Supporting Information

How Does the Surface Charge of Ionic Surfactant and Cholesterol forming Vesicles Control Rotational and Translational Motion of Rhodamine 6G Perchlorate (R6G ClO_4)?"

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1. Preparation of Cholesterol and Surfactant Containing Nano-aggregates:

At first, Milli-Q water was filtrated through syringe filter (0.2 μm) to remove the dust particles. The stock solutions of aqueous SDS and CTAB (concentration of 20 mM, which was well above their cmc) were prepared using appropriate amount of each surfactant in different volumetric flask. Afterwards, a specific amount (volume) of stock solution was added into different glass bottle and then, required amount of cholesterol was added in each glass bottle to vary Q values (Q = [cholesterol]/[surfactant]) from 0 to 1.0. The solution mixtures were then sonicated by using an ultrasonic probe sonicator (Processor SONOPROS PR-250 MP, Oscar Ultrasonics Pvt. Ltd. India) containing titanium probe (frequency ~20±3 kHz for 15 min). Finally, the solution mixtures were kept for 24 hours to stabilize the system before each measurement.

2. Instrumentations:

2.1. 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 instrumental software according to following equation:

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

where k_B, T, D and η represent Boltzmann constant, temperature, diffusion coefficient and viscosity, respectively.

2.2. 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.

2.3. Transmission Electron Microscopy (TEM) Measurements:

Transmission electron microscopy (TEM) measurements were performed for the structural characterization of cholesterol-CTAB vesicles at Q=1.00 by JEOL model JEM 2010 transmission electron microscope (operating voltage of 200 kV) using 0.5 wt % of uranyl acetate as the staining agent.

2.4. Time Resolved Anisotropy Measurements:

In this TCSPC set up, a pico-second diode laser (IBH, UK, Nanoled) was used as excitation source, and a motorized polarizer was used on the emission side. The emission decays were collected through motorized polarizer using a Hamamatsu MCP PMT (3809U). The time resolution of the set up was ~90 ps. During anisotropy measurements, samples containing R6G molecules were excited by vertically polarization pulse using 440 nm laser. The emission decays for parallel $I_{\parallel}(t)$ and perpendicular $I_{\perp}(t)$ polarizations were accumulated alternatively until a certain peak difference between $I_{\parallel}(t)$ and $I_{\perp}(t)$ decays were reached. The anisotropy decays of R6G containing solutions were analyzed using IBH DAS, version 6, and decay analysis software. In steady state absorption, emission and time resolved anisotropy measurements, the concentration of the R6G was kept ~10^{-6} M in each solution mixture.

2.5. Fluorescence Correlation Spectroscopy (FCS) Measurement:

Fluorescence correlation spectroscopy (FCS) measurements of cholesterol-SDS and cholesterol-CTAB solution mixtures were carried using DCS 120 Confocal Laser Scanning Microscope (CLSM) system (Becker & Hickl DCS-120) with inverted optical microscope of Zeiss (Carl Zeiss, Germany). Water-immersion objective (40X, NA = 1.2) was used. A drop of solution

mixture was placed on a glass cover slip and a 488 nm picosecond diode laser (bh BDL-SMC) was used as excitation source. The laser was operated in the CW mode. Scanning of the sample was controlled by bh GVD-120 scan controller. The obtained fluorescence signal was separated from the excitation light by using a main dichroic filter. The fluorescence signal was then splited into two channels after focusing onto a 50/50 beam splitter through a pinhole and finally collected by two single photon avalanche diodes (SPADs) connected with two channels. A correlator card was used to record the fluorescence autocorrelation traces from the signals of two detectors. During FCS measurement, the concentration of the R6G was kept ~50-60 nM in each solution.

The correlation function G(τ) which was used to describe the temporal fluctuation of fluorescence intensity is defined as[34,35]

$$G(\tau) = \frac{\langle \delta F(t) \delta F(t+\tau) \rangle}{\langle F(t) \rangle^2} \qquad (2)$$

where $\delta F(t)$ represents the fluctuation of fluorescence signal $F(t)$ as deviations from the temporal average of the signal $\langle F \rangle$ at time t. Therefore, $\delta F(t) = F(t) - \langle F \rangle$. For the diffusion of a single component system, a three dimensional (3D) fitting model can be defined as

$$G(\tau) = \frac{1}{N}\left[1 + \frac{\tau}{\tau_D}\right]^{-1}\left[1 + \frac{\tau}{S^2\tau_D}\right]^{-\frac{1}{2}} \qquad (3)$$

$G(\tau)$ is called autocorrelation function for simple solution containing one diffusing molecule (excluding the contribution of triplet state). In this equation, N is the average number of fluorescent molecules in the detection volume, and τ_D is the average time of fluorescent molecules diffusing in the detection volume. S is the structure parameter which is equal to the

$\frac{\omega_z}{\omega_{xy}}$ where ω_z is the longitudinal radius and ω_{xy} is the transversal or waist radius of the confocal volume. τ_D is related to the translational diffusion coefficient by the following equation

$$\tau_D = \frac{{\omega_{xy}}^2}{4D} \qquad (4)$$

The structure parameter (S) of the excitation volume was determined using R6G in water as a reference sample of known diffusion coefficient (D_t = 426 $\mu m^2 s^{-1}$). The value of structure parameter is found to be 5. This value has been fixed for the analysis of all data obtained in our systems. Fitting of the FCS data of R6G in water resulted in the diffusion time of ~73.6 μs which has been used to calculate the transverse radius and the confocal volume of our set-up. The calculated transverse radius is ~354 nm and the confocal volume is 1.39 fL.

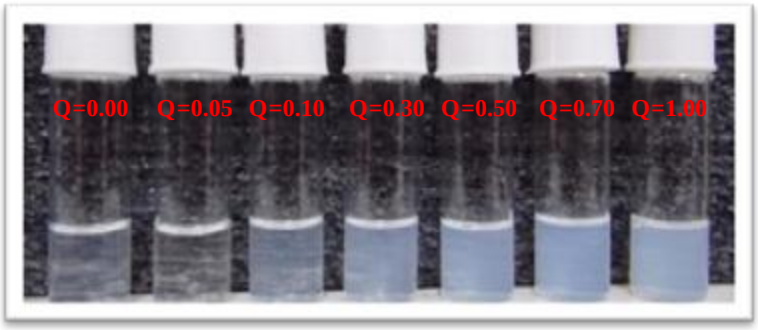
Table S1: Diffusion Coefficients (D_t) of R6G in Water and Aqueous Solution of Cholesterol-surfactant aggregates at Different Q values.

System	Q value	D_t ($\mu m^2/s$)[a] of R6G
Water		426 (100%)
Cholesterol-CTAB	0.00	74 (42%)
	0.05	84 (43%)
	0.10	87 (40%)
	0.30	142 (46%)
	0.50	281 (66%)
	0.70	391 (100%)
	1.00	410 (100%)
Cholesterol-SDS	0.00	54 (51%)
	0.05	53 (52%)
	0.10	36 (51%)
	0.30	35 (50%)
	0.50	26 (49%)
	0.70	16 (60%)
	1.00	13 (67%)

[a] Experimental error ~8%

Figures

(a) Cholesterol-SDS



(b) Cholesterol-CTAB

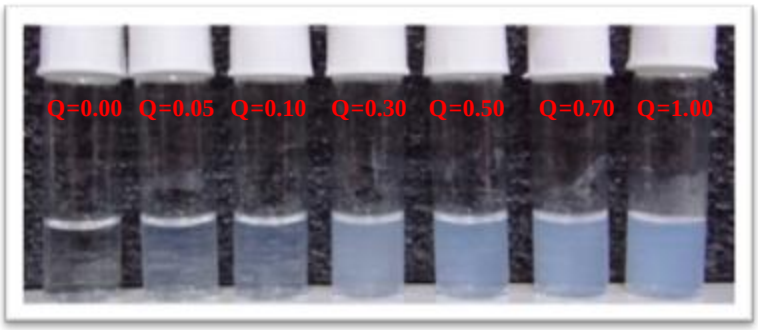


Figure S1: Visual Observations (optical micrographs) of aqueous (a) Cholesterol-SDS mixture with increasing cholesterol concentration, i.e., at different Q values. For the sake of comparison, optical micrographs are also taken in (b) Cholesterol-CTAB mixture at different Q values.

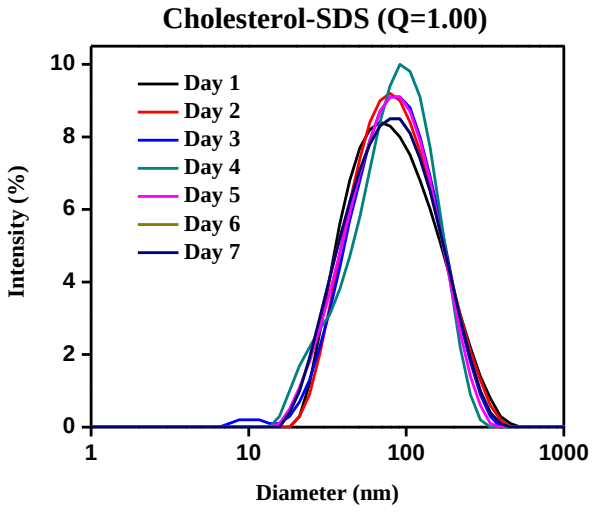


Figure S2: Intensity-size distribution profiles of cholesterol-SDS aggregates at Q=1.00 (monitored for 7 days).

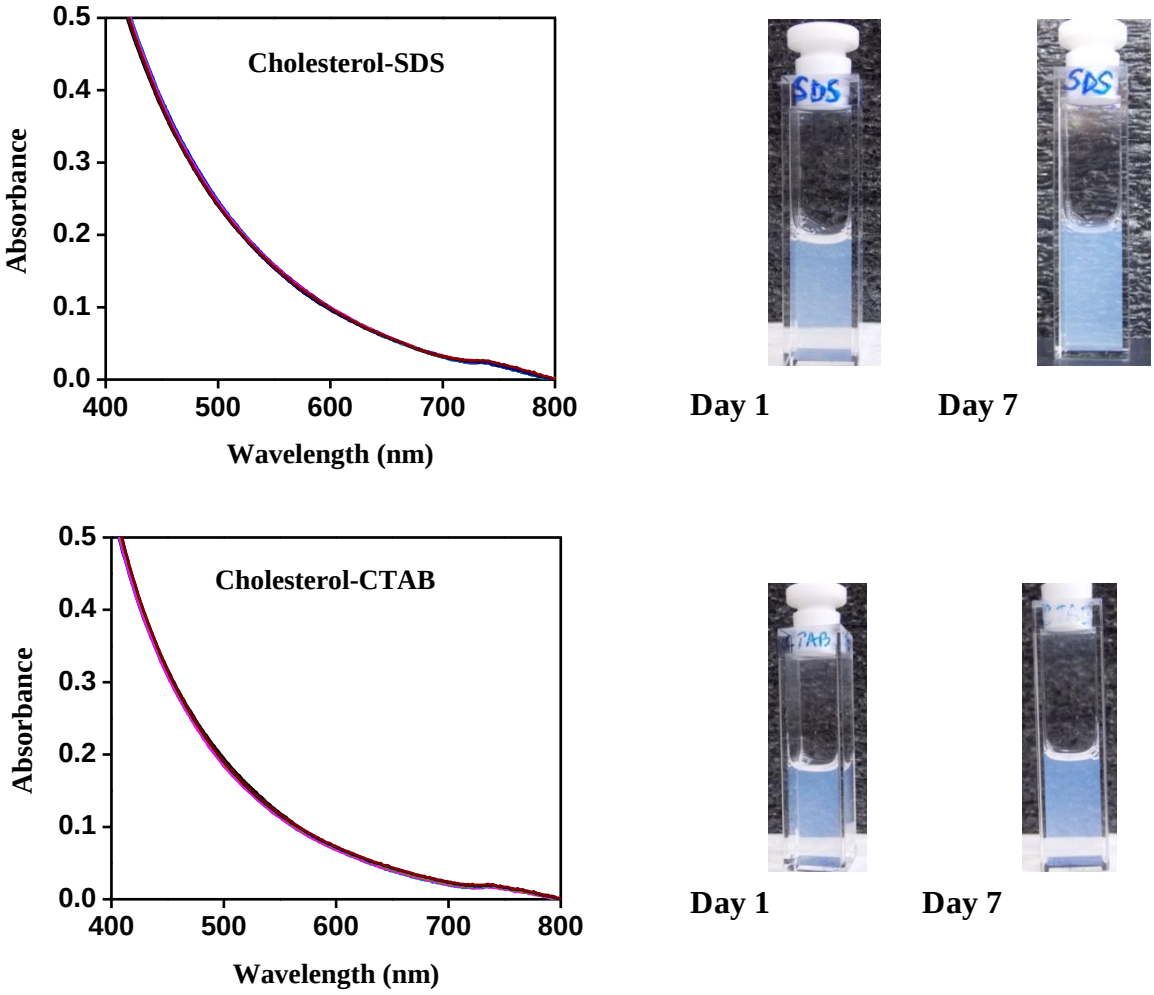


Figure S3: UV-Vis absorption spectra of cholesterol-SDS and cholesterol-CTAB at Q=1.00 (monitored for 7 days)**.** Photographs of the solution mixtures are also depicted for day 1 and day 7.

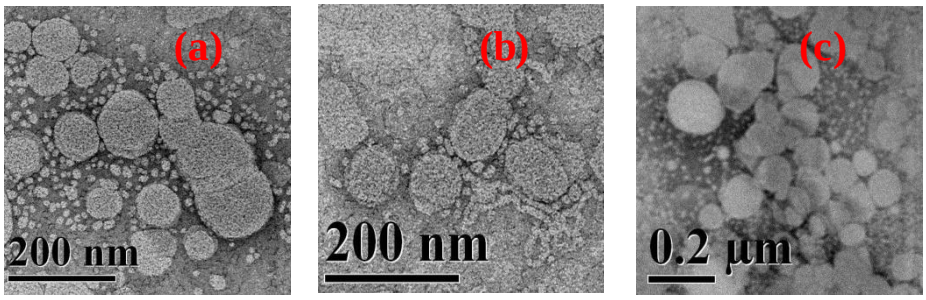


Figure S4: TEM images (a, b, c) of cholesterol-CTAB aggregates at Q=1.00.

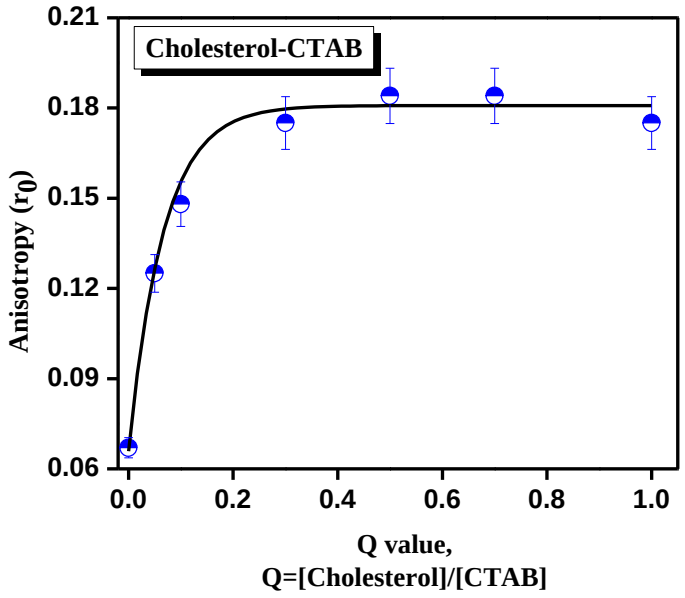


Figure S5: Variation of steady state anisotropy (r_0) of DPH in supramolecular assemblies of CTAB/Cholesterol mixtures in water at different Q ([cholesterol]/[CTAB]) values.

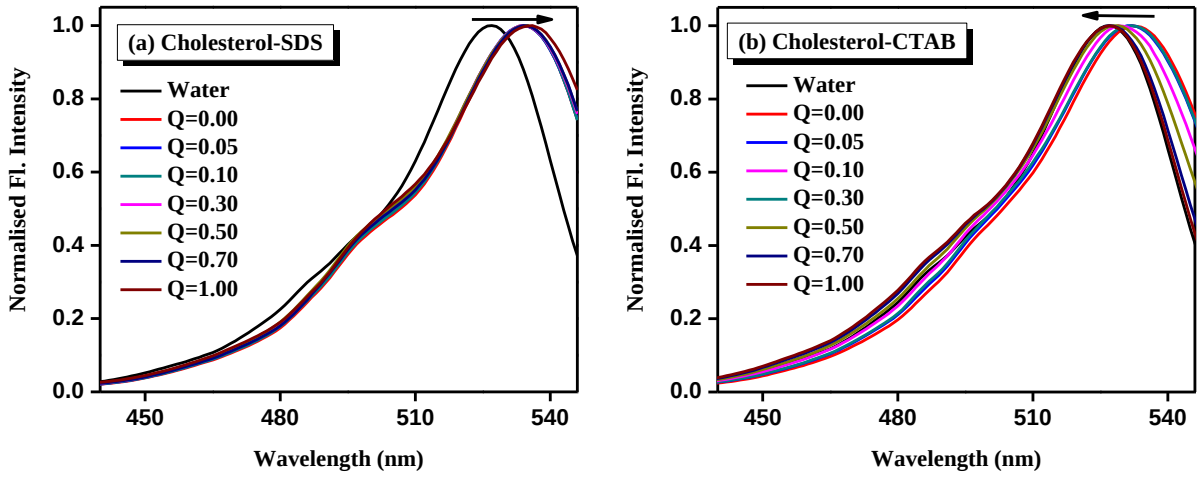


Figure S6: Excitation spectra of R6G in (a) Cholesterol-SDS and (b) Cholesterol-CTAB supramolecular aggregates at different Q values. For the sake of comparison, an excitation spectrum of R6G in water is also shown.

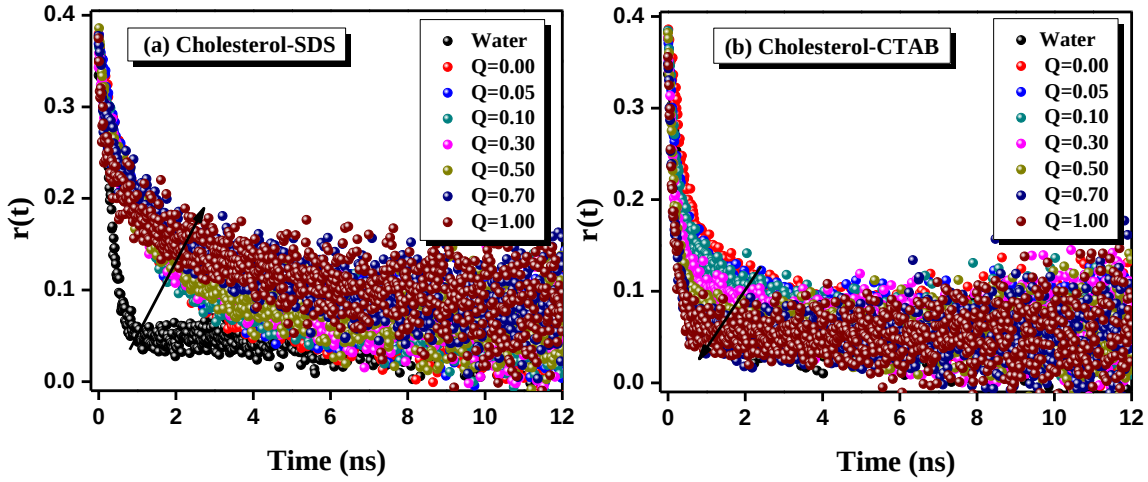


Figure S7: Anisotropy decays of rhodamine 6G in (a) cholesterol-SDS, and (b) cholesterol-CTAB solutions at different Q values. The anisotropy decay of R6G also included in Figure a, b for comparison.

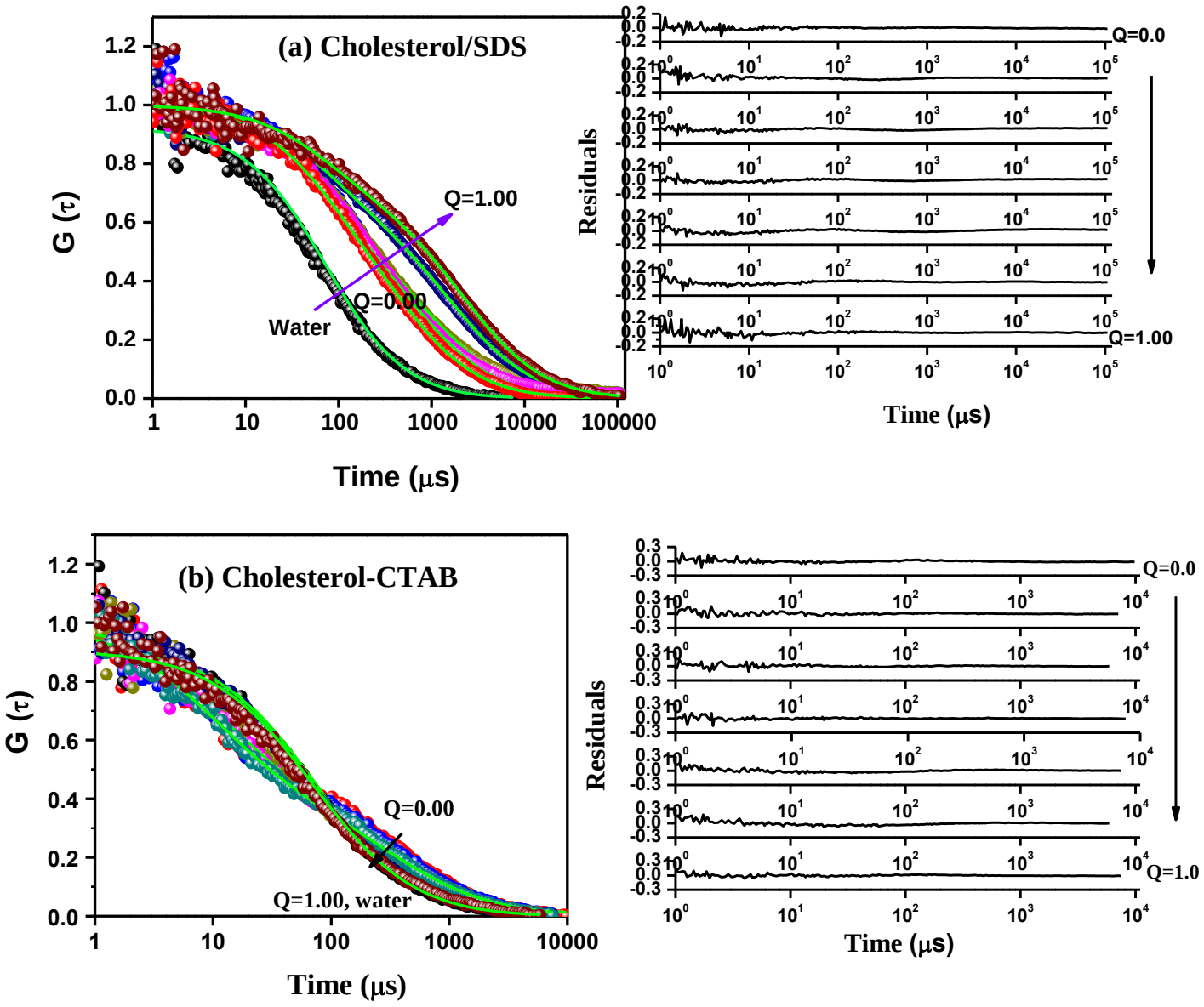


Figure S8: FCS traces of R6G with fitted line and residuals, (a) Cholesterol-SDS and (b) Cholesterol-CTAB.