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Förster resonance energy transfer among a structural isomer of adenine and various Coumarins inside a nanosized reverse micelle

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ABSTRACT

In this article we have studied Förster Resonance Energy Transfer (FRET) using 2-aminopurine (2-AP), a structural isomer of adenine as donor and various Coumarins as acceptors inside AROSOL-OT (AOT)-water reverse micelles (RM) using steady-state and time-resolved fluorescence spectroscopies. We have used three sets of FRET and all the pairs except 2-AP–Coumarin-480 exhibited quite efficient FRET. For the efficient pairs, overlap integral $J(\lambda)$ and Förster distance (R_0) are of high values but the rate constant of energy transfer (k_{ET}) are quite low. The rate is gradually amplified with increase in water content for the 2-AP–Coumarin-440 pair while the reverse is observed for 2-AP–Coumarin-460. In future our FRET pair can be used in more modified and sophisticated confined media such as biomembranes of varying size, physical properties and chemical compositions etc.

1. Introduction

More than 30 years ago, Stryer [1] first demonstrated that 2-aminopurine, a structural isomer of adenine (A), was strongly fluorescent and could be selectively excited in the presence of natural bases. In contrast to brilliant dyes, like fluorescein and cyanin, 2-AP is a generally non-perturbing substituent since it is structurally similar to adenine (6-aminopurine) and will form thermodynamically equivalent base pairs with thymine in DNA helices and uracil in RNA helices [2]. It is a sensitive fluorescent probe by changing its spectral features depending on the environment, e.g., changes in its quantum yield imply a subtle conformational change in nucleic acids [3,4]. It was shown that 2-AP can undergo charge transfer reactions that can be used to investigate various aspects, such as the mechanism of DNA-mediated charge transfer. Kelley et al. have used 2-AP to measure the distance dependence of charge transfer and the influence of stacking [5]. Moreover it has been proved that the biological function remain conserved after replacement of adenine by 2-AP [6]. Recently 2-AP has been employed as an electron donor or an electron acceptor in different DNA assemblies [7]. A few works related to systems involving energy transfer between 2-AP and 2-AP containing systems have been published [8,9]. At room temperature, the nucleic acid bases can act as energy donors and energy acceptors. Excited state FRET in between nucleic base pairs inside DNA helix was thoroughly investigated [10–12]. Processes have been reported that involve singlet–singlet energy transfer from the bases to added probes [13]. Murphy et al. have used Coumarin-102 for replacing a base pair in B-DNA double helix and they obtained substantial shifts in the absorption and fluorescence spectra of Coumarin incorporated into DNA relative to free in aqueous solution which clearly demonstrated that the Coumarin is incorporated within the interior the DNA helix [14]. Coumarins are also important compounds having the properties of inhibition of ATPase reaction of DNA gyrase in addition to their extensive use as fluorescent dye [15,16]. In this context we have done an energy transfer study between 2-AP and a series of Coumarin molecules inside the nanocavity of AOT-H_2O RM system by using steady-state as well as time-resolved fluorescence spectroscopy. Our main objective in this present work to establish a new series of donor–acceptor (D–A) set which can be used effectively for DNA dynamics as well as conformational study merely by replacing DNA bases with our probe molecules in desired location inside a helix. It will be a rigorous field to explore but here we simply have done some model work to check whether FRET is possible between 2-aminopurine and different Coumarin molecules inside nanosized RM core.

Fluorescence resonance energy transfer techniques have long been used successfully as 'spectroscopic ruler' to study proximity relationships in biomolecular systems [17] with high temporal resolution and sensitivity. FRET has been employed to measure distances between fluorescent tags on proteins to observe the kinetics of conformational changes in RNA through

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time-resolved studies [18] and to elucidate the structure and dynamics of polymer–polymer interfaces [19,20]. A series of good articles have been published on various aspect of FRET [21–24]. Recently Bhattacharya et al. have reported the FRET study in various confined media such as triblock copolymer, reverse micelle, micelle [25,26]. In this work we have tried to focus the applicability of the used donor and acceptors as an efficient FRET pair inside reverse micelles.

2. Experimental: materials and methods

All laser grade Coumarin dyes were obtained from Exciton and used as received. 2-AP and AOT surfactant [sodium bis(2-ethylhexyl) sulfosuccinate] were purchased from sigma chemicals. AOT was dried under vacuum for 24 h at ~60 °C before used. The structures of all the Coumarin dyes, 2-AP and the surfactant are given in Scheme 1 (abbreviations also given). We have used n-heptane as dispersed solvent and water as core solvent. The solution was prepared using the procedure used by Levinger et al. [27]. The size of the reverse micelles is usually given by AOT reverse micelles can be formed from w_0 1 up to 70 in a range of nonpolar solvents. Many studies have shown that AOT reverse micelles formed in ternary solutions of water, AOT, and nonpolar solvent display a spherical form. Because the water defines the volume while the AOT surfactant defines the surface area; for spherical reverse micelles; w_0 is directly proportional to the micellar radius; where w_0 = [water]/[AOT].

The concentration of AOT was kept at 0.09 M for all the measurements. During the preparation of RM, measured amount of 2-AP containing water was added and then to observe FRET, we have gradually added Coumarins in microlitre amount. Absorption and emission spectra were measured with Shimadzu (model UV 1601) UV–vis spectrophotometer and Jobin Yvon-fluoromax-3. All the fluorescence spectra were corrected for the wavelength sensitivity of the detection system. Fluorescence decay transients were measured and fitted by using a commercially available spectrophotometer (LifeSpec–ps, Edinburgh Instruments, UK) with a 60 ps instrument response function (IRF). In all cases 2-AP was excited at 280 nm by using the third harmonic laser beam of the 900 nm from a mode-locked Ti-sapphire laser with 80 MHz repetition rate (Tsunami, Spectra Physics), pumped by a 10 W Millennia (Spectra Physics) followed by a pulse peaker (rate 8 MHz). Optical path length of our UV and fluorescence cell was 1 cm and fluorescence signals were collected in magic angle (54.7°) polarization.

We have used the following rate expression for FRET derived by Förster.

$$K_T(r) = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6 \tag{1}$$

where r is the distance between the donor and the acceptor, R_0 is the Förster distance at which $k_T(r)$ is equal to the decay rate of the donor in the absence of the acceptor, τ_D is the lifetime of the donor in the absence of acceptor. R_0 is the Förster distance which can be defined as

$$R_0^6 = \frac{9000(\ln 10)\kappa^2 Q_D}{128\pi^5 N n^4}\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \tag{2}$$

where κ^2 is the orientation factor of two interacting dipoles, Q_D is the fluorescence quantum yield of the donor in the absence of acceptor, n is the average refractive index of the medium in the wavelength range where spectral overlap is significant, $F_D(\lambda)$ is the fluorescence spectra of the donor normalized so that $\int_0^\infty F_D(\lambda)d\lambda = 1$ and $\varepsilon_A(\lambda)$ is the molar absorption coefficient of the acceptor which is typically in units of $M-1\,cm^{-1}$, N is Avogadro's number. The value of κ^2 is very significant, and it may vary from 0 (mutually perpendicular transition dipoles) to 4 (collinear dipoles). It is not possible to determine the actual value of κ^2 [28]. Several methods have been proposed for solving this problem, but it is possible to determine the upper (k^2_{max}) and lower (k^2_{min}) values using steady-state fluorescence anisotropy and time-resolved anisotropy measurement as [29].

$$K^2_{min} = \frac{2}{3}\left[1 - \left(\frac{d_D^x + d_A^x}{2}\right)\right] \tag{3}$$

$$K^2_{max} = \frac{2}{3}[1 + d_D^x + d_A^x + 3d_D^x d_A^x] \tag{4}$$

where d_i denotes the ratio of square root of the steady-state fluorescence anisotropy (r_i^{ss}) and the initial value of anisotropy (r_i^0) in the anisotropy decay of the ith species (donor or acceptor). The value of κ^2 is very significant, multiplying the absolute distance between 0 and $4^{1/6}$. For this range of kappa squared values, the distance calculated using the Förster model may vary less than 20%. We therefore used $\kappa^2 = 2/3$ (random orientation) for the calculation of R_0. Steady-state fluorescence anisotropy have been measured by using the following equation

$$r_0 = \frac{I_{VV} - G\cdot I_{VH}}{I_{VV} + 2G\cdot I_{VH}} \tag{5}$$

Where G is the correction factor. I_{VV} and I_{VH} are steady state fluorescence spectra polarized parallel and perpendicular to the polarization of the excitation light, respectively.

3. Results

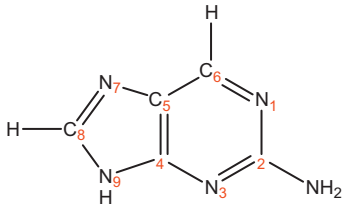
3.1. Steady-state measurement

2-Aminopurine shows absorption peak at 306 and 310 nm in water and methanol, respectively and inside AOT RM (w_0 = 12.0), at 306 nm. 15 nm red-shift in the fluorescence spectra (354.0–369 nm) is observed when w_0 value is changed from 0.0 to 12.0 while in bulk water the emission maximum is at 369 nm. 2-AP is almost insoluble in n-heptane and soluble in water and UV-fluorescence results indicate that there will be preferential transfer of 2-AP to the RM from bulk solvent. All the Coumarins have low solubility in n-heptane as well as in water compared to AOT reverse micelle. 5 nm redshift is observed in the absorption spectra of C-440 during change in w_0 value from 0.0 to 12.0 but no shift is observed in case of C-460. Similar trend is also observed in case of emission spectra of C-440 and C-460. The emission maximum of the former is red-shifted by 30 nm while that of the latter remains unaffected on changing the w_0 value from 0.0 to 12.0. These results indicate that C-440 is located near the water pool and therefore senses the micropolarity after gradual addition of water but C-460 is away from water pool, most probably inside surfactant tail due to hydrophobic interaction. We have calculated the quantum yield of 2-aminopurine embedded inside RM core by using the following gradient method [28].

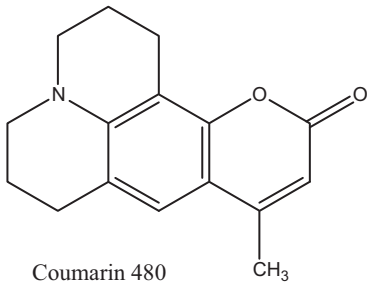
$$\Phi_x = \Phi_{ST}\left(\frac{Grad_x}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right) \tag{6}$$

where Φ_x and Φ_{ST} are the quantum yields of the experimental solution and standard solution taken. Quinine sulfate in 0.1 (N) H_2SO_4 solution was taken as standard solution. Grad is the gradient from the plot of integrated fluorescence intensity vs. absorbance and the η is the refractive index of the solvent.

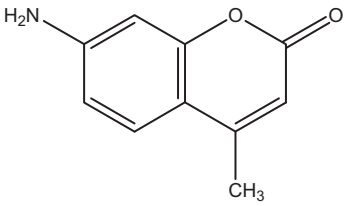
For 2-AP the values of Φ_x obtained are 0.156, 0.162, 0.169 inside RM core at w_0 = 4.0, 8.0, and 12.0, respectively. We have measured steady-state fluorescence anisotropy of 2-AP in AOT RM at various w_0 values and these data are taken at emission maximum of 2-AP. By determining steady-state fluorescence anisotropy, we have



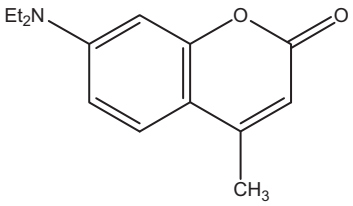
2 AminoPurine



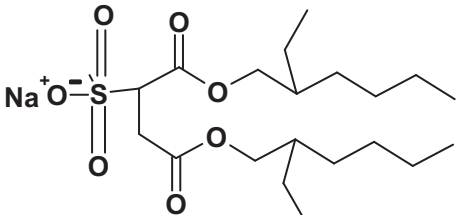
Coumarin 480



Coumarin 440



Coumarin 460



Scheme 1.

obtained a picture of confinement of 2-AP inside RM at different w_0 values (Table 1).

With successive addition of a micro-molar amount of C-440 and C-460, the fluorescence intensities of donor 2-AP in RM (Fig. 1) have been gradually decreased. Among the 2-AP–Coumarin pairs, the 2-AP–C-440 pair was found to be more efficient. The efficiency of energy transfer (E) was calculated by using Eq. (6)

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \tag{7}$$

as where τ_{DA} is the fluorescence lifetime of the donor in the presence of the acceptor and τ_D is the fluorescence lifetime of the donor in the absence of the acceptor. We can also calculate the overlap integral $J(\lambda)$ from the overlap of emission spectra of donor and absorption spectra of the acceptor (Fig. 2) and R_0 values for different systems which are listed in Table 2. The distance r between the donor and acceptor can be calculated as

$$E = \frac{R_0^6}{R_0^6 + r^6} \tag{8}$$

Table 1
Steady-state fluorescence anisotropy (r_0) of 2-AP inside reverse micelle at different w_0 values.

System	r_0 at $w_0 = 4.0$	r_0 at $w_0 = 8.0$	r_0 at $w_0 = 12.0$
2-Aminopurine	0.156	0.134	0.091
C-440	0.139	0.128	0.103
C-460	0.035	0.033	0.032

The calculated r values for different systems are listed in Table 2. To get an idea about the partition of the 2-AP, C-440 and C-460 inside AOT-water reverse micelle we have used method described by Novaira et al. [30]. We prepared sets of solution for each probe molecules and in each solution we kept the concentration of 2-AP at $\sim 5 \times 10^{-6}$ M and 8×10^{-6} M. The first set was the solution of AOT in n-heptane at $w_0 = 0$, i.e., no water and then we observed UV–vis spectra and the fluorescence spectra at different AOT concentration. In the second, third and fourth set, we carried out the same measurement with the variation of AOT molecule but at $w_0 = 4.0$, 8.0, and 12.0, respectively, i.e., in the last three set we kept the water concentration at fixed proportion with the variation of the AOT molecule. Then we plotted the variation of the fluorescent intensity with the AOT concentration and fitted the plot with the following equation

$$I = \frac{I_0(\Phi_f + \Phi_b K_p[\mathrm{AOT}])}{(1 + K_p[\mathrm{AOT}])} \tag{9}$$

where I_0 is the incident light, and I_f and I_b are the fluorescent intensities when the probe is present in the external solvent and the dispersed pseudophase, respectively. I is the fluorescent intensity observed at the AOT concentration considered. Φ_f and Φ_b are the fluorescence quantum yields of probe in n-heptane and bound to the reverse micellar interface, respectively. [AOT] represents the effective AOT concentration in the solution, i.e., total AOT concentration minus the CMC value. K_p represents the partition coefficient value of the probe molecule at that condition. We got a gradual increase in the partition coefficient value with the increase in water

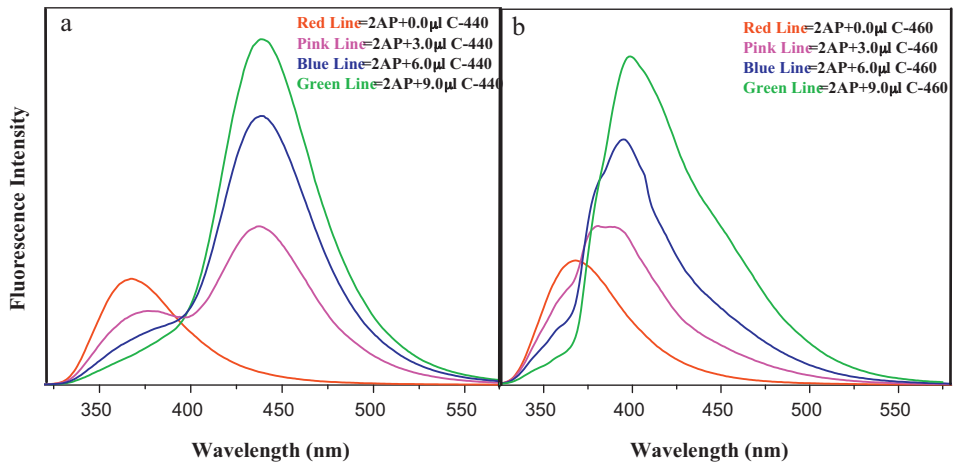


Fig. 1. Steady-state fluorescence quenching spectra of 2-AP in presence of (a) C-440 and (b) C-460 inside AOT-water reverse micelle at $w_0 = 8.0$.

Table 2
FRET parameters for different donor–acceptor pairs at different w_0 values.

Acceptors in AOT-H_2O RM	Donor in AOT-H_2O RM	$J(\lambda)$ (M^{-1} cm^{-1} nm^4)	R_0 (Å)	E (%) (τ_{DA}/τ_D)	r (Å)	Φ_D^0	K_{ET} (s^{-1})×10^{-8}
C-460 at $w_0 = 4.0$	2-AP	1.48×10^{16}	55.48	21.0	69.19	0.156	0.29
C-460 at $w_0 = 8.0$	2-AP	1.43×10^{16}	55.42	21.0	69.15	0.162	0.27
C-460 at $w_0 = 12.0$	2-AP	1.44×10^{16}	55.75	13.0	77.11	0.169	0.14
C-440 at $w_0 = 4.0$	2-AP	1.17×10^{16}	53.37	29.0	61.90	0.156	0.45
C-440 at $w_0 = 8.0$	2-AP	1.13×10^{16}	53.40	35.0	59.11	0.162	0.55
C-440 at $w_0 = 12.0$	2-AP	1.09×10^{16}	53.45	39.0	58.00	0.169	0.63
C-480 at $w_0 = 4.0$	2-AP	2.83×10^{16}	62.00	–	–	0.156	–
C-480 at $w_0 = 8.0$	2-AP	2.72×10^{16}	62.23	–	–	0.162	–
C-480 at $w_0 = 12.0$	2-AP	2.60×10^{16}	61.77	–	–	0.169	–

loading for 2-AP and C-440 but the reverse order is obtained for C-460 (Table 3). Here the plot obtained by fitting with Eq. (8) is shown in Fig. 3.

3.2. Time-resolved study

The rate constant of energy transfer and also the efficiency have been determined by using the lifetime of the donors and acceptor in RM. The fluorescence lifetime was measured at the emission maximum for donor molecule. The decays of 2-aminopurine were found to be biexponential. The lifetime decays in absence of acceptor and

Table 3
Equilibrium constant (K_p) for the partition co-efficient of 2-AP, C-440 and C-460 in AOT/water/n-heptane reverse micelle at 25 °C through steady-state fluorescent measurement.

System	K_p (M^{-1}) at $w_0 = 4.0$	K_p (M^{-1}) at $w_0 = 8.0$	K_p (M^{-1}) at $w_0 = 12.0$
2-Aminopurine	8.98	13.45	15.05
C-440	11.56	15.59	21.20
C-460	9.00	9.11	9.20

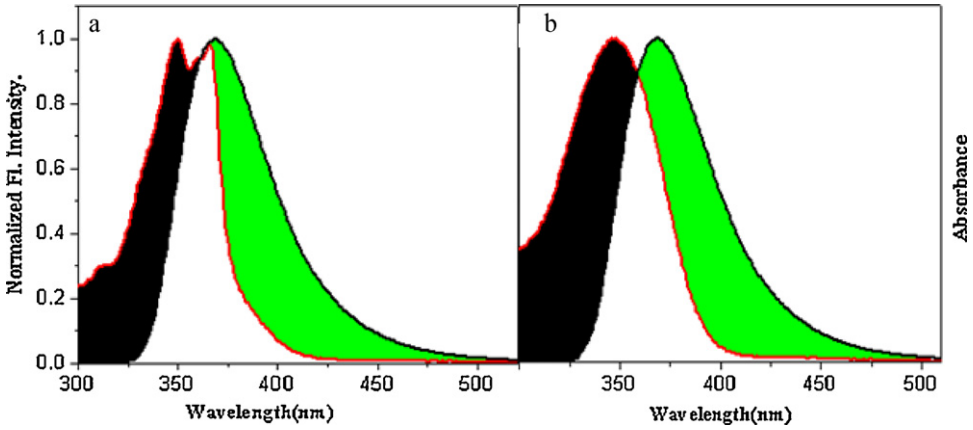


Fig. 2. (a) Overlap plot of emission spectra of 2-AP (donor) and absorption spectra of C-460 (acceptor) and (b) C-440 (acceptor) respectively inside AOT-water reverse micelle at $w_0 = 8.0$.

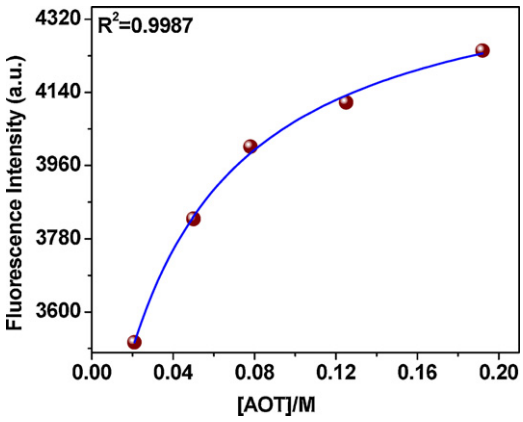


Fig. 3. Variation of fluorescence intensity measured at of C-440 with varying AOT concentration at $w_0 = 12$ in pure AOT/water/n-heptane reverse micelle. Bold line represents the fitting curve using Eq. (9).

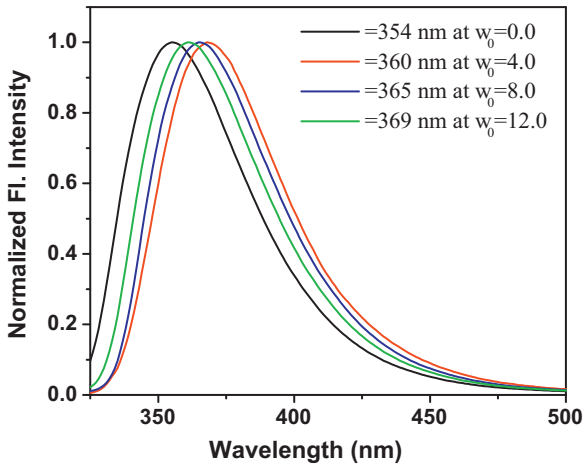


Fig. 5. Overlay plot of normalized emission spectra of 2-AP inside AOT reverse micelle at different w_0 values.

in presence of acceptor are shown in Fig. 4. The average lifetime was calculated by the following Eq. (10)

$$\langle\tau\rangle = \tau_1 a_1 + \tau_2 a_2 \quad (10)$$

where τ_1, τ_2 are the first and second components of decay time of the dye molecules and a_1 and a_2 are the corresponding relative weightage, respectively. In presence of certain micro molar amount of acceptor, lifetime of donor decreases and the values are given in Table 4.

4. Discussion

By analyzing the fluorescent spectral data of 2-AP and a 15 nm red-shift from $w_0 = 0.0$ to $w_0 = 12.0$ (Fig. 5) provide strong support in favor of the assumption that it is most likely located inside RM. Solubility of 2-AP in water is good while it is very poor in n-heptane. Strong red-shift with increased fluorescence intensity of 2-AP clearly indicates that 2-AP is located in such a position where it senses the change in micropolarity after gradual addition of water, i.e., near the water pool. High values of steady-state fluorescence anisotropy of 2-AP inside RM also indicate its confinement. Fluorescence properties of acceptors were also monitored to confirm their location inside RM. C-440 has a tendency to form hydrogen-bond with the negatively charged sulfate group of the surfactant. We have checked absorption spectra and emission spectra of C-440 at different w_0 values, in which 5 nm redshift is observed in case of absorption spectra indicating encapsulation of C-440 inside reverse micelle. This conjecture is again supported by distinct redshift of 30 nm in emission spectra of C-440 during change in w_0 from 0.0 to 12.0. So we can say that C-440 gradually senses a microenvironment of greater polarity inside RM. Again significant increment is observed in red end side in the absorption spectra which indicates that the probe molecules undergo a migration from the bulk n-heptane to reverse micelle where the polarity is higher compared to that in the bulk solvent. In case of C-460 no redshift is observed in absorption spectra as expected because no H-bond formation is possible in case of C-460 and no redshift is observed in emission spectra. Emission spectra of C-460 is almost unchanged during change in w_0 from 0.0 to 12.0 indicating that this probe is located on the hydrophobic surface just away from water pool. The steady sate anisotropy value of C-440 and C-460 also support the fixation of C-440 in its location. In methanol the value of steady-state fluorescence anisotropy is ~0.011 for the Coumarin probes and it is of higher value inside reverse micelle for both the Coumarins. To confirm our supposition we have calculated partition coefficient (K_p) for all the dyes. Partition coefficient value gradually increases with w_0 value for 2-AP and C-440 but it is unaltered in case of C-460. Now it is easy to state that 2-AP and C-440 are located inside RM core while C-460 is located in the hydrophobic region of RM.

Reverse micelles have inner water-pool around which an interface is present formed by water-headgroups of AOT. Actually C-440 is located at that interfacial region by forming H-bond with the negatively charged headgroup near the water-pool, not so far from the water-pool. But C-460 cannot form H-bond while it resides in the hydrophobic region of AOT reverse micelles. This can be explained by the following way: solubility of C-460 in water as well as in n-heptane is very low and it is more hydrophobic in nature

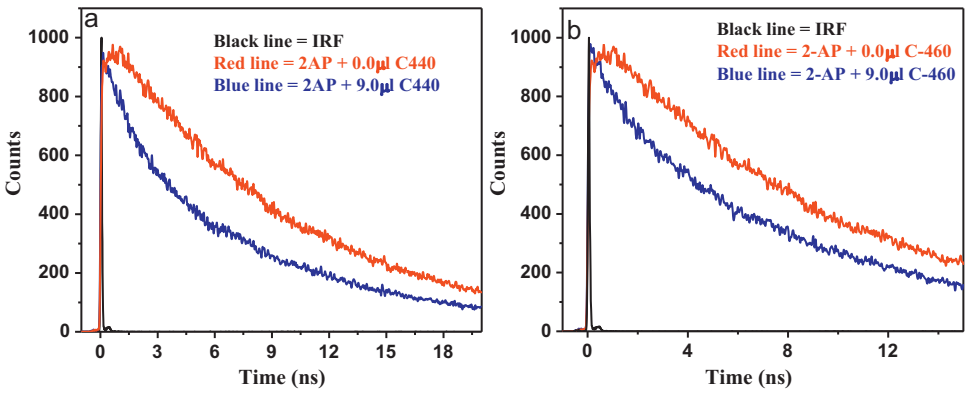


Fig. 4. Time-resolved fluorescence quenching decays of 2-AP in presence of (a) C-440 and (b) C-460 inside AOT-water reverse micelle at $w_0 = 4.0$.

Table 4
TCSPC data for different donor-acceptor pairs at different w_0 values.

Donor–acceptor	Acceptor conc. (μM)	a_1	τ_1 (ns)	a_2	τ_2 (ns)	$\langle\tau\rangle$ (ns)
2-AP + C-460 at w_0 = 4.0	0.0	1.0	9.15	–	–	9.15
2-AP + C-460 at w_0 = 4.0	11.23	0.76	9.20	0.24	1.19	7.23
2-AP + C-460 at w_0 = 8.0	0.0	1.0	9.78			9.78
2-AP + C-460 at w_0 = 8.0	11.23	0.70	9.10	0.30	1.25	7.75
2-AP + C-460 at w_0 = 12.0	0.0	1.0	10.10			10.10
2-AP + C-460 at w_0 = 12.0	11.23	0.69	9.25	0.31	1.50	8.84
2-AP + C-440 at w_0 = 4.0	0.0	1.0	9.15			9.15
2-AP + C-440 at w_0 = 4.0	10.98	0.67	9.28	0.33	1.55	6.53
2-AP + C-440 at w_0 = 8.0	0.0	1.0	9.78			9.78
2-AP + C-440 at w_0 = 8.0	10.98	0.64	9.35	0.36	1.26	6.42
2-AP + C-440 at w_0 = 12.0	0.0	1.0	10.10			10.10
2-AP + C-440 at w_0 = 12.0	10.98	0.60	9.40	0.40	1.59	6.12
2-AP + C-480 at w_0 = 4.0	0.0	1.0	9.15			9.15
2-AP + C-480 at w_0 = 4.0	11.15	0.95	9.50	0.05	1.69	9.11
2-AP + C-480 at w_0 = 8.0	0.0	1.0	9.78			9.78
2-AP + C-480 at w_0 = 8.0	11.15	0.94	10.20	0.06	2.10	9.71
2-AP + C-480 at w_0 = 12.0	0.0	1.0	10.10			10.10
2-AP + C-480 at w_0 = 12.0	11.15	0.90	10.75	0.10	2.99	9.98

compared to C-440. Moreover anisotropy value of C-460 (0.035) in presence of AOT reverse micelles is large compared to that in bulk solution (0.011), which indicates its confinement. This confinement is due to hydrophobic interaction between C-460 and alkyl chain of AOT. But the anisotropy values are lower compared to C-440 because strength of H-bonding is strong enough compared to hydrophobic interaction. Additionally the location of C-460 can also be rationalized by using fluorescence results. C-460 shows emission maxima 465 in water, 415 in methanol, and 406 nm inside AOT reverse micelle where as C-440 shows emission maxima in AOT reverse micelle (537 nm) close to methanol (432 nm). These data proves that C-460 is located in a nonpolar region even less polar than polarity of methanol and at the same time it is confined. It is possible only when it is located in the hydrophobic chain of AOT facing the highly nonpolar solvent. In case of C-440, close matching of fluorescence maxima with methanol proves that it resides in such a region which has higher polarity than highly nonpolar solvents but lower polarity than water and it is possible only when it is located around the headgroup region.

High quantum yields of 2-AP (gradient plot is given in Fig. 6) at all the w_0 values make it suitable for serving as a good donor inside RM core. Time-resolved results give us useful information about the efficiency for any pair and we have observed that 2-AP–C-440 pair is more efficient than 2-AP–C-460 pair. Moreover an opposite trend in variation of efficiency is recorded for these two pairs. In all systems we have found that $J(\lambda)$ is of the order of 10^{16} M^{-1} cm^{-1} nm^4.

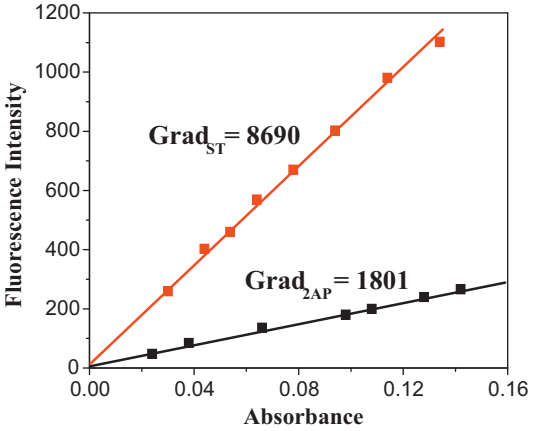


Fig. 6. Plot of gradient for rhodamine 6G (as standard, ST) and gradient for 2-AP.

Due to high value of extinction coefficient of acceptor (10^4) we got a high value of $J(\lambda)$. But the rate of energy transfer (k_{ET}) is not sufficiently high for all systems. In this study we have got Förster distance (R_0) 5–6 nm and D–A distance (r) 6–7 nm, respectively. All the Coumarins do not seem to be a suitable candidate as an acceptor for such system although good overlap between donor and acceptor as well as high value of extinction coefficient is present. There is no fluorescence lifetime quenching for 2-AP–C-480 pair. This fact can be attributed by the considerable solubility of C-480 in n-heptane in presence of AOT as well as different structural feature [31]. As a result of which C-480 cannot reach the location necessary in order to show FERT.

At various w_0 values, we have calculated all the FRET parameters and it was observed that as water content increases $J(\lambda)$ decreases. Gradual increment of FRET efficiency with increase in w_0 value is observed for 2-AP–C440 pair while efficiency is lowered in case of 2-AP–C-460 pair. This conclusion can be realized by considering the increased partition of 2-AP and C-44O with increase in water content in RM polar pool. This result is also an indication of non-identical location of these two acceptors. For both the pairs 2-AP–C-440 and 2-AP–C-460, Förster distance (R_0) value remains almost same at different w_0. Absolute distance(r) is decreased by ~4 Å during w_0 = 4.0 to w_0 = 12.0 for 2-AP–C-440 pair whereas this parameter is increased for the other pair. One possibility is that C-440 is located in such a position where its movement is more hindered (H-bonding between C-440 and AOT headgroup) than that of C-460, i.e., inside RM at the interfacial region around headgroups while C-460 is entangled on the hydrophobic surface just away from water pool. Indeed this idea is supported by their anisotropy values where r_0 of C-440 is greater than C-460 at corresponding w_0 values. Now the opposite trend of variation in absolute distance(r) for the two pairs can be demonstrated by considering some key parameters that are operating in parallel way for 2-AP–C-440 and differently for the other. With increase in water content, partition of both 2-AP and C-440 into the RM core increases and both are located in a well-defined location by their H-bond formation with the headgroup of the surfactant. As a consequence efficient FRET is observed for this pair with increase in water content. The increase in size of the RM is not so important for 2-AP–C-440 due to their firm entrapment inside RM interface while it becomes a key factor for 2-AP–C-460 pair to show different nature with respect to other pair. As demonstrated previously, C-460 is located on the hydrophobic surface and gradually it moves far away from 2-AP with increase in size of the RM. Unaltered partition of C-460 inside RM core along with greater separation of donor and acceptor with

increase in water content makes their FRET parameters less efficient with increase in size of the RM.

The size of RM at $w_0 = 4.0$ to $w_0 = 12.0$ is 3.0–5.0 nm [32,33] which are less than the D–A distance (6–7 nm). So the donor and acceptor can be located in two different RM. The lower value of energy transfer rate constant may be due to their existence inside two different RM cores which are less quickly diffused to each other. As a consequence they cannot acquire a suitable position to show FRET between themselves even after being confined in a RM.

In conclusion we can see that 2-AP and Coumarins are verified as successful FRET pair inside AOT–H_2O reverse micelle which share many fundamental properties of bio-membranes such as the dominance of interfacial effects on their behavior and the existence of an ordered array of oriented molecules making them well-suited to act as membrane models [33]. Moreover our examined FRET pairs show distinct nature in their FRET parameters according to their structural features. In future these can now be used in more modified and sophisticated confined media such as biomembranes with varying size, physical properties and chemical compositions etc.

5. Conclusion

During FRET measurement we have found the absolute distance between 2-AP and the Coumarin dyes are greater than the diameter of the RM, implying that the donors and acceptor are located in different RM, i.e., inter-micellar FRET is observed. The partition coefficients of 2-AP and C-440 get enhanced with increase in water content of RM while it is lowered for C-460. With increase in size of RM, the trend of variation of FRET parameters are opposite to each other for 2-AP–C440 and 2-AP–C460. This difference in behavior is due to their different locations in RM solution which in turn is solely due to their different structural properties. All these results prove that 2-AP and Coumarin are quite good candidates for use as FRET pairs inside a confined media. It is also a comparative study between an H-bond forming acceptor and non H-bonding acceptor with same donor inside AOT reverse micelle. These pairs can also be used as extrinsic fluorescent probes inside DNA containing system to conduct FRET study as well as conformational study in DNA-protein assembly.

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