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Picosecond Electronic Energy-transfer Studies in Sodium Dodecyl Sulfate Micelles

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Electronic energy-transfer between several pairs of extrinsic organic probes incorporated in SDS micelles was studied in the picosecond domain by following the donor fluorescence decay. Deviations to kinetic models where independent spatial distribution is assumed for donor and acceptor are attributed to probe induced perturbations of the micellar structure that result in an attractively correlated spatial distribution for the donor–acceptor pair.

The explicit distance dependence of dipolar electronic energy transfer (r^{-6}) allows its use as a tool to infer about the spatial distribution of molecules solubilized in micelles,[1] and therefore on aspects of the molecule–micelle interaction.

The use of foreign molecules to probe the micellar structure has been the subject of some debate, and severe criticisms were expressed, on the grounds of induced perturbations, namely regarding water penetration.[2] In spite of this, little is known on the nature or importance of such perturbations, especially for low occupancy numbers.[3]

There is experimental evidence for strong perturbation of sodium dodecyl sulfate (SDS) micelles by probes possessing long alkyl chains such as the *n*-(9-anthroyloxy)stearic acid series (*n*-AS)[1,4] or functionalized dyes.[1,5] Perturbation of other small globular micelles by similar probes was also reported.[6] Observed effects in SDS micelles are: induced size growth for a family of tris(bipyridyl)ruthenium(II);[5] formation of large (albeit unknown) structures for the *n*-AS family, in the absence of salts and for probe to surfactant ratios as low as 1 : 18;[4] closer-than-random relative distance distribution of pairs of probes, *e.g.* Triton X-100 monomer and *n*-AS molecule.[1]

In this work, the correlation of locations effect reported in ref. 1 is studied by time-resolved fluorescence in the picosecond domain for probes constituting energy transfer donor–acceptor pairs.

The effect of chain possession by the probe is investigated by using pairs where one or both of the partners is devoid of such a part.

Models for Energy-transfer Kinetics

Energy transfer is assumed to occur by the dipolar (Förster) mechanism. Neglecting the intrinsic decay, the donor survival probability S for a single pair at a distance r is[7]

$$S(r) = \exp\left[-\frac{3}{2}\overline{k^2}(t)\left(\frac{\bar{R}_0}{r}\right)^6 \frac{t}{\tau}\right] \quad (1)$$

where τ is the lifetime of the donor, $\bar{R}_0$ is the isotropic, dynamically averaged Förster radius for the pair and $\overline{k^2}$ is the finite time average of the orientational factor.

$$\overline{k^2}(t) = \frac{1}{t}\int_0^t k^2(t')\,\mathrm{d}t' \quad (2)$$

For a large number of pairs, the average survival probability is

$$\langle S(r)\rangle = \int_0^4 f(\overline{k^2})S(r)\,\mathrm{d}\overline{k^2} \quad (3)$$

where $f(\overline{k^2})$ is the time-dependent distribution of the $\overline{k^2}$. Two limiting cases are now considered for randomly oriented molecules: frozen and fast-compared-to-transfer rotation, *i.e.* the so-called static and dynamic regimes.[8] The rationale for considering randomly oriented pairs will be given in the Discussion section.

In the static situation, one has[7,8]

$$f_s(\overline{k^2}) = f(k^2) = \frac{1}{2\sqrt{(3k^2)}}\{\ln(2+\sqrt{3}) - H(k^2-1)\ln[\sqrt{(k^2)} + \sqrt{(k^2-1)}]\} \quad (4)$$

H being the Heaviside function. On the other hand, in the dynamic regime

$$f_d(\overline{k^2}) = \delta(\overline{k^2} - 2/3) \quad (5)$$

where δ is the delta function.

The macroscopic decay law is obtained from eqn. (3) by appropriate weighting with respect to distance and number of pairs. Two limiting distance distributions are considered for donors and acceptors independently distributed:[1] donor and acceptor on concentric spherical surfaces (Fig. 1) corresponding to well defined radial locations in the micelle (a for the inner probe, R_s for the outer one) and a uniform distribution of each within a sphere (of radius R_s), corresponding to totally undefined locations in the micelle. Taking into account the Poissonian distribution of acceptors amongst the micelles with a mean occupation number μ (μ = [acceptor]/[micelle]) the macroscopic decay ρ is obtained as[1]

$$\rho = \exp(-t/\tau)\exp[-\mu(1-J)] \quad (6)$$

where $J(t)$ is given by

$$J = \int g(r) < S(r) > \mathrm{d}r \quad (7)$$

$g(r)$ being the distance distribution function.

For the radial location model, $g(r)$ is given by[9]

$$g_r(r) = \frac{r}{2aR_s}; \quad r\varepsilon[R_s - a, R_s + a] \quad (8)$$

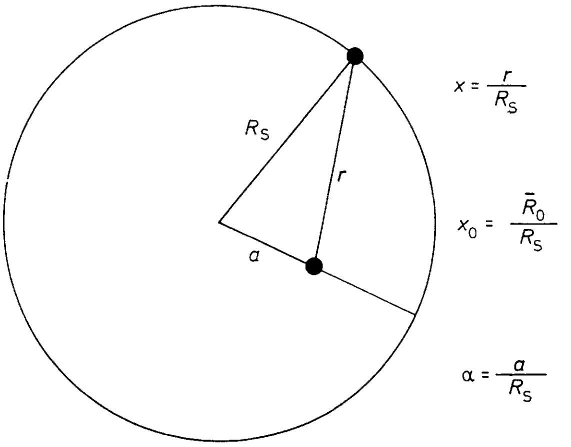


Fig. 1 Geometry and adimensional parameters of the system for the R and D model

while for the uniform distribution model it is[9]

$$g_u(r) = \frac{3r^2}{16R_s^3}\left(\frac{r}{R_s} - 2\right)^2\left(\frac{r}{R_s} + 4\right); \quad r\epsilon[0, 2R_s] \qquad (9)$$

Four different theoretical decay laws are thus obtained namely, radial and static (R and S), uniform and static (U and S), radial and dynamic (R and D) and uniform and dynamic (U and D). The static models always correspond to lower efficiencies of transfer and exhibit smaller 'transients' as shown in Fig. 2 and also discussed in ref. 1 for the steady state. In other treatments of energy transfer in spherical geometry[10,11] the static case was not dealt with adequately, the results for three-[10] or two[11]-dimensional infinite medium being used, these constituting poor approximations for this restricted geometry situation. Note that even for systems without rotational mobility, the kinetics of energy transfer may be close to the dynamic case owing to mixed polarization in the donor emission and/or acceptor absorption in the overlap region.[1]

It is important to estimate theoretically the time region where deviations to single exponential (due to energy transfer) are noticeable in the decay. It is shown in Fig. 3 that this region covers a timescale comparable to the donor's intrinsic lifetime.

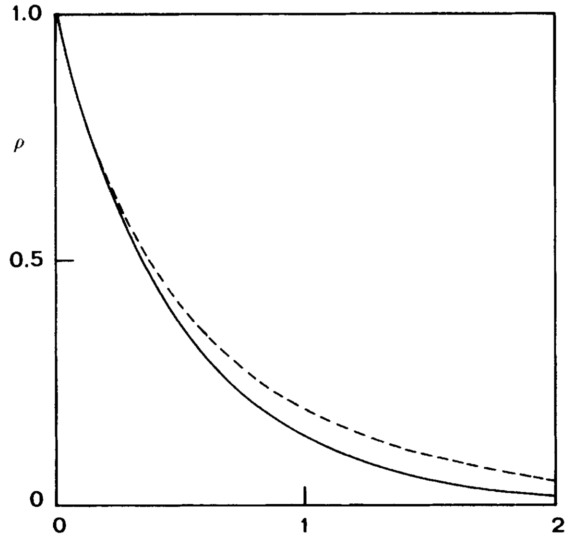


Fig. 2 Theoretical decay for the donor with an acceptor at a distance R_0 in the static (– – –) and dynamic (——) regimes; $\theta = t/\tau$

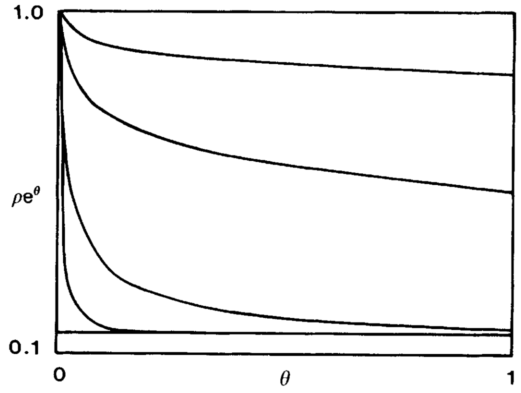
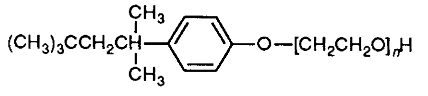


Fig. 3 pe^θ *vs.* θ (R and D model) for $\alpha = 1$, $\mu = 2$ and, from the top, $x_0 = 0.5$, 1, 2 and 3; $\theta = t/\tau$

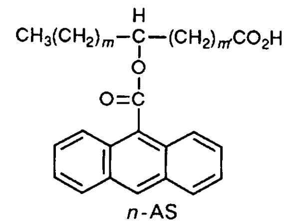
Materials and Methods

Chemicals

The surfactants Triton X-100 (BDH, scintillation grade), sodium dodecyl sulfate (SDS, Merck, p.a.) and the probes *n*-(9-anthroyloxy) stearic acids (*n*-AS; n = 3, 6, 9, 12, Molecular Probes, Eugene, Oregon) were used as received. The scintillator 2,5-diphenyloxazole (PPO, Nuclear Enterprises, scintillation grade) and acridine orange (AO, Fluka for microscopy) also underwent no further purification. Molecular structures are shown below.

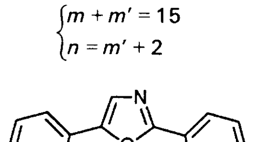


Triton X-100 ($\bar{n}$ = 9–10)

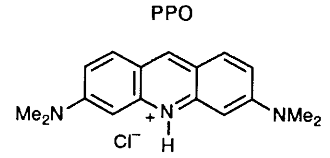


n-AS

$$\begin{cases} m + m' = 15 \\ n = m' + 2 \end{cases}$$



PPO



AO

Absorption and Fluorescence Measurements

SDS micellar solutions were prepared in a 0.1 mol dm^{-3} NaCl solution, the aggregation number being $\upsilon = 91$ and the c.m.c. = $(1.4\text{–}1.6) \times 10^{-3}$ mol dm^{-3}. The experiments for the donor–acceptor system PPO–AO, were carried out adding no salt to the SDS micelles (c.m.c. = 8.2×10^{-3} mol dm^{-3} [13]). *n*-AS was dissolved by adding an ether solution to a hot (45 °C) and well stirred micellar solution.

Low micellar concentrations (10^{-4} mol dm^{-3}) and low occupancy numbers μ ($\mu < 2.1$) were used, and the solutions were allowed to stand for two days before measurements.

The donor occupancy number was always $\mu \approx 0.01$ to prevent energy migration.

Fluorescence spectra were obtained at 25 °C using Perkin-Elmer MPF-3 or MPF-44A spectrofluorimeters in a right-angle geometry, and excitation and emission bandwidths of 4 nm. Spectral correction was performed with a calibration curve obtained from fluorescence standards (MPF-3)[14] or using a DCSU spectral correction unit (MPF-44A).

The isotropic, dynamically averaged, critical radius of transfer $\bar{R}_0$ was calculated from eqn. 10:[1]

$$\bar{R}_0 = 0.2108\left(k^2\phi_0 n^{-4}\int_0^{\infty} I(\lambda)\varepsilon(\lambda)\lambda^4\,\mathrm{d}\lambda\right)^{1/6} \qquad (10)$$

with $\bar{R}_0$ in Å, where k^2 is the effective orientational factor (2/3), ϕ_0 is the donor quantum yield in the absence of acceptor, n is the refractive index of the medium, $I(\lambda)$ is the normalized fluorescence spectrum of the donor, $\varepsilon(\lambda)$ is the molar absorption coefficient (dm^3 mol^{-1} cm^{-1}) and λ is in nm. There is some uncertainty regarding the value of the effective refractive index n to be used in eqn. (10), namely the sodium D line values for water ($n_D = 1.33$) and a long-chain hydrocarbon ($n_D = 1.40$) could be considered. In any case knowledge of the precise value of the refractive index is not critical, owing to the weak dependence of R_0 on n, and to its variation within narrow limits.

Absorption spectra were recorded with a Perkin-Elmer Lambda 5 spectrophotometer.

Time-resolved Measurements

The fluorescence decays were recorded using a Spectra Physics mode-locked synchronously pumped and cavity-dumped dye laser system whose output was frequency doubled with a KDP crystal to give excitation pulses with a pulse width of 15 ps at a repetition rate of 825 kHz. The fluorescence was detected using a Hamamatsu 1564 U microchannel plate after passing through a polarizer set at the magic angle and a Jobin Yvon H10 monochromator (4 nm bandpasses). The electronic components were those usually employed in single-photon timing.[15] The experiments were performed using a resolution of 21.6 ps per channel and 1024 channels in the multichannel analyser.

A total of 20000 counts were usually accumulated in the maximum channel. Excitation wavelengths were chosen in the range 290–320 nm. In all cases donor fluorescence was collected at its maximum emission wavelength. Temperature (25 °C) was controlled to within 0.2 °C. The adequacy of the fit to the decay data was determined by the inspection of the weighted residual plots, the reduced χ^2 and also the Durbin–Watson parameter.[16]

Data treatment was carried out on a Digital PDP 11/73 computer.

For each value of the radial parameter $\alpha = a/R_s$ (fixed) the minimum reduced χ^2 was obtained by direct search by varying the paramer $x_0 = \bar{R}_0/R_s$. The integral $J(t)$ [eqn. (7)] was computed with 51 equally spaced points. Analysis was carried out by fitting over the decay portion beginning at the maximum of the decay curve and ending in the channel where the counts were 0.5% of the maximum (typically 100 counts). Background noise was subtracted by averaging over the first 70–80 channels. In one of the systems (Triton X-100 as a donor) correction for scattered light had to be applied and in the following way: After background correction, the experimental decay curve can be written[16] as $D = AL \otimes \rho + BL$, L being the convolution of the excitation pulse with the instrumental response, wavelength independent in our case, ρ being the delta-pulse response decay and A and B being fitting parameters. Application of the least-squares minimization yields A and B as a function of α and x_0 and hence the relative weight of scattered light (B/A). Beginning the analysis a few channels after the maximum while fixing $B = 0$ always yielded identical results, thus confirming that no rising edge effects were present other than scattering.

Results

All the experiments for the donor–acceptor pairs described in Table 1 were performed in SDS micellar solutions.

The systems for the energy-transfer study verify the following criteria, imposed by the model assumptions: (i) complete absence in the aqueous phase, *i.e.* strict micellar solubility of donor and also of acceptor (definition of μ); (ii) single exponential donor decay in the absence of acceptor; (ii) short or moderate donor lifetime (no significant diffusional contribution); (iv) $R_0 \approx R_s$. Were the critical radius of transfer, R_0, too large, an active sphere of quenching mechanism would be operative, and no information on probe location could be obtained.[1]

In Table 1 are also presented the donor's quantum yield, ϕ_0, and the relevant Förster critical radius, R_0.

Absorption and emission spectra of PPO and AO are shown in Fig. 4. Spectral data for the other probes, Triton X-100 (monomer), and n-AS were previously reported.[1]

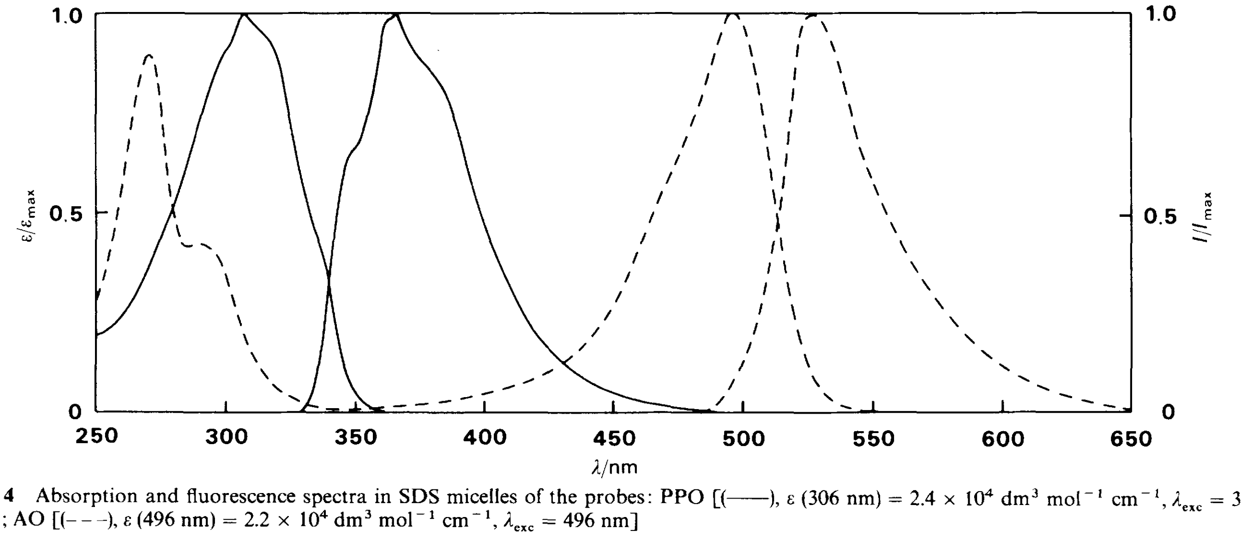


Fig. 4 Absorption and fluorescence spectra in SDS micelles of the probes: PPO [(——), ε (306 nm) = 2.4 × 10^4 dm^3 mol^{-1} cm^{-1}, λ_{exc} = 341 nm]; AO [(– – –), ε (496 nm) = 2.2 × 10^4 dm^3 mol^{-1} cm^{-1}, λ_{exc} = 496 nm]

Table 1 Fluorescence quantum yields ϕ_0 of the donors, and Förster critical radius $\bar{R}_0$ [eqn. (10), $k^2 = 2/3$ and $n = 1.33$], for the donor–acceptor pairs solubilized in SDS micelles

donor	acceptor	ϕ_D	$\bar{R}_0$/Å
PPO	AO	1.00^a	29
PPO	*n*-AS ($n = 3, 6$)	1.00^a	31
Triton X-100 (monomer)	*n*-AS ($n = 6, 9, 12$)	0.30^b	19

^{a} Determined *vs.* PPO in cyclohexane, $\phi_F = 1.0$.[17] ^{b} Determined *vs.* naphthalene in cyclohexane, $\phi_F = 0.23$.[17]

Fluorescence Decay of the Probes

As required for the model application, the decay of both donors, PPO (Fig. 5) and Triton X-100 (Fig. 6) is single exponential, with lifetimes of 1.84 and 6.42 ns, respectively.

No transient effects due to oxygen quenching were therefore observed in the solutions.[18]†

In order to obtain additional evidence regarding the strict solubility of the probes in the micelles, a study of its fluorescence decay was carried out. For monomeric Triton X-100 in water (10^{-5} mol dm^{-3}), well below its c.m.c. = 2.4×10^{-4} mol dm^{-3},[19] the decay is almost single exponential with a lifetime of 4.5 ns; Above the c.m.c. the lifetime increases up to 6.4 ns. The other probe, AO, when in micelles of SDS ($\mu = 0.05$), is also single exponential ($\tau = 3.28$ ns).

The *n*-AS decay is complex, wavelength and viscosity dependent, and in general can only be fitted by a sum of three exponentials.[20] This behaviour is attributed to the intramolecular torsional relaxation in the excited state,[20,21] and this fact prevents its use as a donor in a time-resolved study.

Energy-transfer Studies

Donor decay data were analysed according to the dynamic models, assuming radial (R and D) or uniform (U and D) distribution of the probes.

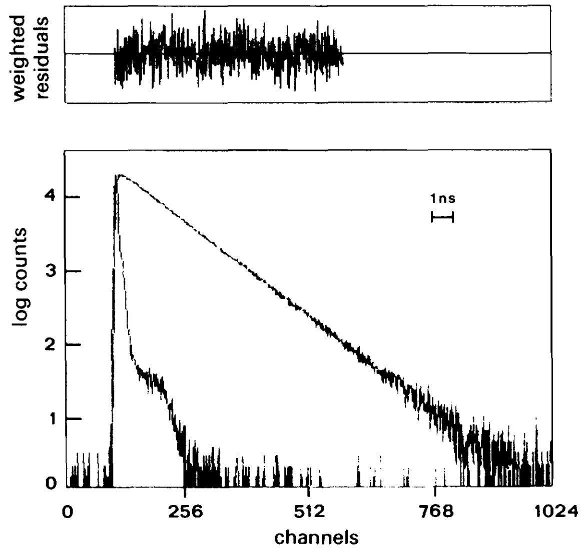


Fig. 5 Fluorescence decay and plot of the weighted residuals of PPO incorporated in SDS micelles. $\chi^2 = 1.06$, DW = 2.08, $\tau = 1.84$ ns

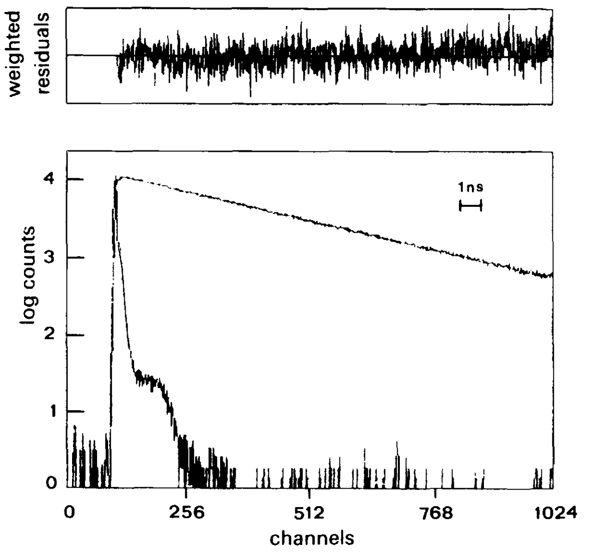


Fig. 6 Fluorescence decay and plot of the weighted residuals of Triton X-100 (monomer), incorporated in SDS micelles. $\chi^2 = 1.22$, DW = 1.80, $\tau = 6.42$ ns

System PPO–AO

Upon increasing the mean occupancy number μ of the acceptor (AO), the decay of PPO becomes increasingly complex and can only be fitted by a minimum of three exponentials,

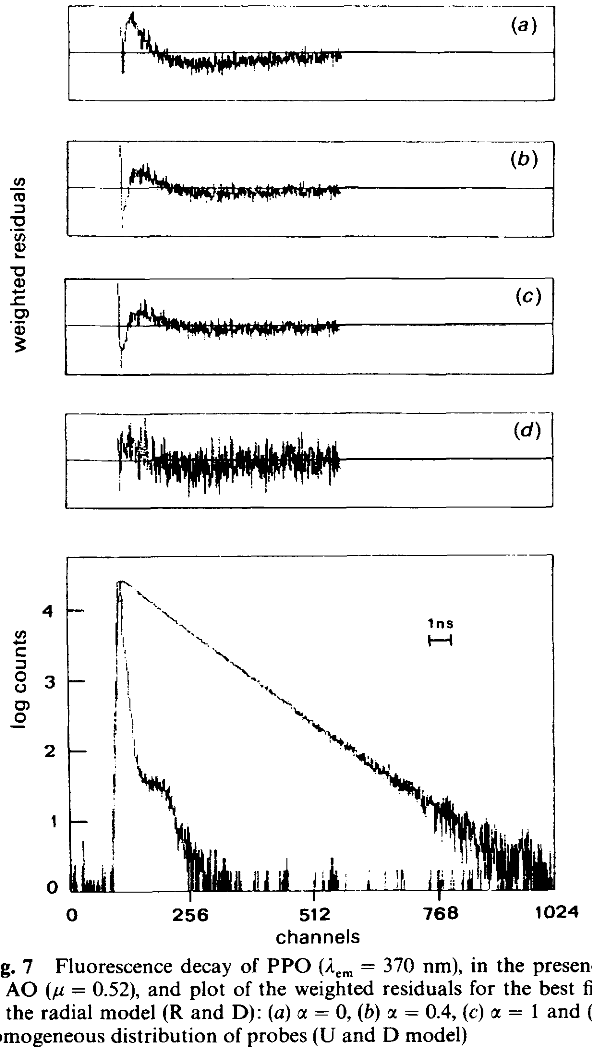


Fig. 7 Fluorescence decay of PPO ($\lambda_{em} = 370$ nm), in the presence of AO ($\mu = 0.52$), and plot of the weighted residuals for the best fits to the radial model (R and D): (*a*) $\alpha = 0$, (*b*) $\alpha = 0.4$, (*c*) $\alpha = 1$ and (*d*) homogeneous distribution of probes (U and D model)

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† From the reported oxygen solubility in SDS micelles it can be estimated that under atmospheric conditions $\mu(O_2) = 0.02$, *i.e.* 98% of the micelles are O_2 free. Quenching from oxygen dissolved in the bulk water is also negligible in this case.

Table 2 Values of x_0 and χ^2 (in parenthesis) corresponding to the fit of PPO decay for the indicated values of radial parameter α (R and D model) and two mean occupancy numbers μ of acceptor, AO, in SDS micelles

	α					
μ	0	0.2	0.4	0.6	0.8	1
0.52	1.61 (6.96)	1.61 (5.66)	1.69 (4.26)	1.84 (3.64)	2.03 (3.40)	2.24 (3.34)
1.04	1.59 (31.14)	1.58 (24.07)	1.64 (15.91)	1.76 (12.31)	1.94 (10.94)	2.14 (10.61)

Table 3 Values of x_0, χ^2, and Durbin–Watson parameter (DW) corresponding to the fit of PPO decay (U and D model), for two mean occupancy numbers μ of acceptor, AO in SDS micelles

μ	x_0	χ^2	DW
0.52	1.13	1.29	1.62
1.04	1.15	4.65	0.42

no physical meaning being attributed to the recovered lifetimes and pre-exponential coefficients.

The experimental decay was then fitted to the dynamic (R and D; U and D) models for energy transfer, Fig. 7 and Table 2 and Table 3.

The model recovered value of x_0 is largely independent of the mean occupancy number, but the goodness of fits judged, *e.g.* by the χ^2 value, is worse for the higher value of μ.

Although the fit improves upon increasing the α value, it is not satisfactory even for the maximum allowed value $\alpha = 1$. The deviation is greatest in the short-time region, where the experimental decay is always faster than the theoretical one.

For the U and D model the recovered x_0 values are also μ independent, but χ^2 is again worse for the higher value. The plot of residuals Fig. 7(*d*) is qualitatively similar to the ones obtained for the R and D model.

*System PPO–*n*-AS* (n = *3*, *6*)

A trend of variation similar to the previous system was observed. The complex decay inherent to the energy transfer mechanism, is not satisfactorily fitted by either models (Tables 4 and 5), while it improves when a radial position of both chromophores is assumed ($\alpha = 1$). Again the value of x_0 shows no significant variation with μ, and is identical within error for both probes. Regarding the fit to the U and D model, the arguments described for the PPO–AO system apply.

*System Triton X-100–*n*-AS* (n = *6*, *9*, *12*)

As described for the previous systems, the deviations in the model fitting are significant at the shorter range of times of the donor decays, when in the presence of any of these acceptors. The x_0 value is again μ independent, and also identical for all the probes, *i.e.* does not depend on the substitution of the chromophore on the aliphatic chain, Table 6.

Table 5 Values of x_0, χ^2, and Durbin–Watson parameter (DW), corresponding to the fit of PPO decay (U and D model), for two mean occupancy numbers μ of acceptor [n-AS (n = 3, 6)] in SDS micelles

μ	x_0	χ^2	DW
	3-AS		
0.50	1.27–1.29	1.15	1.65
0.99	1.33	1.25	1.37
	6-AS		
0.50	1.48	0.99	1.99
0.99	1.40–1.41	1.39	1.60

The unsatisfactory fit to the U and D model is shown by the data in Table 7.

Discussion

Fluorescence Decay of the Probes

The lifetime of Triton X-100 (monomer), when in micelles of SDS, $\tau = 6.42$ ns, is similar to the one obtained for Triton X-100 above its c.m.c., $\tau = 6.4$ ns, this value being higher than the one reported in the literature $\tau = 4.0$ ns.[22]

For the monomer in water, the measured value of $\tau = 4.5$ ns, is in agreement with the published value.[22,23]

For AO in the pre-micellar region of SDS micelles (5×10^{-3} mol dm^{-3}), biexponential decay of 0.98 ns (51%) and 3.20 ns (49%) is found in the literature.[24] The long component is identical to our value of 3.20 ns, the short component probably arising from AO complexes with a few molecules of SDS.[24]

It can be concluded from the above that all the probes are indeed incorporated in the SDS micelles.

Energy-transfer Studies

Both models for energy transfer imply decay laws that are not strictly verified. In all cases, the statistical criteria for fitting (weighted residuals plot, reduced χ^2 and Durbin–Watson parameter) point to significant deviations. In addition, the fit was always better for $\alpha = 1$, and some of the recovered x_0 values were out of an expected range, being too high. A global explanation for these observations is the existence of a decay faster at shorter times than the theoretically

Table 4 Values of x_0 and χ^2 (in parenthesis) corresponding to the fit of PPO decay for the indicated values of radial parameter α (R and D model) and mean occupancy number of acceptor, n-AS (n = 3, 6), in SDS micelles

	α					
μ	0	0.2	0.4	0.6	0.8	1
			3-AS			
0.50	1.51 (2.81)	1.54 (2.24)	1.66 (1.82)	1.84 (1.65)	2.04 (1.59)	2.26 (1.57)
0.99	1.52 (6.26)	1.55 (4.32)	1.68 (2.88)	1.86 (2.34)	2.07 (2.15)	2.29 (2.10)
			6-AS			
0.50	1.54 (1.94)	1.59 (1.66)	1.74 (1.46)	1.94 (1.38)	2.16 (1.35)	2.39 (1.34)
0.99	1.52 (6.04)	1.56 (4.30)	1.70 (3.04)	1.89 (2.58)	2.10 (2.41)	2.33 (2.31)

Table 6 Values of x_0 and χ^2 (in parenthesis) corresponding to the fit of Triton X-100 (monomer) decay in the presence of *n*-AS ($n = 6, 9, 12$) acceptors for the indicated values of radial parameter α (R and D model) and mean occupancy numbers μ

	α					
μ	0	0.2	0.4	0.6	0.8	1
			6-AS			
1.03	0.78 (7.80)	0.75 (6.22)	0.78 (2.32)	0.88 (1.23)	1.01 (1.22)	1.13 (1.22)
2.05	0.76 (13.66)	0.75 (10.93)	0.74 (3.44)	0.85 (2.53)	0.98 (2.63)	1.10 (2.64)
			9-AS			
1.01	0.79 (7.73)	0.78 (5.84)	0.80 (1.61)	0.92 (1.13)	1.07 (1.15)	1.21 (1.16)
2.02	0.76 (14.69)	0.75 (11.60)	0.75 (3.08)	0.84 (1.22)	0.98 (1.25)	1.09 (1.26)
			12-AS			
0.96	0.77 (4.64)	0.76 (3.82)	0.78 (1.58)	0.89 (1.25)	1.03 (1.26)	1.15 (1.26)
1.92	0.75 (7.75)	0.75 (6.30)	0.74 (2.09)	0.82 (1.28)	0.96 (1.31)	1.07 (1.31)

expected one. The minimum of the χ^2 observed in all the fits to occur for $\alpha = 1$ can thus be explained by considering that it is for this value of the radial parameter that one has the greatest number of donor–acceptor pairs at short distances, eqn. (8), and therefore the fastest possible decay. The same argument applies to some high x_0 values (when PPO is the donor), *e.g.* the recovered $x_0 = 2.2$ for the PPO–AO system implies $R_s = 13$ Å, too small for an SDS micelle ($R \approx 22$–24 Å),[25] if it is assumed that AO is incorporated at the micellar interface as happens with other charged aromatics. This situation is identical to that observed for the PPO–*n*-AS system where $x_0 \approx 2.3$. In contrast to the Triton X-100–*n*-AS pairs, $x_0 \approx 1.1$, obtained for all the anthroyloxy acceptors, implies $R_s \approx 17$ Å, identical to the one obtained in the steady-state study[1] and reasonable for this micelle.

In Table 8 are presented the recovered R_s values from both dynamic models. With the exception of the described systems when PPO is the donor in the R and D model, the values obtained are within the expected range for this micelle. It should be stressed that in agreement with conclusions derived from a steady-state study of some of these systems,[1] it is almost always possible to obtain a reasonable radius for a micelle with any model. In this way conclusions on probe locations obtained from restricted models[10,11,26,27] are probably insignificant.

Table 7 Values of x_0, χ^2 and Durbin–Watson parameter (DW), corresponding to the fit of PPO decay in the presence of acceptor *n*-AS ($n = 6, 9, 12$) (U and D model), for two mean occupancy numbers, μ

μ	x_0	χ^2	DW
	6-AS		
1.03	1.18	7.75	0.29
2.05	1.17	16.08	0.15
	9-AS		
1.01	1.19	14.01	0.13
2.02	1.17	15.99	0.11
	12-AS		
0.96	1.18	5.75	0.40
1.92	1.16	6.90	0.28

Table 8 Values of R_s/Å obtained from the best fits to the R and D and U and D models

system	R and D model	U and D model
PPO–AO	13	25
PPO–*n*-AS	14	22
Triton X-100–*n*-AS	17	16

The inadequacy of both models used in this study is thought to be due to the failure of the distance distribution functions to describe correctly the relative positions of both donor and acceptor when in the micelle.

The static models discussed earlier give rise to worse fits. As previously stated this happens because they imply even smaller 'transients'.

Other possible causes for the observed deviations between experimental decays and models such as (i) non-Poissonian distribution of probes; (ii) probe translational diffusion within the micelle; (iii) orientational anisotropy of the probes, were discussed in detail and ruled out in a previous work.[1]

All models assume the energy-transfer partners to be randomly oriented, *i.e.* not to have preferred orientations. While for some probes evidence for anisotropic orientation exists, *e.g.* octadecylrhodamine B in Triton X-100 micelles,[28] the opposite was reported for the *n*-AS family of probes used.[29,30] In addition, these probes, used as acceptors, exhibit mixed polarization in their first absorption band,[31] which decreases the orientational dependence of the transfer mechanism.[32] For the Triton X-100, PPO and AO probes, no studies on their orientational distribution in micelles exist. However, in view of the common conclusion obtained from all pairs, anisotropy cannot be responsible for the observed deviations. This agrees with the detailed analysis of the effect of anisotropy on steady-state data.[1]

The radial distributions used in the models are also extreme cases: The radius is either precisely defined (R model) or totally unprecise (U model). NMR studies[2d,4] favour radial distributions peaking at a certain radius, but somewhat broadened, a situation mid-way between the R and U models. However, consideration of moderately broadened radial distributions may not bring further 'transiency' into the donor decay and will not therefore further reconcile experimental results with theory.

The experimental decay points to a significant population at very short distances, *i.e.* donor and acceptors are attractively correlated and an independent location of the probes cannot be assumed. This conclusion can be understood on the basis of structural perturbations induced on the micellar structure upon probe incorporation, *i.e.* the probes are preferentially segregated to a perturbed micellar region.

It should be emphasized that this proposed spatial correlation of the probes does not imply formation of molecular aggregates. No alterations were noticed on the absorption spectra in the present study, and no evidence for dimerization of the dyes was observed in the steady-state study.[1] On the other hand, the deviations are also observed in this transient-state study for very low occupancy numbers ($\mu < 0.5$). In addition, the observed complex decays, with a fast component are not compatible with a fully static mechanism.

Surprisingly, in none of the reported studies of energy transfer in micelles using transient techniques are statistical criteria used,[10,26] and the fit is judged on mere visual inspection. Furthermore Choi *et al.*,[26] in a study of energy transfer between dyes in SDS micelles, used an unnormalized distribution function [eqn. (5) and (7) of ref. 26], thus invalidating any derived conclusions. A similar experimental work was previously reported by Kasatani *et al.*[10] but these authors used a restricted model ($\alpha = 1$) (or a uniform distribution function obtained from Monte Carlo methods), and again no statistical criteria for the fit were used.

The recent work of Kaschke *et al.*[11] supports the conclusions of this study, by a pump-probe technique. Although these authors also used a restricted radial model ($\alpha = 1$), they concluded the existence of induced perturbations and consequent attractive correlation of the probes, their results comparing well with our previous steady-state study,[1] and with the present time-resolved one.

Conclusions

Electronic energy transfer between extrinsic probes incorporated in SDS micelles was studied in the picosecond time domain by the single-photon timing technique.

Donor decays were analysed according to four models in which orientational dynamics and distance distribution are varied. In all cases deviations are observed, their trend being rationalized on the basis of probe-induced perturbations of the micellar structure resulting in a closer-than-random distance distribution. The mentioned effect was observed for pairs both with and without long aliphatic chains.

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