Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary information

Determination of equilibrium and rate constants for complex formation by Fluorescence Correlation Spectroscopy supplemented by Dynamic Light Scattering and Taylor Dispersion Analysis

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S. 1 Quantum yields ratio

The quantum yield measurements were performed by using Time-Resolved Fluorometer (Edinburgh Analytical Instruments, FL900CDT) at 25°C. The quantum yield equals the ratio of photons emitted (P_{em}) to photons absorbed (P_{abs}) through the fluorescence

$$Q = \frac{P_{em}}{P_{abs}} \qquad (1)$$

In the experiment, we record the fluorescence spectra of rhodamine 110 in pure water and in cetyltrimethylammonium chloride (CTAC) solutions at the excitation wavelength of 499, 500 and 501 nm, respectively. The ratio of quantum yields of rhodamine 110 in the CTAC aqueous solution (Q_B) and in pure water (Q_C) is calculated via

$$\frac{Q_B}{Q_C} = \frac{P_{em}^{B}}{P_{em}^{C}} = \frac{A_{em}^{B}}{A_{em}^{C}} \qquad (2)$$

where A_{em} denotes the area of the fluorescence emission spectrum. We only observe negligible changes in the quantum yield of rhodamine 110 in the CTAC solution as a function of the surfactant concentration (Fig. S1).

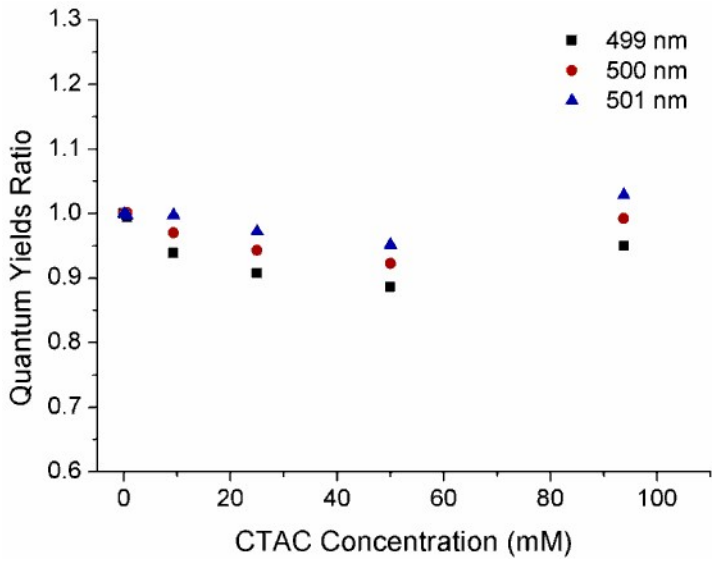

Fig. S1 Ratio of quantum yields of rhodamine 110 in the CTAC surfactant solutions and in pure water as a function of surfactant concentration.

S. 2 Diffusion coefficient of surfactant micelles measured by dynamic light scattering (DLS)

DLS measurements were performed using a Brookhaven BI-200SM goniometer equipped with Contin software. The experiments were conducted at 25°C using a stable argon-ion laser with a wavelength of 514 nm. All samples were filtered through a 0.22 μm filter before the analysis. Each sample was measured at 5 scattering angles at least, ranging from 30° to 150°.

In DLS experiments, the autocorrelation function $G_2(q,t)$ of the light intensity $I(q,t)$ scattered along vector q is recorded. $G_2(q,t)$ is related to the normalized field correlation function $G_1(q,t)$, by the Siegert relation:

$$G_2(q,t) = \beta[G_1(q,t)]^2 \quad (3)$$

where β is the experimental coherence factor. For simple Brownian diffusion, $G_1(q,t)$ can be expressed by the one-component diffusion mode,

$$G_1(q,t) = \exp(-t/\tau) \quad (4)$$

where τ is the decay time of the signal. Combining Eqs. (3) and (4), we obtain

$$G_2(q,t) = \beta\exp(-t/\tau)^2 \quad (5)$$

Fitting the experimental data by Eq. (5) allows us to determine the decay time of the autocorrelation function.

Next, we use the decay time to extract the cooperative diffusion coefficient of micelles (D_c) via

$$1/\tau = D_c q^2 \quad (6)$$

with

$$q = \frac{4\pi n}{\lambda}\sin(\frac{\theta}{2}) \quad (7)$$

where λ is the wavelength of the incident light, n is the refractive index of the sample and θ is the scattering angle.

The self-diffusion coefficient at infinite dilution, D_0, is determined by the relation:[1]

$$D_c = D_0(1 + kC_m) \quad (C_m \rightarrow 0) \quad (8)$$

where C_m is the concentration of micelles and k is a constant. D_c is the cooperative diffusion coefficient that depends on the concentration of micelles due to mutual interactions (attraction or repulsion). The hydrodynamic radius, R_h, is related to D_0, through the Stokes-Sutherland-Einstein equation.

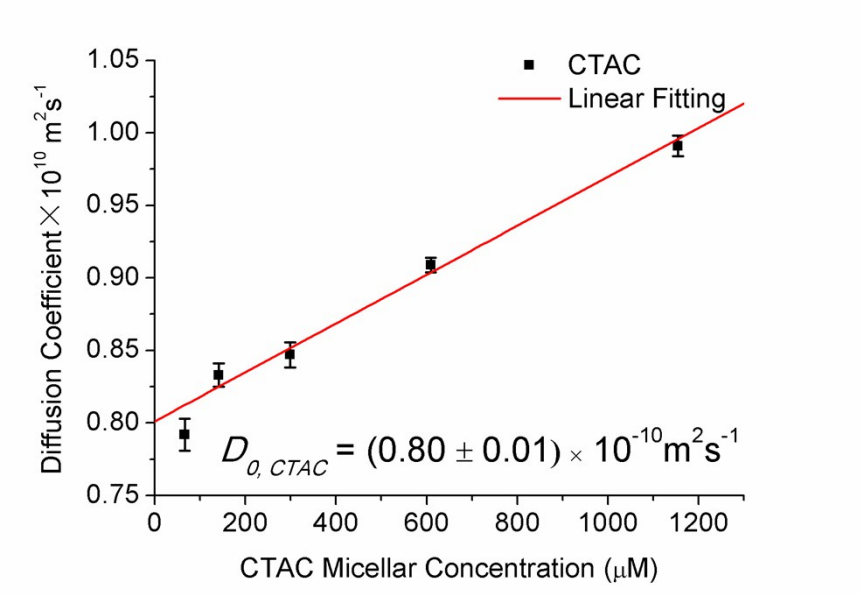

Fig. S2 Linear fit of the cooperative diffusion coefficient of CTAC micelles in aqueous solutions via Eq. (8) Extrapolation concentration to infinite dilution gives the self-diffusion coefficient of CTAC micelle.

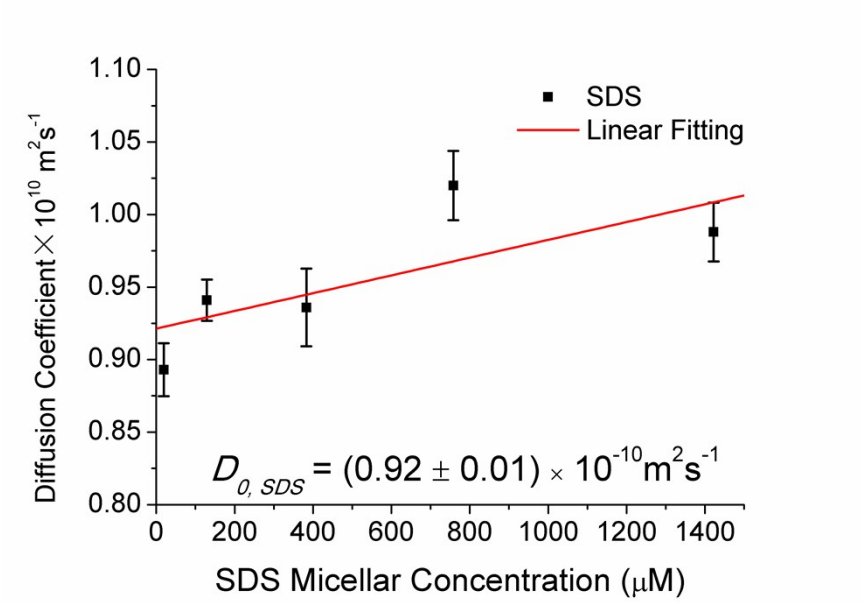

Fig. S3 Linear fit of the cooperative diffusion coefficient of SDS micelles in aqueous solutions via Eq. (8) Extrapolation concentration to infinite dilution gives the self-diffusion coefficient of SDS micelle.

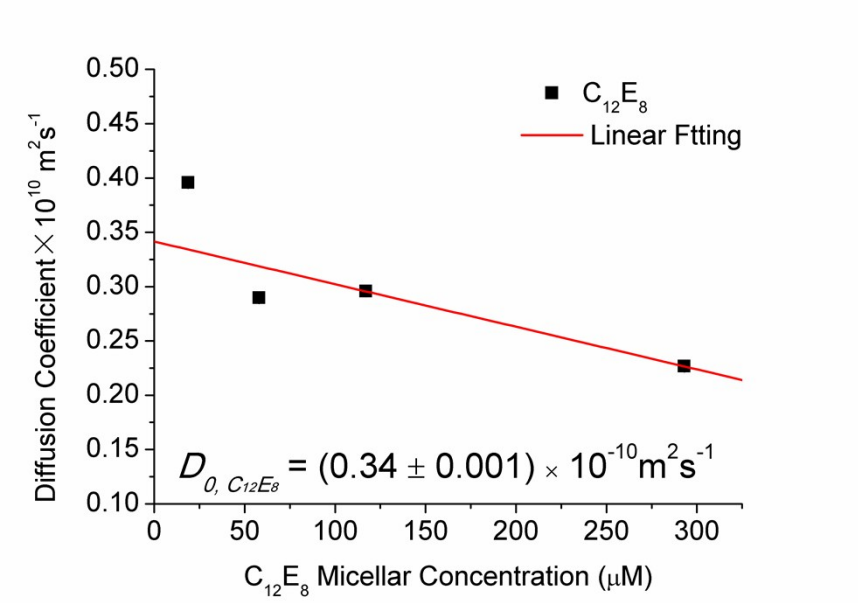

Fig. S4 Linear fit of the cooperative diffusion coefficient of $C_{12}E_8$ micelles in aqueous solutions via Eq. (8) Extrapolation concentration to infinite dilution gives the self-diffusion coefficient of $C_{12}E_8$ micelle.

Extrapolating the micellar concentration to infinite dilution, we obtain the values 0.80×10^{-10} m^2s^{-1}, 0.92×10^{-10} m^2s^{-1} and 0.34×10^{-10} m^2s^{-1}, for the self-diffusion coefficients of CTAC, SDS and $C_{12}E_8$ micelles in aqueous solutions, respectively. In FCS experiments, we only observe the self-diffusion coefficient D_0 (concentration-independent quantity)[2] for all micelles as shown in Figs. S2-S4, because concentrations of the studied dye-micelle complexes are in nanomole range which practically corresponds to the limit of zero concentration in the DLS experiments. Hence, we applied the D_0 of all the studied micelle (from DLS measurements) in analyzing FCS data.

S. 3 Equilibrium constant of Rh110-CTAC interaction determined by Taylor Dispersion Analysis (TDA)

Determination of the equilibrium constant of the dye-micelle interaction by TDA is based on the differences in diffusion coefficients of free dyes in water, free surfactant micelles in water and dyes in micellar solutions. Due to the fact that surfactants do not absorb UV-vis light, we have measured the diffusion coefficient of CTAC micelles by DLS (see Fig. S2).

In TDA experiments, we use the "Flow Injection Method" in long, coiled capillaries to measure the diffusion coefficients of rhodamine 110 in water and in CTAC solutions. The measurements were performed at high flow rates of the carrier phase (31 cm/s). The following scaling equation was used to determine the values of the diffusion coefficients:

$$D = -\frac{1}{48}\frac{u^2R^2}{\sigma_c A \cdot LambertW\left(-1, -\frac{1}{192}\frac{r\,\mu\, e^{-B/A}}{R\,\rho\,\sigma_c A}\right)} \quad (9)$$

where ρ is the density of the carrier phase, u is the average velocity of the flow (averaged across the capillary), μ is the viscosity of the carrier phase, R is the inner radius of the capillary, r is the radius of curvature of the coiled capillary, and σ_c is the dispersion coefficient in a coiled capillary. LambertW is the LambertW function, where A = 0.87 ± 0.02 and B = -3.8 ± 0.2 are fitted parameters.[3]

Then the equilibrium constant K is determined from the following equation:[4, 5]

$$K = \frac{\left(D^{eff} - D_B\right)}{C_m\left(D_A - D^{eff}\right)} \quad (10)$$

where D_B is the diffusion coefficient of the dye, D_A is the diffusion coefficient of the micelle and D^{eff} is the effective diffusion coefficient of the dye-micelle complex. C_m is the concentration of micelles determined by

$$C_m = \frac{C_s - CMC}{N_{ag}} \quad (11)$$

where N_{ag} is the mean number of aggregated surfactant molecules that form one micelle (80 for CTAC)[6] and C_s is the surfactant concentration. CMC is the critical micelle concentration (1.1×10^{-3} M for CTAC).[6]

We have used two solutions of CTAC of the surfactant concentrations 2.50×10^{-3} M and 6.20×10^{-3} M, which correspond to the micelle concentrations (according to Eq. (11)): C_m = 1.74×10^{-5} M and C_m = 6.35×10^{-5} M, respectively. The concentration of the dye in both experiments was 1.10×10^{-4} M.

The equilibrium constant K, calculated via Eq. (10), amounts to 3.61×10^{4} M^{-1}, for the sample with C_m = 1.74×10^{-5} M, and 4.95×10^{4} M^{-1}, for the one with C_m = 6.35×10^{-5} M. The mean value of K is **4.28×10^{4}** M^{-1}.

Table S1. Diffusion coefficients of free rhodamine 110 and free CTAC micelles in water, and the effective diffusion coefficients of rhodamine 110 in CTAC micellar solutions with two different concentrations.

	Rhodamine 110 in water	**CTAC[1] (C_m = 1.74×10^{-5} M)**	**CTAC[2] (C_m = 6.35×10^{-5} M)**	**Rhodamine 110 in CTAC[1]**	**Rhodamine 110 in CTAC[2]**
Diffusion coefficient (D, m^2/s)	3.70×10^{-10}	0.80×10^{-10}	0.84×10^{-10}	2.58×10^{-10}	1.53×10^{-10}

S. 4 Theoretical model of the autocorrelation function

Here we sketch the derivation of the autocorrelation function used to fit the FCS experimental data. More details will be presented elsewhere. The starting point is the model of Elson and Magde[7, 8](see also the review by Krichevsky and Bonnet[9]). The binary reaction $A + B \rightleftarrows C$ is characterized by the equilibrium constant: $K = \frac{k_+}{k_-} = \frac{[C]^{eq}}{[A]^{eq}[B]^{eq}}$, where k_+ and k_- are the association and dissociation rate constants, respectively, and $[A]^{eq}$, $[B]^{eq}$, $[C]^{eq}$ denote the equilibrium concentrations of the components. The set of linearized reaction-diffusion equations for the fluctuations of local concentrations:

$\delta C_A(\vec{r}, t) = C_A(\vec{r}, t) - [A]^{eq}$, $\delta C_B(\vec{r}, t) = C_B(\vec{r}, t) - [B]^{eq}$, $\delta C_C(\vec{r}, t) = C_C(\vec{r}, t) - [C]^{eq}$,

assumes the following form:

$$\frac{\partial \delta C_A}{\partial t} = D_A \nabla^2 \delta C_A - k_+ [B]^{eq} \delta C_A - k_+ [A]^{eq} \delta C_B + k_- \delta C_C \quad (12)$$

$$\frac{\partial \delta C_B}{\partial t} = D_B \nabla^2 \delta C_B - k_+ [B]^{eq} \delta C_A - k_+ [A]^{eq} \delta C_B + k_- \delta C_C \quad (13)$$

$$\frac{\partial \delta C_C}{\partial t} = D_C \nabla^2 \delta C_C + k_+ [B]^{eq} \delta C_A + k_+ [A]^{eq} \delta C_B - k_- \delta C_C \quad (14)$$

where D_A, D_B, D_C denote the diffusion coefficients of the components *A, B* and *C*, which in our case correspond to the micelles, dyes and dye-micelle complexes, respectively, and we

assume that $D_A = D_C = D$. To solve the above set of equations the standard methods of Fourier transform and normal modes are used. The latter are characterized by the eigenvalues:

$$\lambda_0 = -q^2 D, \quad \lambda_\pm = -\frac{1}{2}\left[q^2(D + D_B) + R\right] \pm \frac{1}{2}\sqrt{q^4|\Delta|^2 + 2\varepsilon q^2|\Delta|R + R^2} \tag{15}$$

and the corresponding eigenvectors,[7] where q is the magnitude of the wave vector. $R = k_+([A]^{eq} + [B]^{eq}) + k_-$ is the chemical relaxation rate, $\varepsilon = 2\beta - 1$, $\beta = k_+[A]^{eq}/R$, and $|\Delta| = D_B - D$. In FCS, the correlations of concentration fluctuations, $\langle \delta C_j(\vec{r}, 0)\delta C_l(\vec{r}', t)\rangle$, are monitored, where j and l run over the fluorescent components. The autocorrelation function of intensity fluctuations, $G(t)$, is defined as a convolution of the auto- and cross-correlation functions of these concentration fluctuations with the illumination intensity, $I(\vec{r})$, which is usually assumed Gaussian:

$$I(\vec{r}) = I_0 exp\left[2(x^2 + y^2)/L^2 - 2z^2/H^2\right] \tag{16}$$

where H and L are the sizes of the beam waist in the direction of light propagation and in the perpendicular direction, respectively. Our approximation for $G(t)$ is based on the application of the double-tangent construction to $\lambda_\pm(q^2)$, which become linear functions of q^2 in the limits $q \to 0$ and $q \to \infty$, hence we assume that

$$\lambda_+ \approx \begin{cases} -D_+ q^2, & q < q_c \\ -Dq^2 - R(1-\beta), & q > q_c \end{cases} \tag{17}$$

$$\lambda_- \approx \begin{cases} -D_- q^2 - R, & q < q_c \\ -D_B q^2 - R\beta, & q > q_c \end{cases} \tag{18}$$

where $q_c^2 = R/|\Delta|$ is the intersect of the tangents at $q = 0$ and $q = \infty$, and $D_+ = D\beta + D_B(1-\beta)$, $D_- = D(1-\beta) + D_B\beta$ are the effective diffusion coefficients. A similar approximation is also applied to the amplitudes of the exponential factors $e^{t\lambda_\pm}$. This leads to the following formula for $G(t)$, normalized to unity at $t = 0$:

$$G(t) \approx \frac{y\beta}{1-y+y\beta}h\left(\frac{t}{\tau_A}\right) + \frac{1-y}{1-y+y\beta}\left\{h\left(\frac{t}{\tau_+}\right)\left[1-e^{-R\tau_\Delta\left(1+\frac{t}{\tau_+}\right)}\right] + \beta h\left(\frac{t}{\tau_A}\right)e^{-R\tau_\Delta\left(1+\frac{t}{\tau_+}\right.}\right. \quad (19)$$

where $h\left(\frac{t}{\tau}\right) = \left(1+\frac{t}{\tau}\right)^{-1}\left(1+\frac{t}{\omega^2\tau}\right)^{-1/2}$, $\omega = H/L$, τ denotes one of the relaxation times: $\tau_A = \frac{L^2}{4D}$, $\tau_B = \frac{L^2}{4D_B}$, $\tau_\pm = \frac{L^2}{4D_\pm}$ or $\tau_\Delta = \frac{L^2}{4|\Delta|}$, and $y = K[B]^{eq}/(1+K[B]^{eq})$.

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