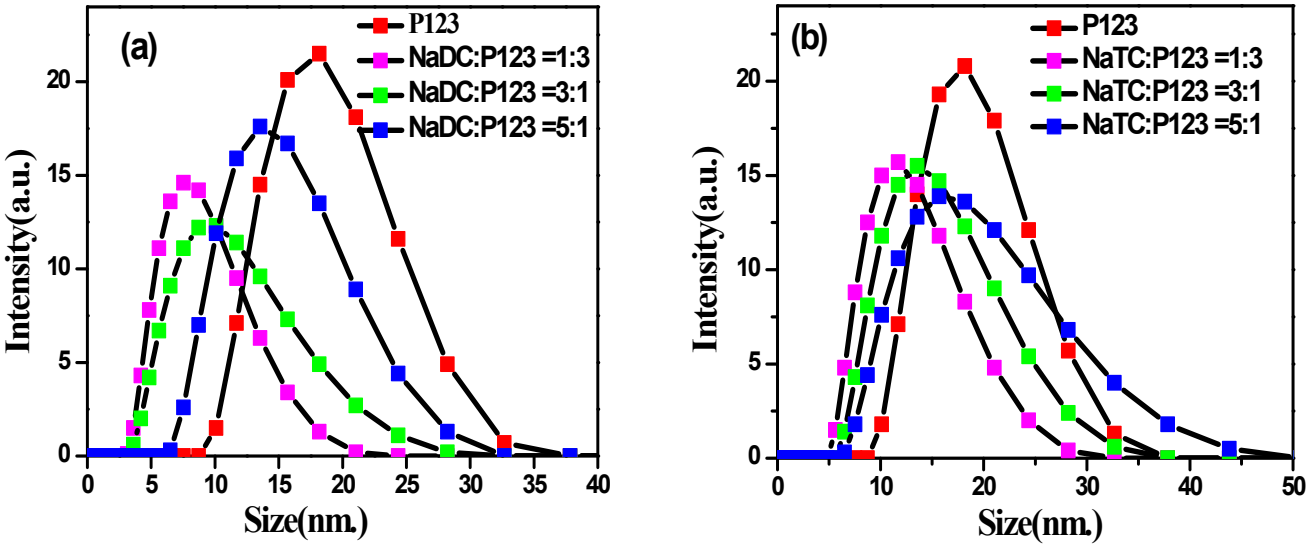


Figure S1: DLS intensity-size distribution of P123 micelle and at different molar ratios of (a) NaDC:P123 and (b) NaTC:P123 mixed micelles.

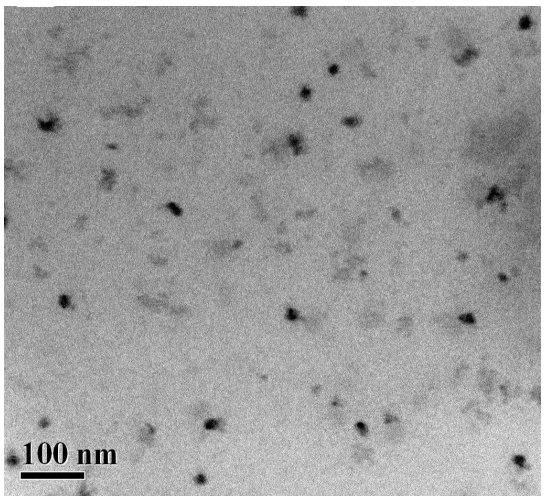


Figure S2: HR-TEM image of P123-bile salt mixed micelle 5 wt % P123 (~9 mM) and in mixed micelle of bile salts and P123 at 5:1 molar ratio.

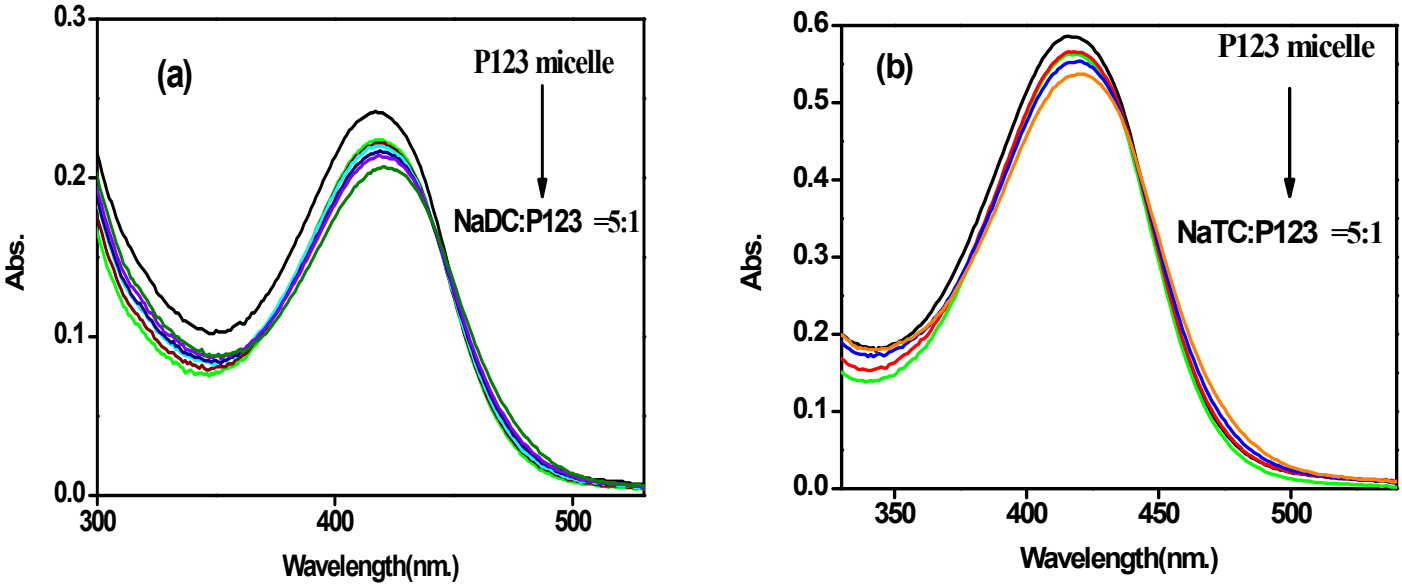
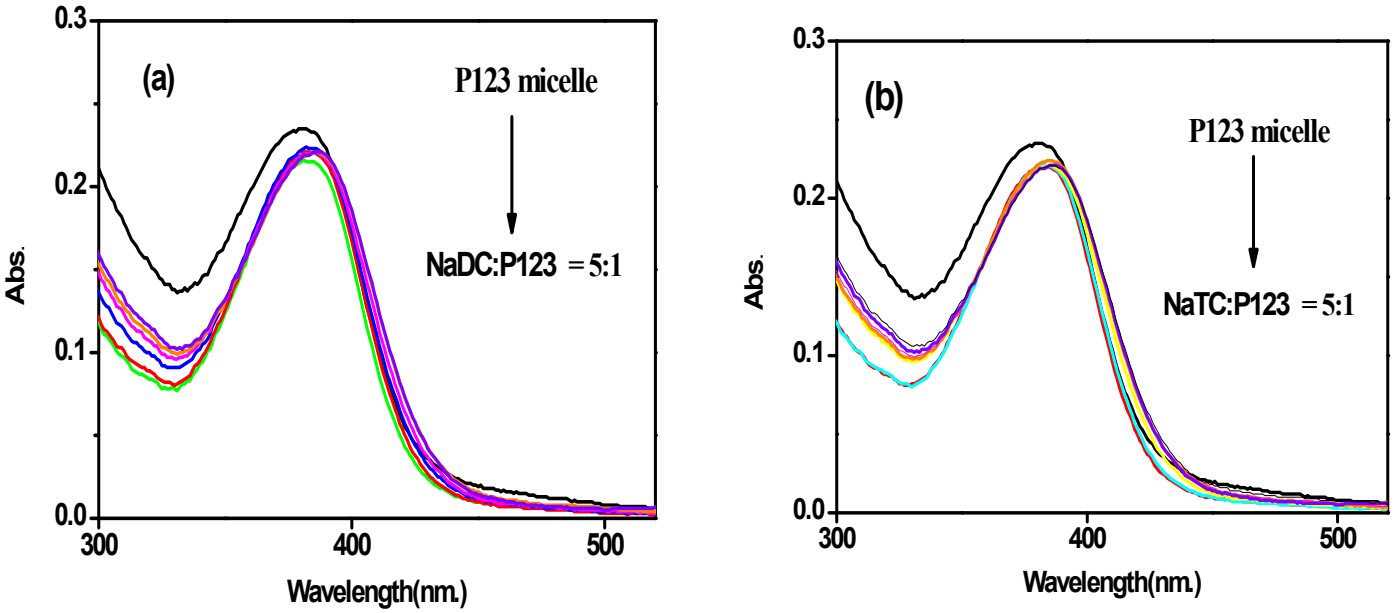


Figure S3: Absorption spectra of C153 in P123 and in presence of (a) NaDC, (b) NaTC at different molar ratios.



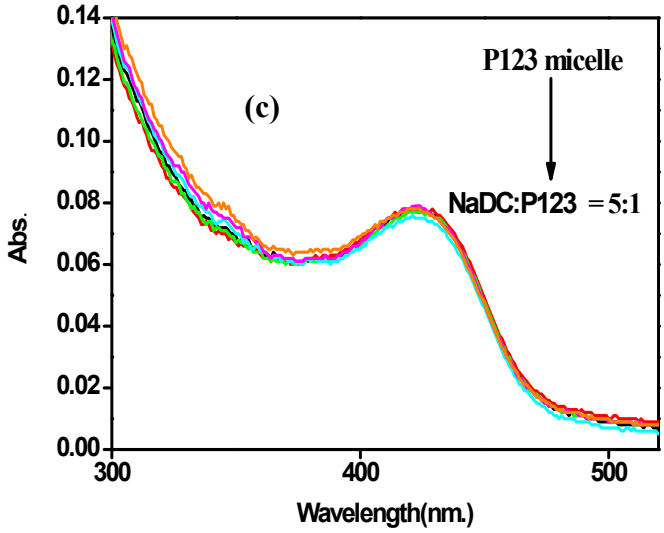


Figure S4: Absorption spectra of C480 in P123 and in presence of (a) NaDC, (b) NaTC at different molar ratios. (c) Absorbance spectra of C343 in P123 and in presence of different molar ratios of NaDC and P123.

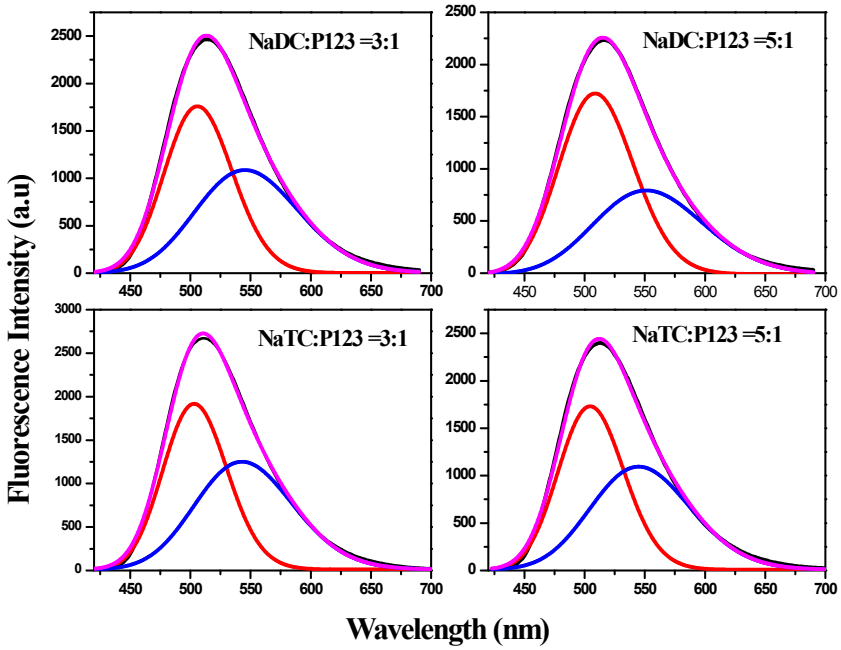


Figure S5. Fluorescence spectra of C153 and their log-normal fit for the different P123-bile salt mixed micelles. The red and blue line spectra indicate copolymer-rich and bile salt-rich micelles, respectively. The overall spectra are shown by the pink lines. The black line indicates experimental data set.

.

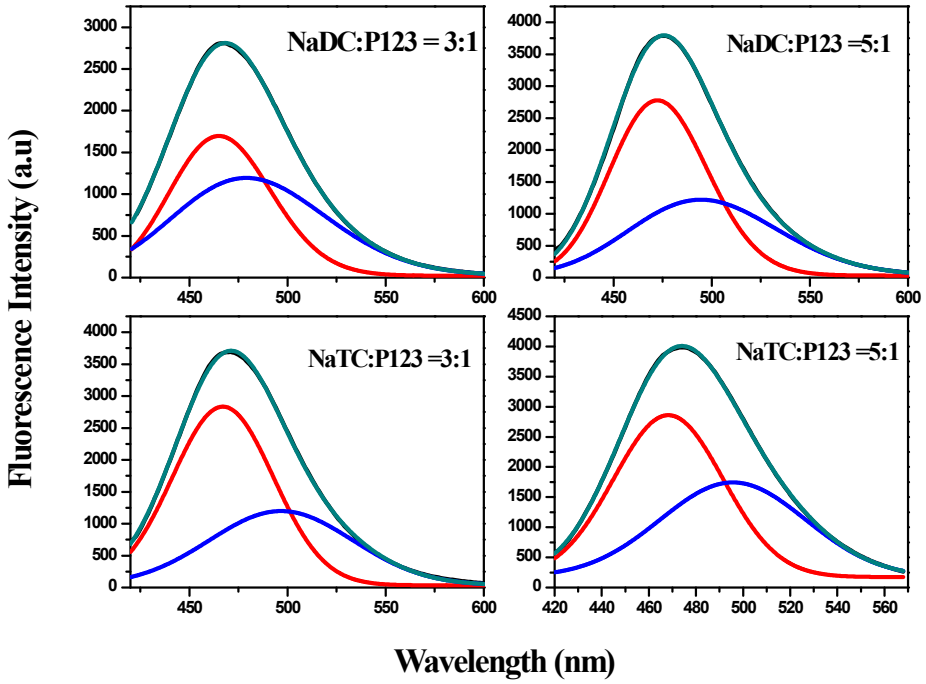


Figure S6. Fluorescence spectra of C480 and their log-normal fit for the different P123-bile salt mixed micelles. The red and blue line spectra indicate copolymer-rich and bile salt-rich micelles, respectively. The overall spectra are shown by the gray lines. The black line indicates experimental data set.

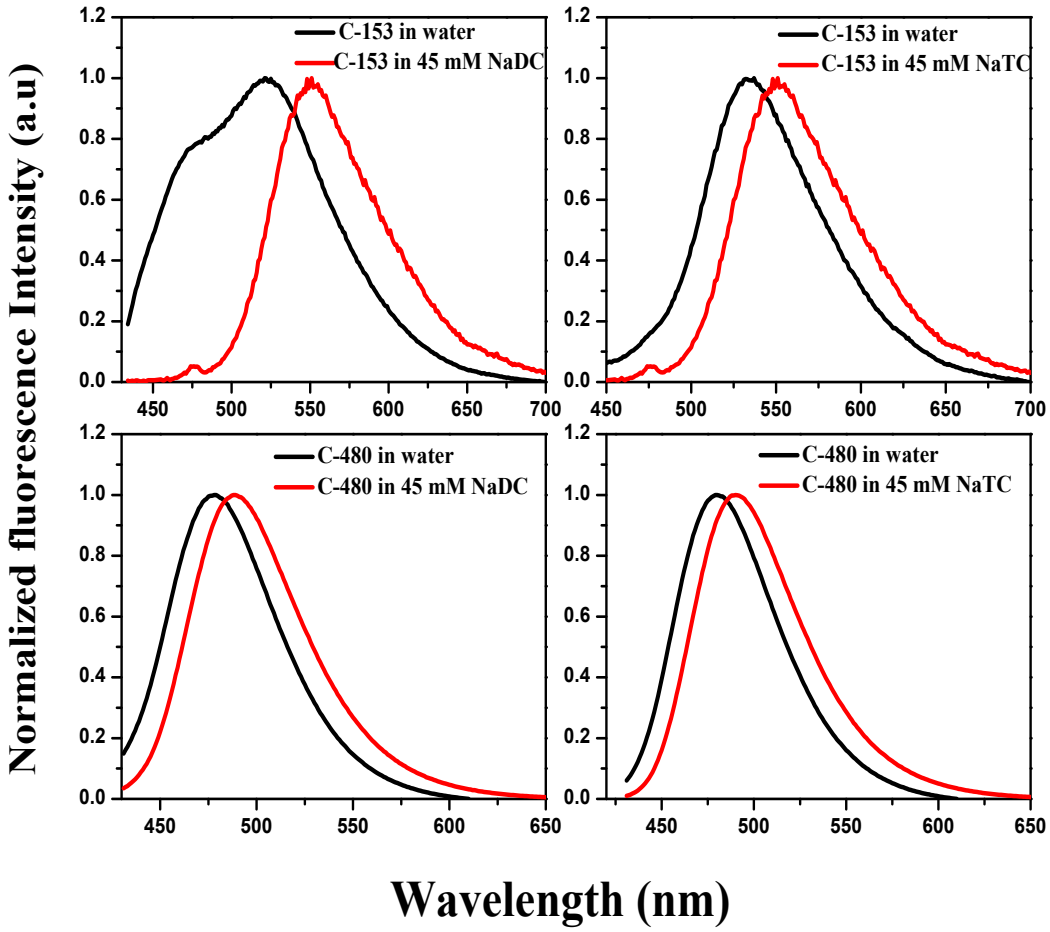


Figure S7. Normalized fluorescence Spectra of C-153 and C-480 in water and different bile salt aggregates.

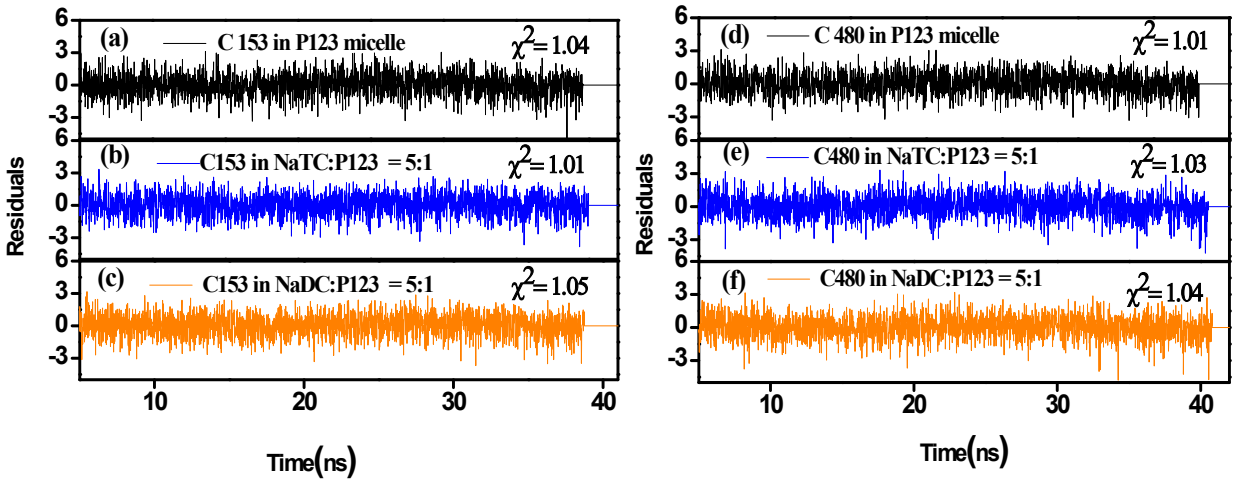


Figure S8: The residuals between the experimental data and the fitted data are shown for the lifetime decays of C153 (a,b,c) and C480 (d,e,f) in pure P123 micelle and at bile salt:P123 = 5:1

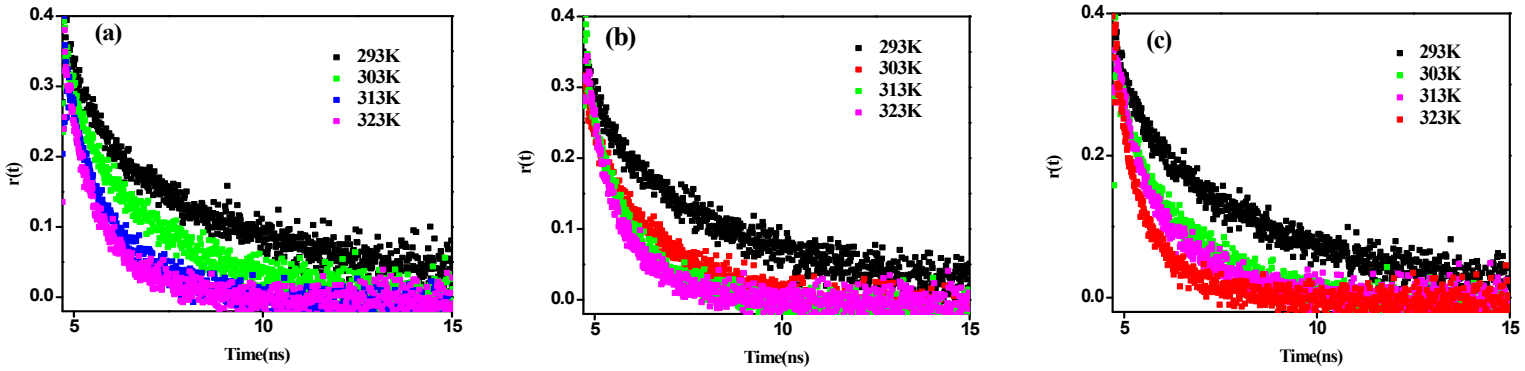


Figure S9: Anisotropy decays of C153 in (a) P123, (b) at NaDC: P123 molar ratio 5:1, (c) at NaTC:P123 molar ratio 5:1 at different temperatures.

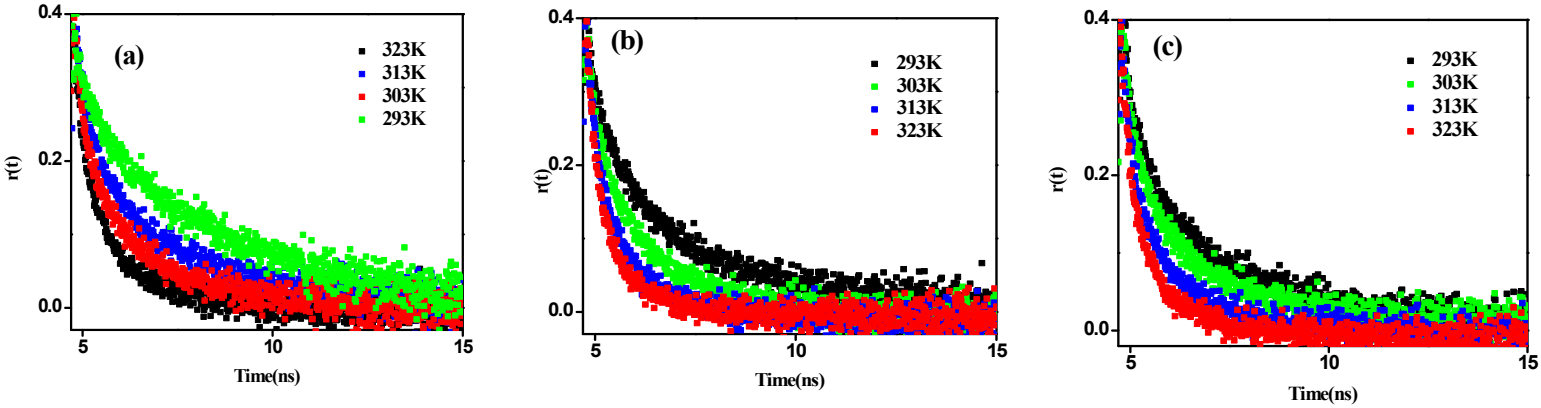


Figure S10: Anisotropy decays of C-480 in (a) P123, (b) at NaDC:P123 molar ratio 5:1, (c) at NaTC:P123 molar ratio 5:1 at different temperatures.

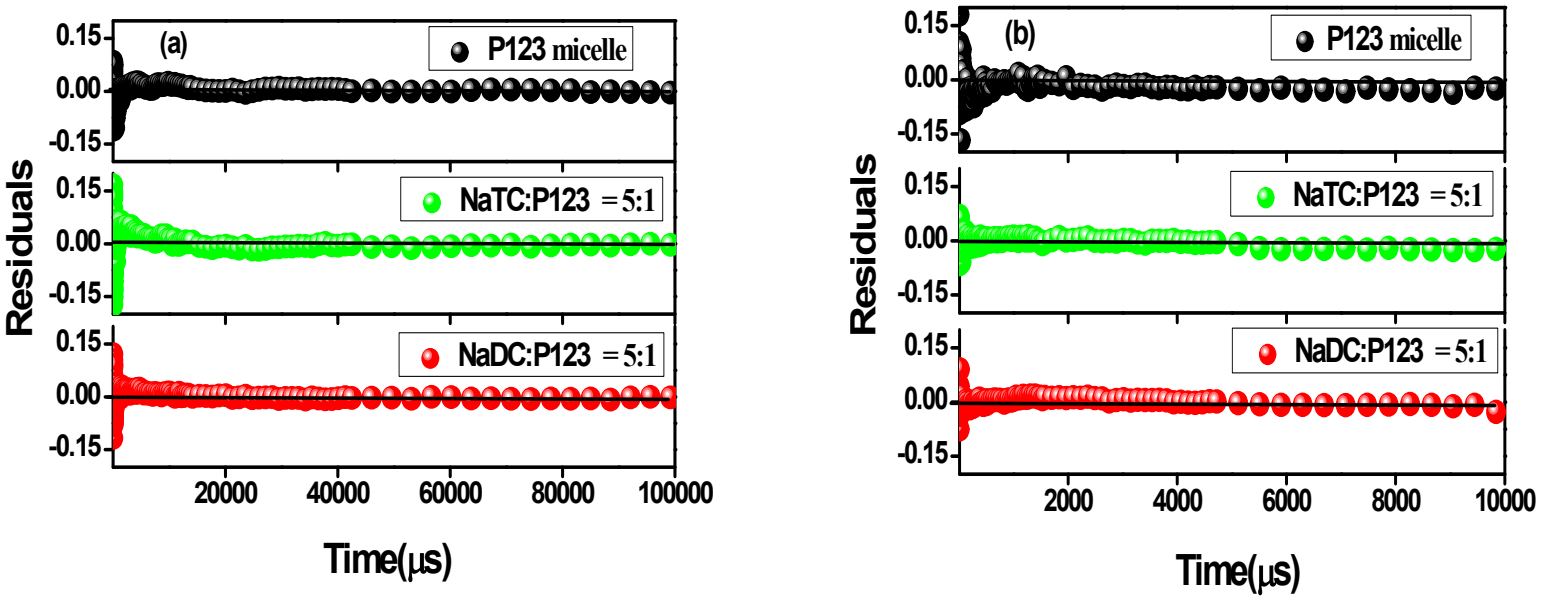


Figure S11: FCS residuals of (a) DCM and (b) R6G in pure P123 micelle and bile salt-P123 mixed micelles of 5:1 molar ratio.