

Ultrafast relaxation dynamics of a biologically relevant probe dansyl at the micellar surface

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Abstract

We report picosecond-resolved measurement of the fluorescence of a well-known biologically relevant probe, dansyl chromophore at the surface of a cationic micelle (cetyltrimethylammonium bromide, CTAB). The dansyl chromophore has environmentally sensitive fluorescence quantum yields and emission maxima, along with large Stokes shift. In order to study the solvation dynamics of the micellar environment, we measured the fluorescence of dansyl chromophore attached to the micellar surface. The fluorescence transients were observed to decay (with time constant ~ 350 ps) in the blue end and rise with similar timescale in the red end, indicative of solvation dynamics of the environment. The solvation correlation function is measured to decay with time constant 338 ps, which is much slower than that of ordinary bulk water. Time-resolved anisotropy of the dansyl chromophore shows a bi-exponential decay with time constants 413 ps (23%) and 1.3 ns (77%), which is considerably slower than that in free solvents revealing the rigidity of the dansyl-micelle complex. Time-resolved area-normalized emission spectroscopic (TRANES) analysis of the time dependent emission spectra of the dansyl chromophore in the micellar environment shows an isoemissive point at 21066 cm^{-1} . This indicates the fluorescence of the chromophore contains emission from two kinds of excited states namely locally excited state (prior to charge transfer) and charge transfer state. The nature of the solvation dynamics in the micellar environments is therefore explored from the time-resolved anisotropy measurement coupled with the TRANES analysis of the fluorescence transients. The time scale of the solvation is important for the mechanism of molecular recognition.

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1. Introduction

Fluorophores commonly used to probe structural, functional and dynamical information of a biomolecule are divided into two general classes, intrinsic and extrinsic. Intrinsic fluorophores are those, which occur naturally in biological macromolecules. In proteins, the dominant intrinsic fluorophore is the indole group of tryptophan. The emission spectrum of indole is highly sensitive to solvent polarity. Recently, a series of publi-

cations [1–3] followed by a review [4] from Zewail's group describe the ultrafast Stokes shift of the fluorescence from a single tryptophan at the surface of a protein to probe the dynamics of the water layer in the close vicinity of the protein molecule (hydration). In many cases, intrinsic fluorophores are not suitable for the dynamical studies of protein due to unavailability of proper excitation source or inconvenient fluorescence in UV–Vis spectrum. A wide variety of extrinsic probes have been developed for labeling the biological macromolecules in such cases [5]. These are also widely used to probe guest dynamics of nanocavities [6]. Dansyl chloride (DC, 5-dimethylamino-1-naphthalene sulfonyl chloride) is one of the widely used extrinsic probes in

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biological labeling [1,7]. DC reacts with free amino groups of a protein that fluoresce at the green-orange region of the visible spectrum.

Upon UV excitation, in polar media dansyl chromophore undergoes a twisted intramolecular charge transfer (CT) reaction [7,8]. For non-polar solvents, in the steady state, the emission is strong and is mostly from locally excited (LE) state (i.e. before charge separation). In polar solvents, the fluorescence decreases and is dominated by emission from the CT state. The solvent polarity and rigidity of the environment around the probe determine the wavelength and yield of emission. That is why dansyl is a useful biological probe. A molecular picture of solvation for the probe has been detailed in [7]. The study used femtosecond resolved laser spectroscopic technique and confirmed that in polar solvent, upon UV excitation the probe undergoes a CT reaction along with reorganization of solvent molecules around the probe (solvation). In the bulk polar solvent and with the protein Histone I, the time scale of twisting (8–11 ps) was found to be longer than that of solvation (<1 ps). Thus it was concluded that during the transformation from the initial LE state to the CT state, the polar solvent can “immediately” respond to the new configuration of the solute.

Recently, an extensive study [9] on the functionality of an enzyme α -chymotrypsin (CHT) upon complexation with cetyltrimethylammonium bromide (CTAB) micelle indicates that the activity of the enzyme increases by a factor of 2.5 compared to that in bulk water (buffer). The study concluded that the higher catalytic activity results from significant conformational change of the micelle-bound enzyme. However, another study of the enzyme-micelle complex from our group [10] involving a different substrate shows retarded (by a factor of 7) activity of the enzyme. In order to explore the effect of the environmental relaxation dynamics around the enzyme upon complexation with the micelle, the study used dansyl chromophore covalently attached to the surface of the enzyme. A significant retardation (nanosecond time scale) of the dynamics in the complex compared to that in free enzyme (picosecond time scale) was found.

For bulk polar solvents it is known that there is a time scale separation between the CT reaction and solvation dynamics [7], the former being an order of magnitude slower than later. However, if the two processes are having comparable time scale, particularly in a biomolecular environment, the use of DC as a probe of solvation dynamics requires further cautions. The important point is to find out if a probe undergoing a twisted CT reaction in the excited state is suitable for interrogating the environmental relaxation (solvation) or not. Secondly, if there is a different emissive species due to CT reaction in the excited state, whether it is possible to get the signature of the species. To address the above-mentioned questions, we have studied a cationic

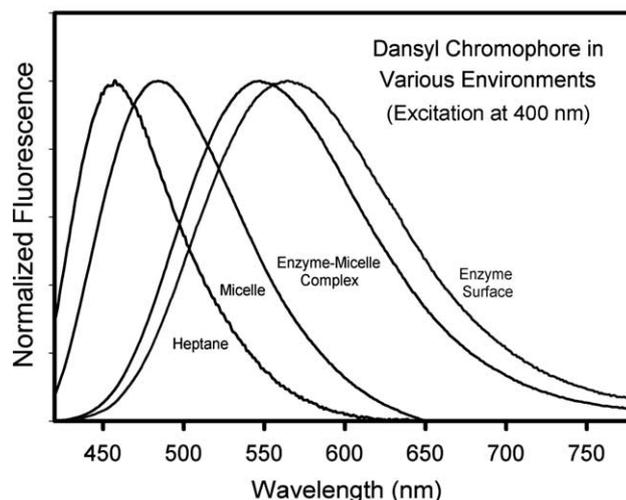
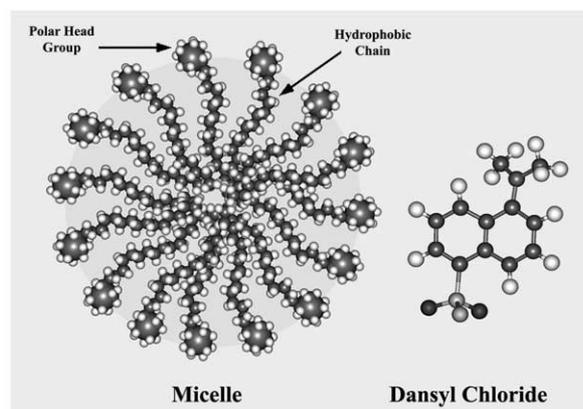


Fig. 1. A schematic of cationic CTAB micelle structure (upper, left). Molecular structure of dansyl chloride is shown in upper right. Lower, the normalized steady state fluorescence spectra of the probe dansyl in chemical and biological environments (see text). Note the huge solvatochromic shift in emission.

micelle (CTAB) using dansyl chromophore (Fig. 1, upper) as a fluorescent probe. The micelle is often used to mimic the surface effect of proteins [7,11–13]. However, the dynamical time scale of the environmental relaxation at micellar surface is significantly different from that at the protein surfaces [1]. By observing picosecond to nanosecond dynamics of population and polarization-analyzed anisotropy for the micelle-probe complex, we elucidate the nature of local environmental relaxation and the rigidity of binding structure of the complex. To confirm the coexistence of LE and CT excited states in the emission of the dansyl probe we also used time-resolved area-normalized emission spectroscopic (TRANES) method [14–16].

2. Experimental details

Steady-state absorption and emission were measured with Shimadzu Model UV-2450 spectrophotometer and

Jobin Yvon Model Fluoromax-3 fluorimeter, respectively. All transients were taken by using picosecond-resolved time correlated single photon counting (TCSPC) technique. The commercially available setup was a picosecond diode laser pumped time resolved fluorescence spectrophotometer from IBH, UK. The picosecond excitation pulse from the diode laser was used at 405 nm. By using liquid scatterer the FWHM of the instrument response function (IRF) was found to be ~ 225 ps. The fluorescence from the sample was detected by a photomultiplier after dispersion through a double grating monochromator. For all transients the polarizer in the emission side was adjusted to be at 54.7° (magic angle) with respect to the polarization axis of excitation beam.

DC from Molecular Probes, and CTAB from Fluka were used as received. Aqueous solutions were prepared in phosphate buffer (0.1 M, pH 7) and the DC-micelle complexes were prepared by mixing of DC (0.2 mM) with CTAB (50 mM) in a neutral buffer solution and kept the mixture at room temperature for 3 h.

3. Results and discussion

3.1. Solvation of the dansyl chromophore

Fig. 1, (lower) shows steady-state emission spectra of dansyl probe in various environments. At the surface of an enzyme α -chymotrypsin (CHT) dansyl (covalently bound) shows a fluorescence peak at 564 nm. For the dansyl-bonded enzyme and the CTAB-micelle complex, the emission maximum shifts to 547 nm. The fluorescence spectrum of dansyl probe with the CTAB micelle shows a peak at 484 nm. In all the cases the excitation wavelength was maintained at 400 nm. The emission spectrum of the dansyl chromophore in bulk *n*-heptane, a nonpolar solvent is also shown for comparison. Fluorescence maximum at 457 nm (excitation at 350 nm) is obtained, which is 27 nm blue shifted compared to that in the micellar environment. From the steady-state spectra, the solvatochromic shift of the fluorescence towards longer wavelengths with the increase in environmental polarity is evident. Note that the emission spectra of the systems shown here are studied in detail and reported elsewhere [10].

In a micellar solution, there are three possible locations of the probe, bulk water, inner hydrocarbon core and the peripheral Stern layer (at the surface). Dansyl is sparingly soluble in water and after solubilization in micelle the time-resolved anisotropy, $r(t)$ shows a decay (see below) which is much slower than that in bulk solvent (~ 50 ps) [7], revealing a clear indication of the attachment of the probe with the micelle. Thus the first possibility can be ruled out. In the hydrocarbon core the emission maximum is expected to be similar to that in *n*-

heptane, which is not the case here. Moreover dansyl molecules staying in the non-polar hydrocarbon core of the micelle is not expected to contribute to the observed solvation dynamics. Thus the dynamics is exclusively due to the dansyl molecules at the surface Stern layer.

The transients observed at three characteristic wavelengths, from the blue to the red side of the fluorescence spectrum of dansyl in the micelle, are shown in Fig. 2. The emission transients detected in the blue region of the fluorescence spectrum are characterized by an instant rise (IRF) and a picosecond decay component (~ 350 ps). When detection is done in the red region, the decay part slows down until eventually an initial rise on a picosecond time scale (~ 350 ps) is observed. A nanosecond decay was found to be almost 12 ns, which is present at all wavelengths with different contributions is the lifetime of dansyl chromophore in the relaxed equilibrium state [7]. These overall features are well recognized as being characteristics of solvation [11].

The solvation dynamics is described by the response function $C(t)$, which is defined as: $C(t) = [v(t) - v(\infty)]/[v(0) - v(\infty)]$, where $v(0)$, $v(t)$ and $v(\infty)$, denote the observed emission energies (in wavenumbers) at time 0, t and ∞ , respectively. To construct the dynamical change of the spectra at various times, t , and the solvent response function, $C(t)$, we have adopted the procedure

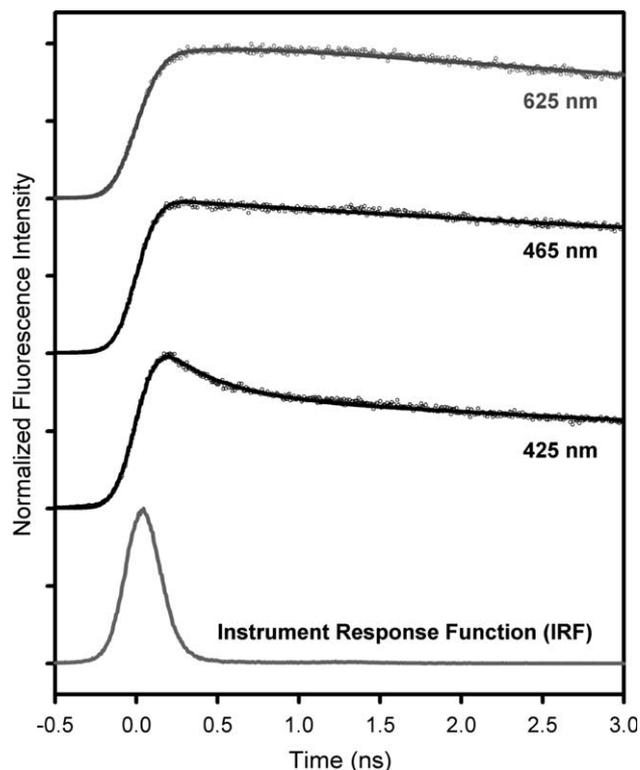


Fig. 2. Picosecond-resolved transients at three characteristic wavelengths of the dansyl probe in micelle. Transients are normalized for comparison.

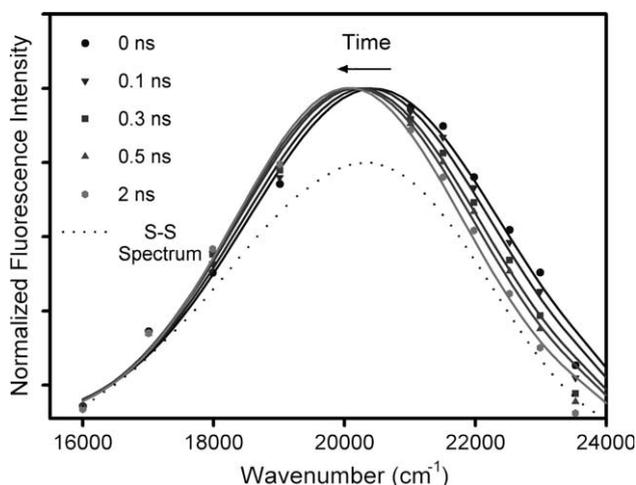


Fig. 3. The normalized spectral evolution (TRES) at five delay times from $t = 0$ for dansyl in the micellar environment. The dotted line indicates steady-state spectrum of the probe dansyl in micelle.

described in [17]. Using the parameters of best fit to the emission transients and the steady-state emission spectrum, time-resolved emission spectra (TRES) were constructed. The time constants for solvation were determined from the temporal evolution of $C(t)$. Fig. 3 depicts the TRES curves of dansyl in the CTAB micelle. The steady-state fluorescence spectrum is shown in the Fig. 3 (dotted line) for comparison with infinite time (2 ns in this case) spectrum.

The time evolution of $C(t)$ is shown in Fig. 4. The decay curve of the $C(t)$ was fitted to an exponential function, giving a time constant of 338 ps where the 50 ps or less component was not resolved. The net dynamical spectral shift is 374 cm^{-1} (from 20441 to 20067 cm^{-1}) over a time span of 2 ns. The temporal

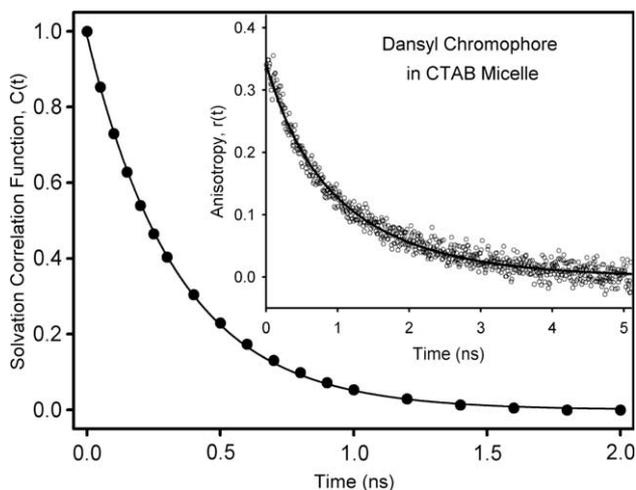


Fig. 4. The solvation correlation function, $C(t)$ of the micellar environment probed with the dansyl chromophore. Inset shows time-resolved anisotropy, $r(t)$ decay of the dansyl probe in the micellar environment.

behavior of the $C(t)$ is very similar to the dynamics observed recently using the DCM (4-(dicyanomethylene)- α -methyl-6(p-dimethylaminostyryl) 4H-pyran) as solvation probe [11]. The study [11] reported a biexponential nature of the decay of $C(t)$ with time constants 170 ps (50%) and 630 ps (50%); estimated average solvation time and net spectral shift were found to be 400 ps and 550 cm^{-1} , respectively, consistent with our observations.

3.2. Solvation vs. twisting dynamics

To study the degree of orientational rigidity of the dansyl probe in the micelle and the nature of spectral shift in the solvation process, we obtained the fluorescence anisotropy decay at the wavelength 488 nm for the excitation wavelength of 405 nm. For this, we took emission with parallel and perpendicular polarizations and obtained anisotropy as a function of time according to the formula $r(t) = [I_{\text{para}} - G \cdot I_{\text{perp}}] / [I_{\text{para}} + 2 \cdot G \cdot I_{\text{perp}}]$. The magnitude of G , the grating factor of the emission monochromator of the TCSPC system is found by using a coumarin dye in methanol by following the longtime tail matching technique [18] to be 2.1. The $r(t)$ function is the sum of two exponentials (inset of Fig. 4) with time constants 413 ps (23%) and 1.3 ns (77%) with $r(t) = 0.34$ at $t = 0$ ns. The faster time constant (413 ps; 23%) is similar to the solvation time constant 338 ps obtained from $C(t)$ decay. Thus an entanglement of conformational changes, which should alter the anisotropy to yield twisted CT state in the observed solvation dynamics can not be completely ruled out. However, lesser weight (23%) of the faster time constant (413 ps) of the $r(t)$ decay and resemblance of the observed time constant of solvation dynamics with other study [11] using a different probe DCM reveal that the contribution of conformational dynamics (twisting) to the solvation is *not* significant. The longer time constant (1.3 ns; 77%) could be due to rotational diffusion of the DC chromophore at the micellar surface and/or slow tumbling motion of the micelle as a whole.

3.3. Existence of excited CT state

As mentioned above there is an indication of existence of excited twisted CT in the time resolved emission. To ascertain emission from two excited states, we further followed TRANES technique, which has been developed recently [14–16]. As described in [14–16] TRANES is a model free modified version of TRES mentioned above. A useful feature of the method is that an isoemissive point in the spectra involves two emitting species, which are kinetically coupled either irreversibly or reversibly or not coupled at all.

Fig. 5 shows TRANES spectra of dansyl chromophore in the CTAB micelle. An isoemissive point at

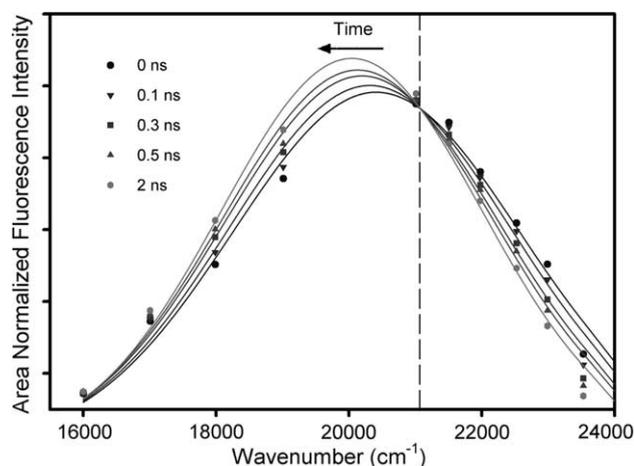


Fig. 5. Time-resolved area-normalized emission spectra (TRANES) of the dansyl probe in micellar environment at five delay times from $t = 0$ ns. Note the isoemissive point at 21066 cm^{-1} (see text).

21066 cm^{-1} is clearly evident in the spectra. One of the possible reasons of existence of two emissive species in the excited state (as indicated by the isoemissive point in the TRANES spectra) is due to the solubilization of the dansyl chromophore at two different sites of the micelle [15]. However, our observation of entanglement of conformational dynamics with the solvation and other steady-state study reported in [8] support the coexistence of the excited CT state with the LE state (before charge transfer).

4. Conclusion

The study demonstrates slow solvation dynamics in a micellar environment (CTAB) by using dansyl chromophore as a probe. The time evolution of TRES shows an exponential decay of time constant 338 ps, which is consistent with other studies of the same micelle using different dye molecule as probe [11]. Picosecond-resolved anisotropy measurement indicates much slower rotational diffusion of the dansyl chromophore in the micelle (1.3 ns) compared to that in bulk solvent (50 ps) [7]. The anisotropy measurement also signifies a possible entanglement of the dynamics of conformational change (twisting; 413 ps) of the probe in the solvation process (338 ps). However, the contribution of the entanglement of twisting in the measured solvation dynamics was found to be insignificant. TRANES analysis of the fluorescence of dansyl chromophore indicates a possible existence of twisted excited CT-state as one of the emissive species.

The dynamical picture of the solvation at the surface of the micelle presented here may have relevance to the function of the micelle in the recognition of the enzyme α -chymotrypsin [9,10]. The labile environment in the close vicinity of the micellar surface would enhance the

interaction with the enzyme. On the other hand dynamically rigid environment is needed around the surface to maintain a 3D structure of the micelle. Thus, the time-scale of the solvation dynamics is crucial to the tradeoff between the micellar structure and its function of molecular recognition [4], and it is possible that the reported time constant (338 ps) is of fundamental importance for such function.

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References

- [1] S.K. Pal, J. Peon, A.H. Zewail, Biological water at the protein surface: dynamical solvation probed directly with femtosecond resolution, *Proc. Natl. Acad. Sci. USA* 99 (2002) 1763–1768.
- [2] J. Peon, S.K. Pal, A.H. Zewail, Hydration at the surface of the protein Monellin: dynamics with femtosecond resolution, *Proc. Natl. Acad. Sci. USA* 99 (2002) 10964–10969.
- [3] L. Zhao, S.K. Pal, T. Xia, A.H. Zewail, Dynamics of ordered water in interfacial enzyme recognition: bovine pancreatic phospholipase A2, *Angew. Chem. Int. Ed.* 43 (2004) 59–63.
- [4] S.K. Pal, A.H. Zewail, Dynamics of water in biological recognition, *Chem. Rev.* 104 (2004) 2099–2123.
- [5] R.P. Haugland (Ed.), *Handbook of Fluorescent Probes and Research Chemicals*, seventh ed, Molecular Probes, Eugene, OR, 1999, pp. 8–13.
- [6] A. Douhal, Ultrafast guest dynamics in cyclodextrin nanocavities, *Chem. Rev.* 104 (2004) 1955–1976.
- [7] D. Zhong, S.K. Pal, A.H. Zewail, Femtosecond studies of protein-DNA binding and dynamics: Histone I, *Chemphyschem* 2 (2001) 219–227.
- [8] B. Ren, F. Gao, Z. Tong, Y. Yan, Solvent polarity scale on the fluorescence spectra of a dansyl monomer copolymerizable in aqueous media, *Chem. Phys. Lett.* 307 (1999) 55–61.
- [9] M.S. Celej, M.G. D'Andrea, P.T. Campana, G.D. Fidelio, M.L. Bianconi, Superactivity and conformational changes on α -chymotrypsin upon interfacial binding to cationic micelles, *Biochem. J.* 378 (2004) 1059–1066.
- [10] R. Sarkar, M. Ghosh, S.K. Pal, Ultrafast surface solvation dynamics and functionality of an enzyme α -chymotrypsin upon interfacial binding to a cationic micelle, *Eur. Biophys. J.*, submitted for publication.
- [11] S.K. Pal, D. Sukul, D. Mandal, S. Sen, K. Bhattacharyya, Solvation dynamics of DCM in micelles, *Chem. Phys. Lett.* 327 (2000) 91–96.
- [12] P.P. Mishra, J. Bhatnagar, A. Datta, Fluorescence monitoring of pH dependent complexation of chlorin p_6 with surfactants, *Chem. Phys. Lett.* 386 (2004) 158–161.

- [13] A. Datta, A. Dube, B. Jain, A. Tiwari, P.K. Gupta, The effect of pH and surfactant on the aggregation behavior of chlorin p_6 : a fluorescence spectroscopic study, *Photochem. Photobiol.* 75 (2002) 488–494.
- [14] A.S.R. Koti, M.M.G. Krishna, N. Periasamy, Time-resolved area-normalized emission spectroscopy (TRANES): a novel method for confirming emission from two excited states, *J. Phys. Chem. A* 105 (2001) 1767–1771.
- [15] A.S.R. Koti, N. Periasamy, TRANES analysis of the fluorescence of Nile red in organized molecular assemblies confirms emission from two species, *Proc. Indian Acad. Sci. (Chem. Sci.)* 113 (2001) 157–163.
- [16] N. Periasamy, A.S.R. Koti, Time resolved fluorescence spectroscopy: TRES and TRANES, *Proc. Indian Natn. Sci. Acad.* 69 A (2003) 41–48.
- [17] M. Maroncelli, G.R. Fleming, Picosecond solvation dynamics of coumarin 153: the importance of molecular aspects of solvation, *J. Chem. Phys.* 86 (1987) 6221–6239.
- [18] D.V. O'Connor, D. Phillips, *Time Correlated Single Photon Counting*, Academic Press, London, 1984.