Supporting Information

Caffeine mediated detachment of mutagenic ethidium from various nanoscopic micelle: An ultrafast FRET study

Soma Banerjee[a], Masanori Tachiya[b] and Samir Kumar Pal[*a]

[a] *Department of Chemical, Biological & Macromolecular Sciences,*

S. N. Bose National Centre for Basic Sciences,

Block JD, Sector III, Salt Lake,

Kolkata 700 098, India

[b]*National Institute of*

Advanced Industrial Science and Technology (AIST), Tsukuba

Ibaraki 305-8565, Japan

[*]Corresponding Authors Email: skpal@bose.res.in (S. K. Pal)

Fax: 91 33 2335 3477,

1. The details of time resolved fluorescence results along with those of fluorescence anisotropy of ethidium (Et) in a wide range of CTAB concentrations (0.2 to 80 mM) both in absence and presence of caffeine:

The critical micelle concentration CMC of CTAB being 0.9 mM [1] our experiments cover both below and above the CMC of CTAB.

Table S1: The fluorescence lifetime components τ_1 and τ_2 represent fluorescence lifetimes of the sample while τ_r represents rotational relaxation time constants of the same and r_0 defines anisotropy at time t=0. Numbers in parentheses show relative contribution. Error ±5%

Et in	τ_1 (ns)	τ_2 (ns)	Fluorescence anisotropy	
			τ_r (ns)	r_0
water	1.6 (100%)		0.110	0.24
100 mM caffeine	2.3 (15%)	7.0 (85%)	0.210	0.23
0.2 mM CTAB	1.6 (100%)		0.100	0.30
0.2 mM CTAB + 100 mM caffeine	1.7 (13%)	6.9 (87%)	0.170	0.34
0.4 mM CTAB	1.6 (100%)		0.110	0.35
0.4 mM CTAB + 100 mM caffeine	1.7 (14%)	7.0 (86%)	0.180	0.34
0.6 mM CTAB	1.6 (100%)		0.110	0.32
0.6 mM CTAB + 100 mM caffeine	1.8 (15%)	6.9 (85%)	0.160	0.37
2 mM CTAB	1.1 (34%)	1.9 (66%)	0.100	0.32
2 mM CTAB + 100 mM caffeine	1.8 (14%)	6.9 (86%)	0.170	0.38
20 mM CTAB	1.2 (45%)	2.1 (55%)	0.110	0.32
20 mM CTAB + 100 mM caffeine	1.8 (14%)	6.9 (86%)	0.180	0.35
40 mM CTAB	1.2 (40%)	2.0 (60%)	0.120	0.29
40 mM CTAB + 100	1.7 (14%)	6.9 (86%)	0.190	0.33

mM caffeine				
80 mM CTAB	1.4 (68%)	2.7 (32%)	0.120	0.33
80 mM CTAB + 100 mM caffeine	1.7 (14%)	6.9 (86%)	0.190	0.32

2. Absorbance, steady state as well as time resolved emission along with the fluorescence anisotropy of Et at different concentrations of SDS and TX-100 both in absence and presence of caffeine:

The results and interpretations are given below separately for SDS and TX-100 and emphasize on the partitioning and location of Et in SDS and TX-100 micelle.

For SDS:

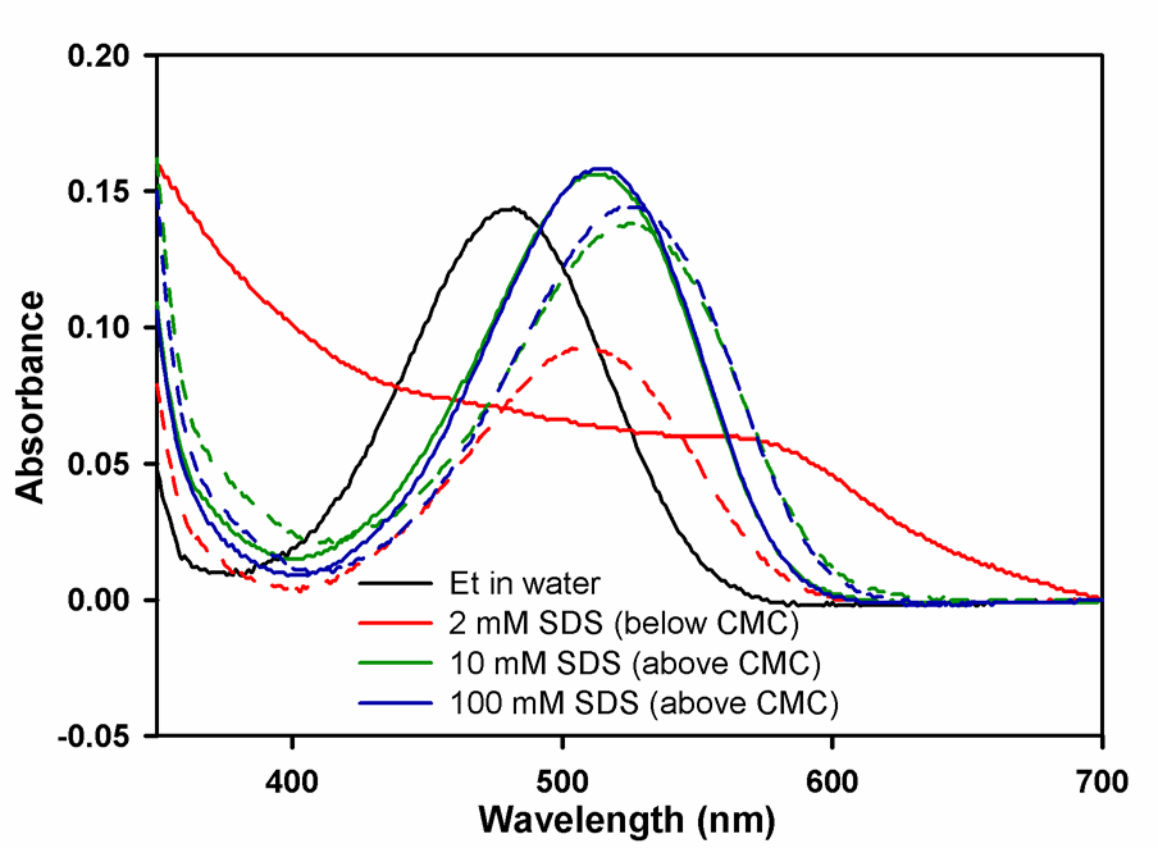

Figure S1: Absorption of ethidium (Et) at different concentrations of SDS with (broken line) and without (bold line) caffeine.

As evident from the above Figure S1, at 2 mM SDS concentration which is below its CMC i.e 8 mM, there is ionic interaction between positively charged Et and negatively charged SDS monomers and produces huge red shift in the absorption peak of Et compared to that in water. The concentration of Et is kept constant in all the solutions at 25 μM. Upon addition of 100 mM caffeine (red broken line), the absorption peak of Et shifts toward the blue end compared to that in 2 mM SDS which indicates that caffeine disrupts the ionic interaction between Et and SDS monomers and forms a complex with Et that produces a characteristic absorption band different from that in water. At SDS concentrations above its CMC i.e. at 10 and 100 mM there is no signature of high ionic interaction as found in case of Et and SDS monomers. However, the absorption peak of Et is significantly red shifted compared to that in water which indicates that Et attaches to SDS micelle with its positively charged moiety towards the negatively charged head group of the SDS micelle and hydrophobic part inside the micelle. The hypochromic effect and the bathochromic shift in the absorption peak of Et in SDS micelle (at 10 and 100 mM SDS concentrations) upon addition of caffeine indicates that caffeine releases a fraction of SDS micelle bound Et and forms a charged complex with the released Et.

From the difference in optical density (O.D) value of Et in SDS micelle in absence and presence of 100 mM caffeine we calculated the amount of Et released from the micelle by caffeine taking the molar extinction coefficient of Et in SDS micelle as 4120 M^{-1} cm^{-1} at 476 nm. It has been found that from 25 μM micelle bound Et, 18.5 μM of Et gets released from the micelle by caffeine while 6.5 μM Et still remains attached to the micelle.

Table S2: The fluorescence lifetime components τ_1 and τ_2 represent fluorescence lifetimes of the sample while τ_{r1} and τ_{r2} represent rotational relaxation time constants of the same and r_0 defines anisotropy at time t=0. Numbers in parentheses show relative contribution. Error ±5%

Et in	τ_1 (ns)	τ_2 (ns)	Fluorescence anisotropy		
			τ_{r1} (ns)	τ_{r2}	r_0
water	1.6 (100%)		0.110		0.24
100 mM caffeine	2.3 (15%)	7.0 (85%)	0.210		0.23
2 mM SDS	1.6 (89%)	0.08(11%)	0.070 (100%)		0.38
2 mM SDS + 100 mM caffeine	1.7 (11%)	6.6 (89%)	0.180 (100%)		0.31
4 mM SDS	1.8 (83%)	0.13 (17%)	0.09 (100%)		0.37

4 mM SDS + 100 mM caffeine	1.8 (13%)	6.8 (87%)	0.18 (100%)		0.29
6 mM SDS	2.4 (87%)	0.64 (13%)	0.28 (100%)		0.23
6 mM SDS + 100 mM caffeine	1.2 (6%)	6.1 (94%)	0.18 (100%)		0.29
10 mM SDS	1.1 (5%)	4.7 (95%)	1.0 (57%)	0.09 (43%)	0.27
10 mM SDS + 100 mM caffeine	1.3 (6%)	6.4 (94%)	1.6 (47%)	0.19 (53%)	0.25
20 mM SDS	1.4 (9%)	4.7 (91%)	3.4 (34%)	0.39 (66%)	0.24
20 mM SDS + 100 mM caffeine	1.5 (8%)	6.4 (92%)	1.3 (46%)	0.08 (54%)	0.32
40 mM SDS	1.4 (10%)	4.8 (90%)	2.3 (40%)	0.36 (60%)	0.19
40 mM SDS + 100 mM caffeine	1.7 (10%)	6.5 (90%)	2.1 (46%)	0.22(54%)	0.29
80 mM SDS	1.4 (9%)	4.8 (91%)	2.2 (42%)	0.38 (58%)	0.27
80 mM SDS + 100 mM caffeine	1.6 (10%)	6.3 (90%)	2.9 (37%)	0.39 (63%)	0.33
100 mM SDS	1.4 (9%)	4.8 (91%)	1.9 (44%)	0.23 (56%)	0.22
100 mM SDS + 100 mM caffeine	1.5 (9%)	6.2 (91%)	2.9 (43%)	0.35 (57%)	0.32

The longer anisotropy lifetime of Et in SDS solution beyond the CMC of SDS is in strong agreement with the proposed model positioning the positive charge of the quarternary Nitrogen of EtBr towards the negatively charged head group of the micelle and hydrophobic part of the Et being buried inside.

For TX-100:

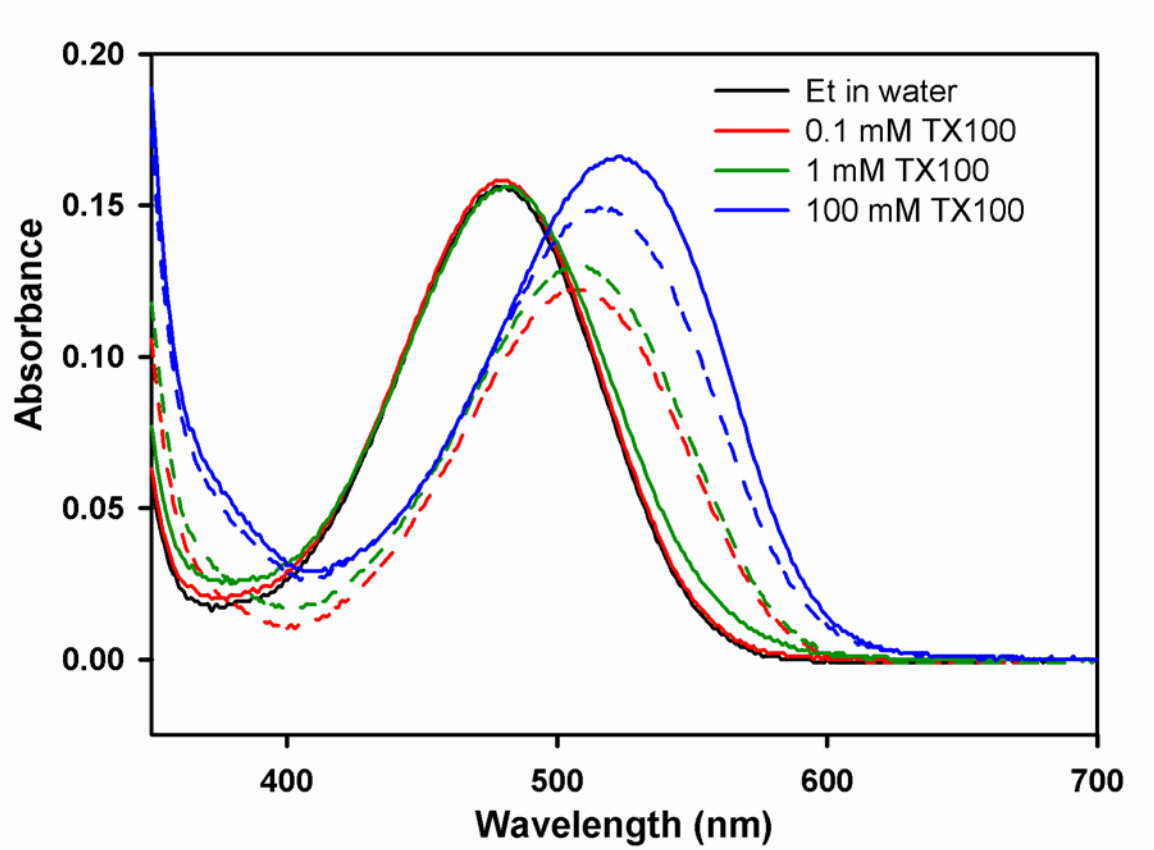

Figure S2: Absorption of ethidium (Et) at different concentrations of TX-100 with (broken line) and without (bold line) caffeine.

As evidenced from the absorption spectra of Et at different concentrations of TX-100, Et neither binds to TX-100 monomers nor to TX-100 micelles at lower concentrations (8.2×10^{-3} mM micellar concentration). However, at high micellar concentration (1mM) we observed bathochromic shift in the absorption peak of Et compared to that in water which reflects association of Et with TX-100 micelle positioning the quarternary Nitrogen of Et towards the hydrophilic head group of the micelle i.e. towards the ethylene oxide part (see the structure of TX-100 given in Figure S7) and hydrophobic part being buried inside. The proposed model collaborates with the fluorescence anisotropy results where we find longer rotational time constant of Et at 100 mM TX-100 concentration. However, detail analysis of the absorption and time resolved spectroscopy results show that caffeine fails to detach Et from TX-100 micelle unlike SDS and CTAB micelles.

Table S3: The fluorescence lifetime components τ_1 and τ_2 represent fluorescence lifetimes of the sample while τ_{r1} and τ_{r2} represent rotational relaxation time constants of the same and r_0 defines anisotropy at time t=0. Numbers in parentheses show relative contribution. Error ±5%

Et in	τ_1 (ns)	τ_2 (ns)	Fluorescence anisotropy		
			τ_{r1} (ns)	τ_{r2} (ns)	r_0
water	1.6 (100%)		0.110		0.24
100 mM caffeine	2.3 (15%)	7.0 (85%)	0.210		0.23
0.1 mM TX-100	1.6 (100%)		0.108		0.35
0.1 mM TX-100 + 100 mM caffeine	2.0 (16%)	7.0 (84%)	0.175		0.36
1 mM TX-100	1.6 (100%)		0.103		0.33
1 mM TX-100 + 100 mM caffeine	1.9 (15%)	6.9 (85%)	0.196		0.30
100 mM TX-100	1.1 (16%)	4.5 (84%)	0.053 (81%)	1.51 (19%)	0.38
100 mM TX-100 + 100 mM caffeine	1.6 (15%)	6.5 (85%)	0.08 (80%)	1.17 (20%)	0.37

3. Resolution of time correlated single photon counting (TCSPC) setup:

The instrument response function (IRF) of TCSPC setup is of 80 ps. However it can resolve time components upto 20 ps. We have plotted the fluorescence decays of H258 in the presence of Et and caffeine in SDS micelle over 1 ns and the Figure S3 given below shows that the decays are distinctly different from the IRF. It has to be noted that, with our time-resolved instrument, we can resolve at least one-fourth of the instrument response time constants after the deconvolution of the IRF.

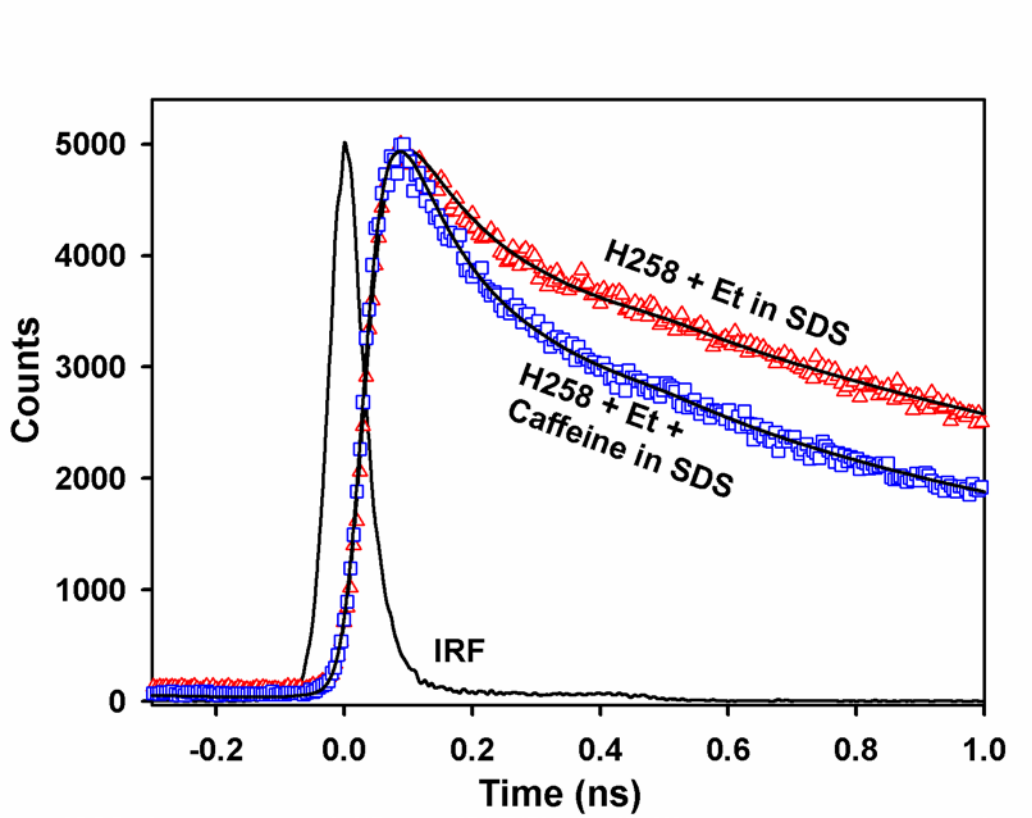

Figure S3: Resolution of TCSPC setup, in order to show distinct fluorescence decays of the probe H258 in SDS micelle in presence of Et and caffeine from the IRF.

4. Förster resonance energy transfer (FRET) between coumarin 500 (C500) and Et on SDS micelle in absence and presence of caffeine:

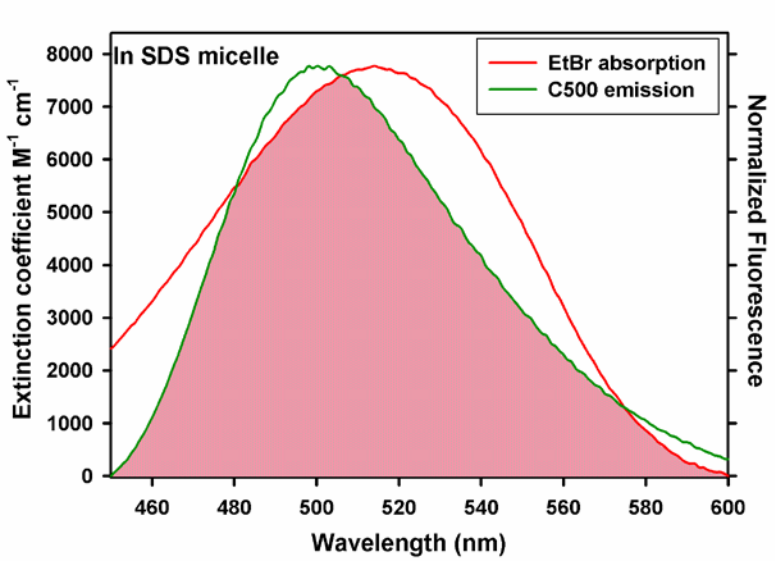

Figure S4: The spectral overlap of C500 emission and Et absorption in (a) 20 mM SDS (at 70^{o}C).

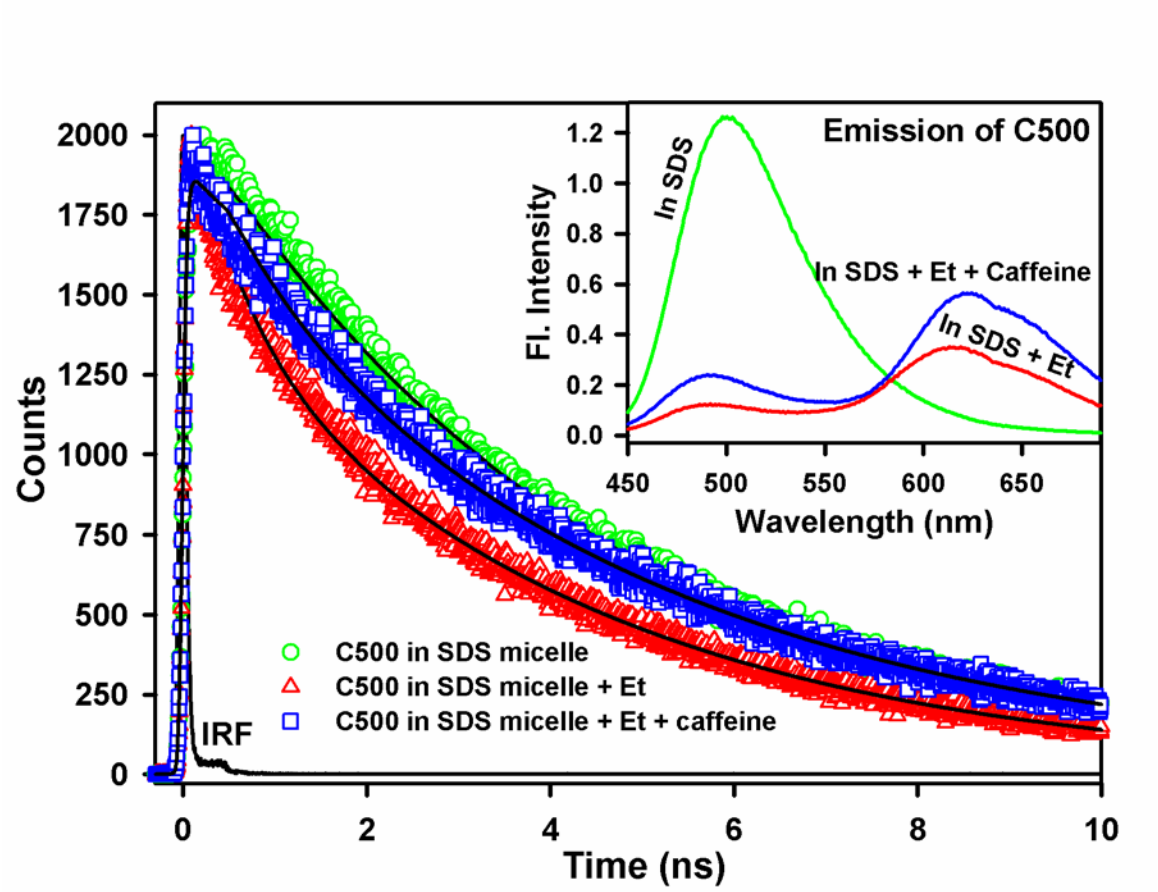

Figure S5: The temporal decays of C500 in SDS micelle at 70°C in presence and absence of acceptor Et ([Et] = 155 μM) and caffeine ([caffeine] = 100 mM) in 20 mM SDS. The corresponding emission spectra are given in inset.

Table S4: The lifetime components of C500 at 70°C in various environments: τ represent the time constants in ns and the numbers in the parentheses represent relative contribution of the components**.** Error ±5%

Sample	τ_1 (ns)	τ_2 (ns)
C500 in SDS	4.33 (100%)	
C500 in SDS + Et	4.33 (67%)	0.56 (33%)
C500 in SDS + Et + caffeine	4.33 (81%)	0.84 (19%)
C500 in SDS + caffeine	4.33 (100%)	
C500 in water	3.34 (100%)	

For FRET calculation, the orientation parameter (κ^2) can be taken as 0.667^2 (see main text). The refractive index (n) of the medium is assumed to be 1.4. Q_D, the quantum yield of the donor C500 in SDS micelle in the absence of acceptor Et, was calculated according to the equation[3].

$$Q = Q_R\left(\frac{I}{I_R}\right)\left(\frac{OD_R}{OD}\right)\left(\frac{n^2}{n_R^2}\right) \quad (3)$$

where Q and Q_R are the quantum yield of C500 in SDS micelle and reference (C500 in water), I and I_R are the integrated fluorescence intensities of C500 in SDS micelle and reference, OD and OD_R are the optical densities of C500 in SDS micelle and reference at the excitation wavelength, and n and n_R are the refractive indices of C500 in SDS micelle and reference solutions. The absolute quantum yield of C500[4] in water was taken to be 0.36. Refractive indices of the solutions are measured by using Rudolph J357 automatic refractometer. The quantum yield of C500 in SDS micelle has been found to be 0.62.

We have also fitted the fluorescence decays of the probe C500 in SDS micelle in presence and absence of the quencher (Et) and caffeine molecules with the Infelta-Tachiya model and again found that the mean number of quencher molecules associated with the micelle (m) reduces in presence of caffeine emphasizing the caffeine mediated release of Et from the micelle.

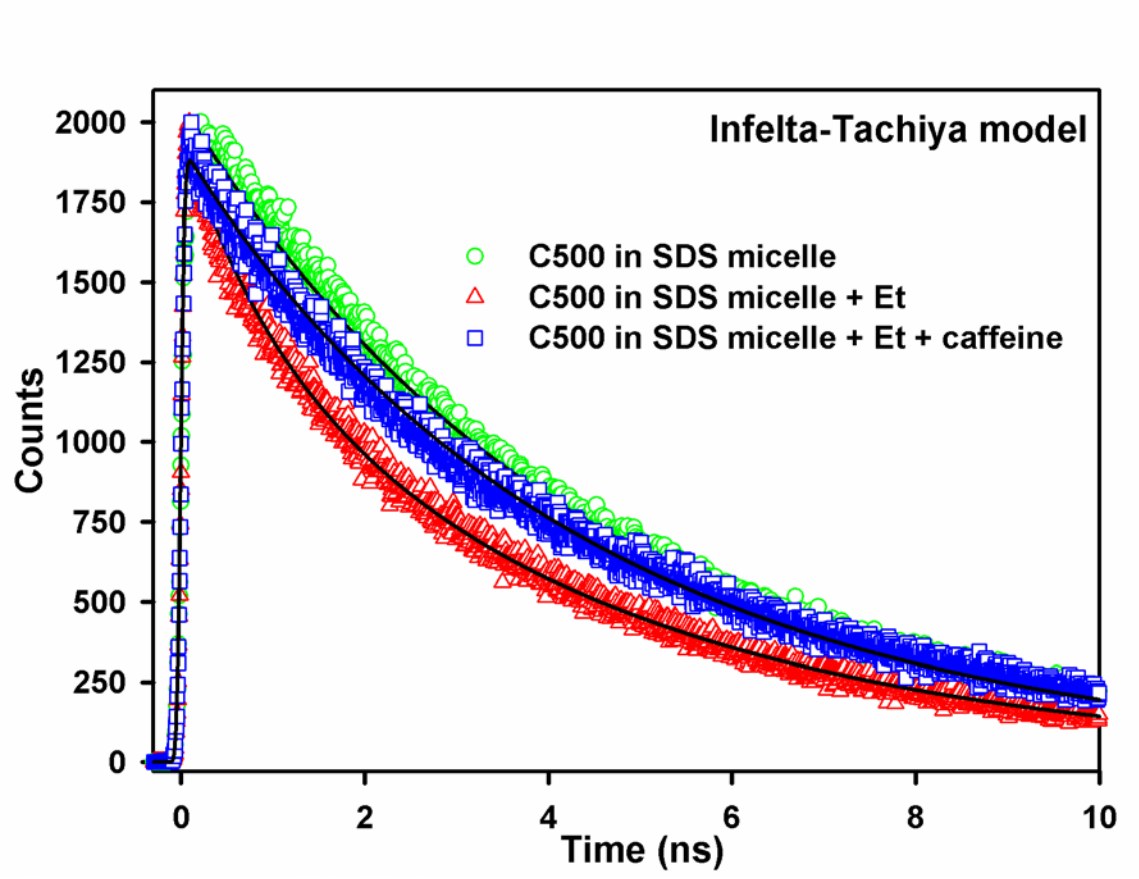

Figure S6. Time resolved fluorescence decay curves of H258 in SDS micelle in absence and presence of caffeine and Et. The bold lines represent the fitting of the curves by the generalised version of the kinetic models developed by Infelta and Tachiya (see main text).

Table S5: Values of the quenching parameters using the simplified version of the model developed by Infelta-Tachiya: Error ±5%

System	k_0	m	k_q
Micelle bound C500	0.23		
Micelle bound C500 + Et	0.23	0.35	0.70
Micelle bound C500 + Et + caffeine	0.23	0.02	0.70

5. Structures of the alkaloid, dyes and surfactants used in the study:

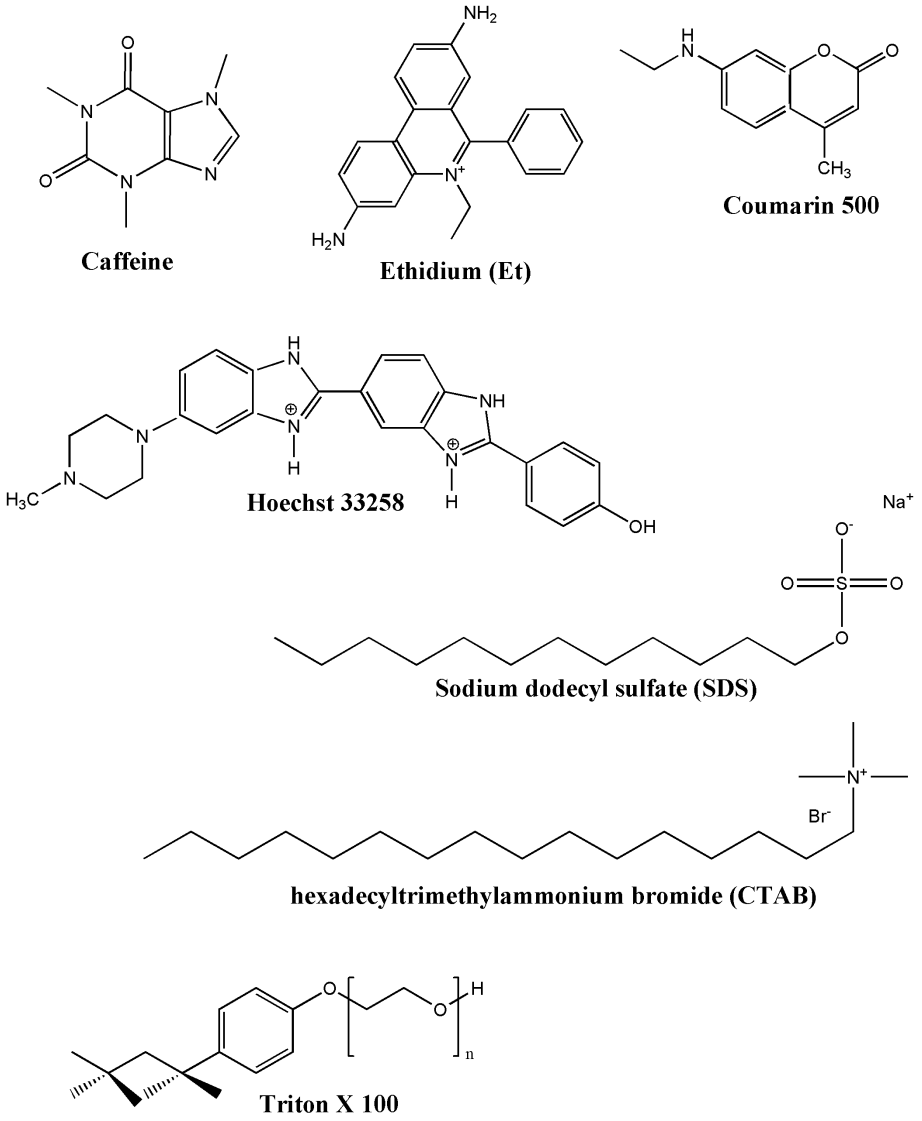

Figure S7: Structures of the caffeine, dyes Hoechst 33258 (H258), Coumarin 500 (C500), ethidium (Et) and surfactants SDS, CTAB and Triton X 100 (TX-100).

References:

(1)Cifuentes, A.; Bernal, J. L.; Diez-Masa, J. C. *Anal. Chem.* **1997**, *69*, 4271-4274.
(2)Lakowicz, J. R. *Principles of fluorescence spectroscopy*; Kluwer Academic/Plenum: New York, 1999.
(3)Goswami, N.; Makhal, A.; Pal, S. K. *J. Phys. Chem. B.* **2010**, *114*, 15236-15243.
(4)Das, K.; Jain, B.; Gupta, P. K. *Chem. Phys. Lett.* **2005**, *410*, 160-164.