Excited-State-Proton-Transfer-Triggered Fluorescence Resonance Energy Transfer: from 2-Naphthylamine to Phenosafranin

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Excited-state proton transfer (ESPT) and fluorescence resonance energy transfer (FRET) have been linearly coupled leading to an efficient pH-sensitive energy transfer from 2-naphthylamine (2NA) to a potentially bioactive cationic phenazinium dye, phenosafranin (PSF). The prototropic product produced exclusively from the photoexcited 2NA in the presence of added alkali serves as the donor for the energy transfer process. The energy transfer process is turned on at pH $\geq$ 12, whereas the process is turned off at a pH lower than that. Within the range of pH 12 to 13, the energy transfer efficiency (E) has been shown to follow a linear relation with the solution pH establishing the governing role of pH of the solution on the energy transfer process. The energy transfer follows a long-range dipole−dipole interaction mechanism. The critical energy transfer distance (R_0) and the distance between the acceptor and the donor (r) have been determined for the ESPT-promoted FRET process at an optimum pH of 13. The present study involving the coupled processes is simple but has its implication due to its potential to be exploited for designing a pH-sensitive molecular switch.

1. Introduction

Phototransformations may be initiated in a molecule through photoexcitation. Intramolecular charge transfer (ICT), excited-state proton transfer (ESPT), and Förster's or fluorescence resonance energy transfer (FRET) are three such photoreactions that have been widely studied because of the potential applicability of these processes in diverse fields ranging from biological chemistry to the development of the electronic switching devices.[1−6] Proton transfer reactions being associated with a charge separation along with a mass transfer gets modified remarkably in the excited state compared with that in the ground state because of the charge redistribution upon photoexcitation.[7] In a number of vital chemical, physical, and biological processes, a primary role is played by this elementary reaction, ESPT. These include acid−base neutralization,[8,9] electrophilic addition, and a score of enzymatic reactions.[10,11] Proton transfer processes also power many of the molecular machines reported so far.[12] Molecular electronic/photonic devices refer to systems in which spatially directed electron, proton, or energy-transfer processes take place in the photoexcited state.[13] Molecular switching has immense importance in biology because functions such as vision, allosteric regulation, and so on operate on the basis of this.[13] A modular construction of light-driven molecular switching is quite difficult to carry out. It would therefore be useful to explore and exploit viable strategies for using light to operate "stand alone" photochemically driven molecular switches.[14] Photoinduced ESPT is a promising avenue in this respect. Realization of the biochemical/biomedical applications of such studies have prompted scientists to get involved in the field. Recently, a color-changing molecular switch that can be monitored by the naked eye has been developed by Chen *et al.*[13] The switch is controlled by changing the pH, implying the impact of pH-sensitive studies.

FRET, because of its explicit distance dependence,[15] is used to characterize a wide variety of macromolecular assemblies such as biological membranes, proteins, DNAs, bichromophoric molecular systems, and so on.[16−19] In this photoprocess, excitation energy is transferred from one molecule (donor) to another molecule (acceptor) loosing the emission from the donor system.[20] The unique properties of FRET, particularly, the high sensitivity toward the proximity of the participating moieties in the nanometer range, both *in vitro* and *in vivo*, makes it a very useful "spectroscopic ruler".[15,20] A small amount of material, even a single cell that contains only picomoles of fluorescent material, may be examined.[21] This powerful technique has also been used to study the protein folding and kinetics of conformational changes in nucleic acids.[22]

These significant excited-state photoprocesses may sometimes be coupled to one another. When such a coupling is done within a molecular system, the excited state events may compete with each other dynamically. Therefore, proper pairing of excited-state processes can provide a score of structural/dynamical information about the participating molecules as well as the medium in which the coupling of transformations takes place.[23,24] To the best of our knowledge, the first work on ESPT-triggered FRET came from our group only recently.[25] In the work, the prototropic product (carbazole anion) produced exclusively from the photoexcited carbazole in the presence of externally added alkali served as the energy donor to a potentially bioactive ketocyanine dye 2-[3-(*N*-methyl-*N*-phenylamino)-2-propenylidene]indanone, MPAPI (acceptor). To establish the possibility of exploiting the ESPT-promoted FRET in a general way, in the present work, we have used another pair of systems to present the same coupled photoprocess. Here ESPT (intermolecular) and FRET have been linearly coupled, leading to an efficient pH-sensitive energy transfer from 2-naphthylamine (2NA) to a potentially bioactive cationic phenazinium dye, phenosafranin (PSF). The prototropic product produced exclusively from the photoexcited 2NA in the presence of externally added alkali serves as the energy donor. Coupling of the two important photoprocesses, namely, ESPT and FRET, projects a viable expansion of the field. Because of the inherent character of ESPT, the pH of the medium regulates the functioning of the

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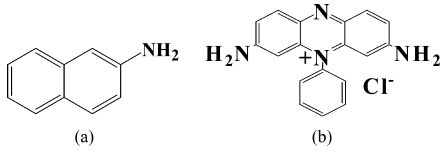
SCHEME 1: Structures of (a) 2NA and (b) PSF



process. Therefore, by controlling the pH of the medium, one can regulate the transfer of energy. Furthermore, ESPT-promoted FRET has the potential to overrule the restriction on the direct energy transfer between the two interacting partners under certain conditions.[25]

It is well known that the acid−base properties of many organic molecules change markedly as a consequence of electronic excitation, and the uniqueness of the proton transfer process lies in the difference between the dissociation constants of the ground and the excited states of the fluorophore.[26,27] 2NA undergoes an ESPT reaction in an alkaline medium resulting in dual emission, one from the neutral and the other from the anion.[28] The ground-state pK of 2NA is >14, whereas the excited-state pK* is reported to be 11.86 from titration method and 12.3 from steady-state fluorescence measurement.[29] 2NA, upon photoexcitation in aqueous alkaline solution at pH ≥ 12, undergoes ESPT reaction.[27−29] 2NA anion, produced exclusively in the photoexcited state, emits at 520 nm. PSF (3,7-diamino-5-phenyl phenazinium chloride) is a cationic dye of the phenazinium group (Scheme 1). The dye has extensively been used in semiconductors, as an energy sensitizer and as a probe for studying various microheterogeneous environments including micelles, reverse micelles, and polymeric matrices.[30−32] It has also been established as a DNA intercalator and is found to form dimeric aggregates at higher concentrations.[33,34] It is red in color with a planar tricyclic phenazinium moiety bearing a positive charge. PSF is a water-soluble dye with a characteristic broad absorption peak in the visible region at ∼520 nm and a broad emission band peaking at ∼580 nm that remains, to a large extent, invariant to the pH of the medium.[30] At a high enough pH (≥13.5), however, its fluorescence property changes because of the deprotonation of the amine groups. Considering the fact that the dye mostly absorbs at a region where the ESPT-produced 2NA anion emits, the fluorophores are judged to be a promising pair for an ESPT-promoted FRET phenomenon.

2NA was photoexcited at 320 nm, where PSF has insignificant absorption. For the FRET process, the donor species, 2NA anion, is generated exclusively in the photoexcited state from its neutral counterpart through ESPT only at a pH ≥ 12. Therefore, with the present system, a pH ≥ 12 turns on the energy-transfer process, whereas the process is turned off at a lower pH. ESPT-triggered FRET, therefore, appears to be aptly modeled for designing pH-sensitive molecular switches. Again, the anion is nonexistent in the dark. Produced in the photoexcited state, it transfers its energy to the acceptor (PSF) in the ground state through a long-range dipole interaction. Therefore, ESPT-promoted FRET provides a promising and convenient bypass route in circumstances where direct energy transfer between the partners is restricted.

2. Experimental Section

2NA and PSF were procured from Sigma Aldrich (USA) and were used as received. Triply distilled water was used to prepare the solutions. Sodium hydroxide (E. Merck, A.R.) was used without further purification. The concentration of 2NA was 8×10^{-6} mol dm^{-3} throughout the experiment. Absorption and steady-state fluorescence measurements were carried out using a Shimadzu UV-2450 spectrophotometer and a Spex fluorolog-2 spectrofluorimeter equipped with DM3000F software, respectively. Fluorescence lifetimes were determined from time-resolved intensity decays by the method of time-correlated single-photon counting (TCSPC) using a nanosecond diode laser at 370 nm (IBH, U.K., nanoLED-05) as the light source and TBX-04 as the detector. The decays were deconvoluted using IBH DAS-6 decay analysis software. Goodness of fit was evaluated by reduced χ^2 criterion and the randomness of the fitted function to the data. All of the experiments were performed at ambient temperature (27 °C) with air-equilibrated solutions.

The fluorescence spectra were resolved into overlapping Gaussian curves using the MS Origin 7.0 fitting algorithm to obtain the minimum number of reproducible components using the adjustable parameters of the center, width, and amplitude for the resolved bands. Multiple attempts to fit the data with different initial parameters generally provided a survey of the extent of statistically equivalent parameter sets. Comparing several resolutions of an overall spectrum, a "good fit" was judged from several criteria including a minimum in the goodness fit parameter χ^2 and the superposition of the convoluted curves on the experimentally obtained spectra.[34,35] From the statistically acceptable fits, a good fit was further judged by the reproducibility in the values of the centers of the Gaussian curves. A final, *albeit subjective*, criterion was to examine the fits for physically plausible results.

3. Results and Discussion

The fluorescence spectrum of aqueous solution of 2NA shows a broadband with a maximum at around 410 nm. As the pH of the solution is increased above 12, the intensity of the 410 nm band decreases with the concomitant development of a new broadband at 520 nm. In this pH range, the absorption as well as excitation spectra remain unaltered, indicating that the proton transfer in 2NA is exclusively an excited-state phenomenon.[27] This new band at 520 nm is ascribed to be due to emission from the 2NA anion produced in the excited state.[27] Thus, 2NA in the excited state has both emitting neutral and anionic forms, the latter being formed through the ESPT process. The absorption spectrum of PSF in aqueous medium shows a broad unstructured absorption band with a maximum at around 520 nm and a broad emission band peaking at around 580 nm.[30] Considering the fact that PSF absorbs mostly at a region where 2NA anion emits, the fluorophores are judged as a matched pair for the ESPT-promoted FRET study.

In aqueous medium, gradual addition of PSF to a 2NA solution at pH 13.0 depicted a gradual depletion of the emission intensity of the fluorophore band of the photoproduced anion at 520 nm with a concomitant development of a new band at ∼580 nm corresponding to PSF with an isoemissive point at 570 nm (Figure 1). The spectra are truncated at 630 nm so as to avoid the second-order scattering. The relative contribution of the 2NA anion band was found to decrease visibly with an associated increase in that of the PSF emission. Interestingly, the intensity of the fluorescence corresponding to the 2NA neutral species does not show a remarkable decrease with the addition of PSF. This implies that the neutral species is not the active participant in the energy-transfer process and reinforces the role of ESPT on the FRET process. However, because the emission band of the 2NA anion partially overlaps with that of PSF, resolution of the spectra is required to get their individual contributions from the observed spectra. In the absence of the added base, excitation of the system at the same wavelength

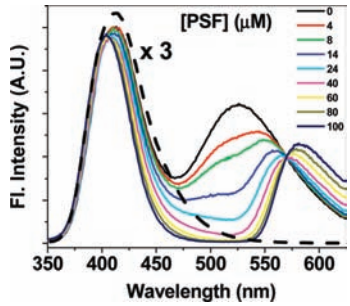


Figure 1. Fluorescence spectra of 2-naphthylamine anion at pH 13 as a function of PSF concentration ($\lambda_{exc} = 320$ nm). PSF concentrations are provided in the legends. Concentration of 2-naphthylamine is 8×10^{-6} M. The dashed line represents the emission spectrum of neutral 2NA.

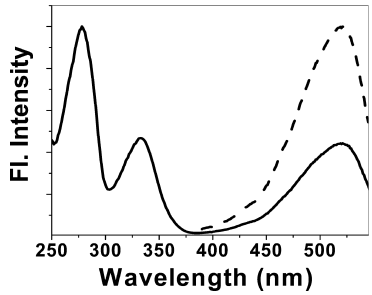


Figure 2. Fluorescence excitation spectrum of a mixture of 2-naphthylamine (8×10^{-6} M) and PSF (10×10^{-6} M) at pH 13 monitoring at 570 nm. Dashed line gives the excitation spectrum of PSF only monitored at 570 nm.

(320 nm) does not yield the 520 nm emission, thereby barring the energy transfer process. The role of pH of the solution in the conjugated process is thus established.[25] In the presence of PSF, the energy of the photoproduced 2NA anion is transferred to the added acceptor, leading to a diminution of the anionic fluorescence at 520 nm.

To verify that the fluorescence of PSF is originating through the FRET process and not by direct excitation, a blank experiment was performed with the same concentration of PSF in water in the absence of 2NA. The solution was excited at 320 nm, and the experimental result was compared with the total fluorescence coming from the mixture of the donor and the acceptor. Insignificant direct excitation of PSF at 320 nm in the blank experiment confirms the occurrence of the FRET process between the chosen pair. Observation of an isoemissive point in Figure 1 reinforces the occurrence of FRET between the present pair of systems, which would have had been absent if the quenching of fluorescence of anion was a simple case of reabsorption. Figure 2 depicts the excitation spectrum in the presence of the donor and the acceptor in aqueous alkaline solution. Neither the absorption nor the excitation spectra (Figure 2) of the mixture of the donor and acceptor systems exhibits any extra band other than the individual bands corresponding to 2NA and PSF. This rules out the formation of any ground-state complex between the donor and the acceptor in the solution.[36−38]

During the ESPT-coupled FRET study, no additional band was observed at longer wavelengths in the fluorescence spectrum of the mixture of the donor and the acceptor (Figure 1). This negates the possibility of the formation of exciplex between the photoexcited donor and the acceptor molecules. Consistent with the reports of Lakowicz,[20] De *et al.*,[37] and our own works,[25,38] the observation, that is, the decrease in the fluorescence intensity of the donor 2NA anion on the gradual addition

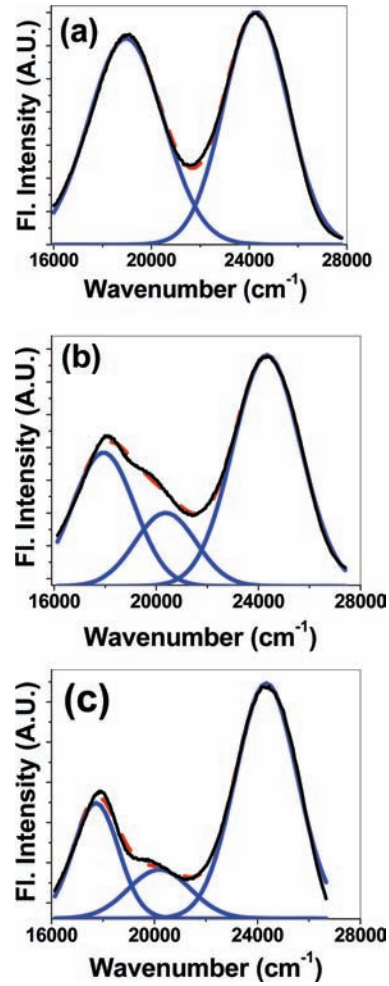


Figure 3. Resolved fluorescence spectra. Concentrations of PSF in (a), (b), and (c) are 0, 8×10^{-6}, and 14×10^{-6} M, respectively. [2NA] $= 8 \times 10^{-6}$ mol dm^{-3}. pH of the solution is 13. Black lines denote the experimental spectra, blue lines denote the resolved spectra, and red dashed lines denote the convoluted spectra from the resolved bands.

of PSF with a concomitant development of the PSF fluorescence, is a confirmed signature of Förster's type resonance energy transfer from 2NA anion to PSF.

In the first work on ESPT-triggered FRET, the emission of carbazole anion (donor) and 2-[3-(*N*-methyl-*N*-phenylamino)-2-propenylidene]indanone, MPAPI (acceptor), were well separated.[25] In the present case, however, there is a reasonable overlap between the emission bands of the donor and those of the acceptor species. This leads to a complication in addressing the quantitative evaluation of the FRET parameters. The problem was resolved by resolution of the spectra (Figure 3) into individual Gaussian curves using the method described in the Experimental Section. The resolutions have been performed in the frequency space because spectral band shape is defined in energy space. For the resolutions, the half-width values and peak maxima were varied slightly on a trial and error basis to obtain the best fit with a minimum in the goodness fit parameter χ^2 and a visual inspection of the superposition of the convoluted curves on the observed spectra. In the absence of PSF, Gaussian resolution of the fluorescence spectrum yields only two bands corresponding to the neutral (at 410 nm) and the anionic species (at 520 nm) of 2NA, respectively (Figure 3a). After the addition of PSF into the solution, Gaussian fits of the emission spectra yield three resolved bands for the system (Figure 3b,c). The first one corresponds to the neutral 2NA emission band with a

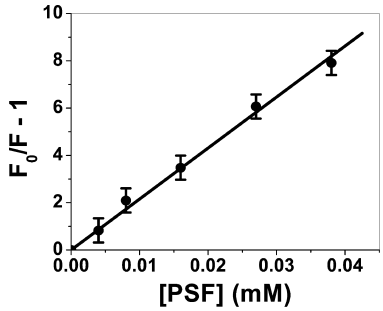


Figure 4. Stern−Volmer plot for the quenching of fluorescence of 2NA anion by PSF at pH 13.

maximum at 410 nm. As already argued, it is not the active participant in the FRET process. The second band corresponds to the 2NA anion appearing at ~520 nm that shows a gradual decrease in its fluorescence intensity with an increase in the PSF concentration, as expected, because of the energy transfer to PSF. The third band appearing at ~575 nm corresponds to the PSF emission and shows a gradual increase in the fluorescence intensity with its increasing concentration, reinforcing the occurrence of the FRET process between the 2NA anion and PSF.

Quenching of the steady-state fluorescence of the donor (2NA anion) in aqueous alkaline solution with the addition of the acceptor (PSF) was followed by Stern−Volmer equation, where the terms have their usual meanings.[20] The individual fluorescence intensities were extracted from the resolved spectra at a specific wavelength (520 nm, the maximum of the donor emission).

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

The linearity of the plot (Figure 4) indicates one type of quenching.[20] The slope of the plot gives K_{SV}, and its value is determined to be 2.2×10^5 M^{-1}.

The determined K_{SV} value falls in the normal range reported earlier for some other FRET processes and is order of magnitude higher than that observed for a normal diffusion-controlled quenching process.[36−38] The high K_{SV} value implies that the dominant mechanism of the fluorescence quenching is the resonance energy transfer occurring through long-range dipole−dipole interaction rather than the simple diffusion-limited process between the excited donor and the ground-state acceptor molecule.

To investigate the role of pH on the ESPT-coupled FRET process, we chose to perform the experiment in different solutions of varying pH (Figure 5). The pertinent point to mention here is that the coupled process is not triggered below pH 12.0 since ESPT does not operate (pK^* is reported to be ~12).[29] Below this pH, the coupled process fails to exhibit because the anion is not generated. Therefore, the experiments were carried out only at and above pH 12.0. Therefore for the present system, the coupled ESPT−FRET process can serve as a model molecular switch that goes on above pH 12 and off below this pH. Considering the fact that the fluorescence of the neutral species of 2NA does not change appreciably upon the addition of PSF (Figure 1), we have normalized the individual spectra at the peak of the neutral species of 2NA to get rid of the slight distortion of the fluorescence spectra because of the dilution effect induced upon addition of PSF.

As is clearly implied from the spectra at various pHs, the intensity of the emission band of 2NA anion is enhanced with

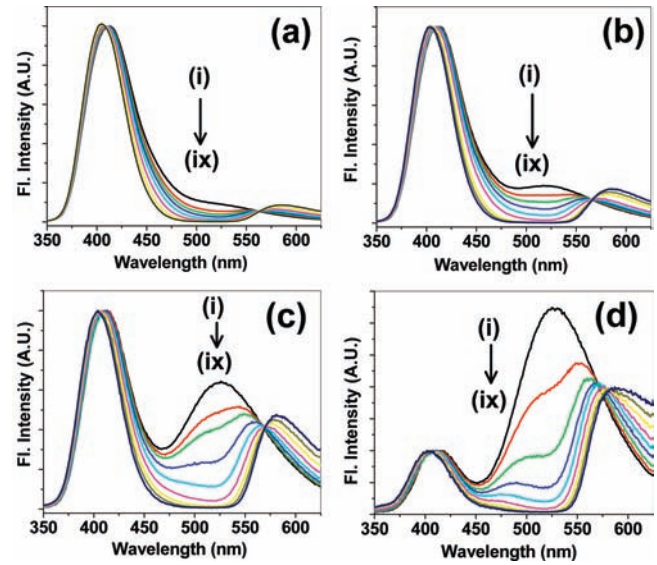


Figure 5. Normalized fluorescence spectra of 2-naphthylamine anion as a function of PSF concentration (λ_{exc} = 320 nm) at pH (a) 12.0 ± 0.1, (b) 12.5 ± 0.1, (c) 13.0 ± 0.1, and (d) 13.5 ± 0.1. PSF concentrations in all of the figures from curves (i) to (ix) are 0, 4, 8, 14, 24, 40, 60, 80, and 100×10^{-6} M. Concentration of 2-naphthylamine is 8×10^{-6} M in each case.

increasing pH. This leads to a more efficient energy transfer at higher pH. This further reinforces the impact of pH in the ESPT-promoted FRET process. However, as the pH of the solution becomes high (≥13.5), the fluorescence property of PSF itself changes because of the deprotonation of its amine protons. Therefore, the experiment loses its significance at very high pH. A pH of 13.0 was found to be the optimum for the quantitative study of the coupled energy transfer process.

For a FRET process, it is important to read the process through a measure of the energy transfer efficiency (E). According to the Förster's nonradiative energy transfer theory,[28,39] the energy transfer efficiency E depends not only on the distance (r) between the donor and the acceptor but also on the critical energy transfer distance (R_0), at which the efficiency of the energy transfer is 50%, by the relation

$$E = R_0^6/(R_0^6+r^6) \quad (2)$$

Energy transfer efficiency can be determined from the following relationship

$$E = 1 - F_{DA}/F_D \quad (3)$$

where F_{DA} is the fluorescence intensity of the donor in the presence of the acceptor and F_D is the fluorescence intensity in the absence of the acceptor. Energy transfer efficiency (E) has been determined to be 0.59 (±0.05) under the 1:1 condition of donor−acceptor concentration using eq 3. Figure 6 presents a plot of E against concentration of PSF in the presence of 8×10^{-6} M of 2NA at pH 13.

The value of R_0 depends on the spectral properties of both the donor and the acceptor systems. R_0 is expressed as follows

$$R_0^6 = 8.8 \times 10^{23}[\kappa^2 n^{-4} \phi_D J(\lambda)] \quad \text{in Å}^6 \quad (4)$$

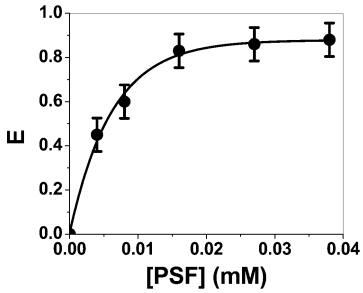


Figure 6. Plot of the energy transfer efficiency (E) against concentration of PSF at pH 13.0.

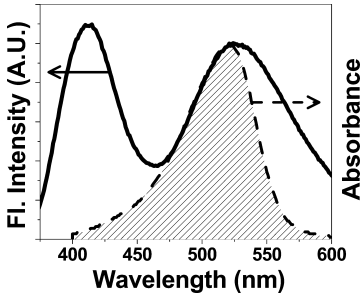


Figure 7. Normalized absorption spectrum of PSF (dashed line) and fluorescence spectrum of 2-naphthylamine (solid line) in aqueous solution at pH 13.

where κ^2 is the factor expressing the relative orientation of the donor to the acceptor molecule, n is the refractive index of the medium (1.333 for water), ϕ_D is the quantum yield of the donor in the absence of the acceptor, and $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor in units of M^{-1} cm^3. In general, κ^2 is assumed to be 2/3, considering the fact that the donor and acceptor randomize by rotational diffusion prior to the energy transfer.[20] The quantum yield of the anion of 2NA in aqueous alkaline medium (the donor here) was determined relative to the quantum yield of the neutral 2NA in water ($\varphi_{water} = 0.47$).[40] While doing this, the refractive indices of water and aqueous NaOH solution (sufficiently concentrated) were taken to be 1.3325 and 1.3628, respectively.[41] The quantum yield of the anion of 2NA was thus calculated to be 0.016. $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor in units of M^{-1} cm^3. The overlap integral expresses the degree of spectral overlap between the emission spectrum of the donor with the absorption spectrum of the acceptor. The greater the overlap, the higher is the value of R_0 and hence the higher the energy transfer efficiency.[20] $J(\lambda)$ is calculated by the numerical integration method. The overlap integral $J(\lambda)$ for a donor−acceptor pair is defined as[19,27,37,38]

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 \, d\lambda \qquad (5)$$

where $F_D(\lambda)$ is the corrected fluorescence intensity of the donor at wavelengths λ to $(\lambda + \Delta\lambda)$ with the total intensity normalized to unity and $\varepsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor at wavelength, λ. The estimated value of $J(\lambda)$ turns out to be 1.88×10^{-13} M^{-1} cm^3. The overlap spectrum is shown in Figure 7.

We have determined the critical energy transfer distance (R_0) and the distance between the donor and acceptor (r) at pH 13, which is found to be the optimum pH for the efficient energy transfer process, accepting that there is an uncertainty in the distance measurement arising principally because of the orientation factor. Critical energy transfer distance has been calculated using eq 4 and found to be 28.7 Å. Knowing the values of E and R_0, using eq 2, we calculate the distance between the donor and the acceptor to be 27.4 Å in aqueous solution under the 1:1 condition of donor−acceptor concentrations. The lower value of r than R_0 is justified from the value of $E > 0.5$ under the 1:1 condition of the donor and acceptor concentrations. We have also estimated the distance between the donor and the acceptor (r) for other concentrations of PSF with varying values of E, and the values are given in Table 1.

TABLE 1: FRET Parameters for Varying Concentrations of PSF

[PSF] (mM)	E	r (Å)
0	0	
0.004	0.45	29.7
0.008	0.59	27.4
0.016	0.83	22.0
0.027	0.86	21.2
0.038	0.88	20.6

TABLE 2: Fluorescence Decay Times of 2NA Anion (λ_{exc} = 370 nm) in the Presence of Varying Concentrations of PSF

[PSF] (mM)	τ (ns)	χ^2
0	5.10 ± 0.5	1.13
0.008	4.90 ± 0.5	1.04
0.024	3.26 ± 0.4	1.05
0.060	3.00 ± 0.3	1.16
0.100	2.43 ± 0.3	1.11

We also made an endeavor to explore the dynamics of the energy-transfer process from time-resolved fluorometry. We monitored the fluorescence decays of the three emitting species at their respective emission positions. The excitation source of our system is a 370 nm nanoLED, and the overall response time of the instrument is ~1 ns. It is pertinent to mention here that the steady-state spectra remain unchanged even upon excitation of 2NA at 370 nm (except for a reduction in the intensity compared with that when excited at 320 nm) because of negligible direct excitation of PSF at 370 nm. The lifetime of 2NA monitored at 410 nm (at pH 13) was found to remain invariant to the addition of PSF and corroborates the inference drawn from the steady-state observation that the neutral species is not the active participant in the coupled energy transfer process.

The decay time of the anion of 2NA (monitored at 500 nm), however, showed a decrease upon the addition of PSF. Its lifetime decreases from 5.10 ns in the absence of PSF to 2.43 ns in the presence of 10×10^{-5} M PSF (Table 2). This is consistent with the normal expectation that the addition of PSF should lead to an enhancement in the energy transfer process and hence the fluorescence lifetime of 2NA anion should decrease. Although a growth in the decay of the fluorescence of PSF originating through FRET is more confirmatory to establish the process, unfortunately, the short lifetime of PSF ($\leq$1 ns)[42] restricted us from observing the growth because of the low instrumental time resolution. However, deconvolution did provide a negative pre-exponent component in the analysis of the decays at 580 nm, suggesting a growth for the PSF fluorescence because of its formation through FRET. Such pre-exponents were absent, while the decays at 410 and 500 nm were analyzed. Although the low time resolution of the instrument forbids us to depend on the fast decay components,

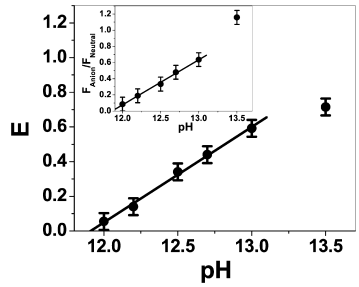


Figure 8. Plot of energy transfer efficiency (E) against solution pH. Inset shows the plot of the ratio of emission intensities of the anionic band to the neutral band of 2NA against pH. Scales for both of the plots are intentionally kept the same so that comparison of the slopes becomes obvious. (For details, see the text.)

the growth does indicate the occurrence of the FRET process through ESPT.

The energy transfer efficiencies under the 1:1 condition of donor−acceptor concentrations have been calculated at various pH values. It was found that the plot of E against pH followed a linear relationship within pH 12 to 13 (Figure 8). As already discussed, at higher pH values, the plot turns out to be nonlinear because the PSF molecule starts to get deprotonated, and the coupled process loses its significance. This rationalizes the absence of the isoemissive point at pH 13.5 (Figure 5d), whereas at other pHs, it is evident. The interesting feature to note here is that the slope of the plot of E versus pH (0.54 ± 0.2) matches well (within experimental error limit) with a plot of the ratio of emission intensity of the anionic band to that of the neutral band of 2NA against pH (0.52 ± 0.2). These experiments confirm that the energy-transfer efficiency of the coupled process is largely governed by the pH of the solution. Therefore, the role of pH in the work is firmly established. The latter plot also turned out to be nonlinear above pH 13, which was ascribed to the onset of OH^--induced quenching of the fluorescence of the neutral species at higher pH.[43,44]

Conclusions

In conclusion, we have successfully coupled ESPT to FRET. The prototropic product produced exclusively from the photoexcited 2NA in the presence of external alkali serves as the energy donor, whereas the cationic phenazinium dye, PSF, acts as the acceptor. The study reveals that the FRET process follows a long-range dipole−dipole interaction. The dependence of the energy transfer efficiency on the pH of the solution establishes the governing role of pH on the overall process. Various parameters associated with the energy transfer process have been determined at an optimum pH of 13. The studied ESPT-triggered FRET process can serve as a molecular switch that goes on and off above and below pH 12, respectively. It is inferred that with a proper choice of the two interacting partners, the coupled reaction, that is, ESPT-promoted FRET, has the potential to be utilized conveniently in pH-sensitive molecular switching. In fact, the wide applicability, generality, and utility of the simple technique of coupling ESPT with FRET, particularly in the fields of designing pH-sensitive molecular switches, is the essence of the work. The demonstration of coupling of the two important photoprocesses, namely ESPT and FRET, both relevant to chemistry and biochemistry, makes the work important from the basic as well as the application points of view.

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