Journal of Fluorescence, Vol. 14, No. 1, January 2004 (©2004)

Probing the Liquid-State Structure and Dynamics of Aqueous Solutions by Fluorescence Spectroscopy

Lorna Dougan,[1,2] Jason Crain,[1,2] Hugh Vass,[1,2] and Steven W. Magennis[1,2,3]

Received September 3, 2003; revised October 17, 2003; accepted October 17, 2003

Time-resolved fluorescence spectroscopy of the solvent-sensitive molecule 1,8-anilinonaphthalene sulfonate (ANS) is used to probe the structure and dynamics of an aqueous methanol solution (mole fraction = 0.5). The intensity decay of ANS in the mixed solvent displays single exponential kinetics under ambient conditions. At low temperature, a simple two-state solvent relaxation model describes the fluorescence decay for ANS in both methanol and the mixed solvent. The temperature dependence of ANS fluorescence in the mixed solvent is attributed to the onset of glassy dynamics in the aqueous component at higher temperature, implying a partial demixing of the water and methanol due to self-association. We discuss the absence of more complicated fluorescence decays in such a heterogeneous solvent system.

KEY WORDS: Water-methanol; fluorescence; 1,8-ANS; structure; dynamics.

INTRODUCTION

The ubiquitous and deceptively simple water molecule continues to receive considerable scientific attention, primarily because of the complexity of its molecular interactions. This anomalous behaviour is especially apparent under non-ambient conditions [1]. It has recently been demonstrated from neutron diffraction studies that the anomalous thermodynamic properties associated with the mixing of a simple alcohol such as methanol with water are due to incomplete mixing at the molecular level [2]. A study of simple aqueous solutions by Brillouin spectroscopy under extreme conditions also suggests that the water network remains intact in the presence of a wide variety of solutes [3]. Studies by other groups using alternative techniques have led to similar conclusions [4]. Such self-association through hydrogen-bonding interactions is of major importance throughout the physical and life sciences, making a study of these simple systems an essential step towards an understanding of more complex structures.

In this context, fluorescence-based techniques can be immensely useful in providing both microscopic and macroscopic-scale structural information, in addition to probing dynamical processes that occur on the timescale of the fluorescence decay. Fluorescent molecules are often extremely sensitive to their local environment with many fluorophores being profoundly influenced by surrounding solvent molecules [5]. The study of solvent-sensitive fluorescent molecules in heterogeneous solvent systems can potentially provide a very powerful means to probe both the underlying structure of the solvent molecules and also the kinetics of processes such as solvent exchange [6].

This paper describes our preliminary studies of a simple aqueous methanol solution using time-resolved fluorescence spectroscopy of the solvent-sensitive 1,8-anilinonaphthalene sulfonate (ANS). The presence of methanol allows access to the low temperature regime, where many of the unusual properties of aqueous systems are manifested.

[1] School of Physics, The University of Edinburgh, Mayfield Road, Edinburgh EH9 3JZ, UK.
[2] Collaborative Optical Spectroscopy Micromanipulation and Imaging Centre, The University of Edinburgh, Mayfield Road, Edinburgh EH9 3JZ, UK.
[3] To whom correspondence should be addressed E-mail: s.magennis@ed.ac.uk.

1053-0509/04/0100-0091/0

EXPERIMENTAL

Materials

All measurements were made with the ammonium salt of ANS (Fluka, used as received). Initial measurements under ambient conditions using either the ammonium salt or the free acid of ANS (Molecular Probes, used as received) were identical. The methanol used in this study was spectroscopic grade from Fluka and was used as received. The water was HPLC grade (Aldrich) and was used as received.

Solutions of ANS (ca. 5×10^{-5} M) were prepared from a stock solution and stored in the dark. The lifetime of ANS fluorescence at room temperature was used as a routine check of sample purity after cooling and after storage. No emission could be detected from the solvents under the instrumental conditions employed. Absorption spectra were measured on a Cary 50 spectrometer using a 1 cm pathlength cell; the absorbance of solutions was between 0.1–0.2 at the excitation wavelength.

Fluorescence Spectroscopy

Time-resolved fluorescence spectroscopy was performed using the technique of time correlated single photon counting (TCSPC) [7]. The aerated solution samples were measured in a 1 cm pathlength cuvette in an Edinburgh Instruments spectrometer equipped with TCC900 photon counting electronics. The excitation source was a Ti-Sapphire femtosecond laser system from Coherent (10 W Verdi and Mira Ti-Sapphire laser) producing pulses of ca. 200 fs at 76 MHz. The output of the Mira was passed through a pulse picker (reducing the rep. rate to 4.75 MHz) and then frequency doubled to give an output at 395 nm. The excitation beam was split, and one portion was used to trigger a fast photodiode. The emission from the sample was collected at right angles to the excitation direction and at the magic angle with respect to the vertical polarisation of the incident beam (excitation polarisation was controlled by a Soleit-Babinet compensator). The light was passed through a monochromator (bandpass 10 nm) then detected by a Hamamatsu MCP-PMT (R3809U-50). The instrument response of the system, measured using a Ludox scatterer, was approximately 50 ps FWHM. Fluorescence decay curves were analysed by iterative reconvolution of the decay with the instrument response function (IRF). Decay curves were recorded with 4096 channels and to 10,000 counts in the peak channel on 5, 20 and 50 ns ranges for the water, water-methanol and methanol solutions respectively. Discrete component analysis was performed with F900 software (Edinburgh Instruments), whilst global analyses were performed using FAST software (Alango Ltd.). The quality of the fits was determined by the value of the reduced chi-squared statistical parameter and by visual inspection of residuals. The fractional intensities (f_i) were calculated from the values of lifetimes (τ_i) and pre-exponential factors (α_i) as follows: $f_1 = \alpha_1\tau_1/(\alpha_1\tau_1 + \alpha_2\tau_2)$ and $f_2 = \alpha_2\tau_2/(\alpha_1\tau_1 + \alpha_2\tau_2)$.

For temperature dependent measurements, a copper holding cell was designed to securely hold the sample cuvette in place in the spectrometer chamber. Windows in the copper cell were positioned in the chamber to give optimum signal for the sample with right-angle detection geometry. The temperature was controlled and adjusted with a flow of cold nitrogen gas through copper tubing around the copper cell. Temperatures were measured with a thermocouple placed directly on the sample cuvette. The copper cell was enclosed in a blackened polystyrene case to provide both insulation and to avoid stray reflections.

RESULTS

Under ambient conditions, the fluorescence decay of ANS was found to be single exponential in pure water and pure methanol and the lifetimes are in good agreement with literature values [8] (Table I). A number of studies of ANS fluorescence in binary solvent systems have been reported, but to the best of our knowledge these have not included the measurement of a water-methanol mixture. Fluorescent lifetime measurements of ANS in a water-ethanol mixture have been reported, though the exact nature of the time-resolved fluorescence decays is not clear. The most recent study indicated that under ambient conditions the fluorescence decay of ANS in water-ethanol mixtures was single exponential [9], in contradiction to an earlier study stating that the decays were clearly non-exponential [10]. We have found that the lifetime of ANS is indeed single exponential under ambient conditions in both water-methanol and water-ethanol mixtures (mole fraction $x = 0.5$) and this is independent of emission wavelength. Global analysis gives a lifetime of 2.2 ns ($\chi^2 = 1.13$) for the water-methanol solution and the quality of the fitted data are evident in Fig. 1, which shows data, fit and residuals for the mixture at an emission wavelength of 510 nm.

The fluorescence decay was measured for ANS in water, methanol and the water-methanol mixture as a function of temperature and the results are summarised in Table I and displayed graphically in Fig. 2. The fluorescence decay times increase upon cooling in agreement with a previous study [11], and this commonly observed

Table I. Fluorescence Decay Parameters of ANS in Water ($\lambda_{em} = 520$ nm), Methanol ($\lambda_{em} = 490$ nm) or Methanol:Water ($x = 0.5$, $\lambda_{em} = 510$ nm)

Solution	Temp (K)	Viscosity (poise) [temp. (K)][a]	Lifetimes (ns)		Preexponential factors		Fractional intensities		
			τ_1	τ_2	α_1	α_2	f_1	f_2	χ^2
Water	298		0.241		1		1		1.09
	283		0.242		1		1		1.19
Methanol	298	0.007 [283]	6.09		1		1		1.06
	248	0.014 [243]	8.47		1		1		1.14
	228	0.017 [233]	9.57		1		1		1.14
	200	0.039 [203]	10.46	0.100	0.841	0.159	0.994	0.006	1.10
	190	0.056 [183]	10.75	0.310	0.817	0.183	0.998	0.002	1.07
Methanol: water ($x = 0.5$)	298	0.019 [295]	2.197		1		1		1.08
	273	0.026 [273]	2.376		1		1		1.09
	248		2.687		1		1		1.05
	238	0.127 [238]	2.875		1		1		1.06
	228		3.153	0.193	0.856	0.144	0.990	0.010	1.01
	218		3.553	0.416	0.831	0.169	0.977	0.023	1.02
	208		4.168	0.769	0.829	0.171	0.963	0.037	1.04
	200	0.904 [200]	4.940	1.357	0.860	0.140	0.957	0.043	1.14

[a] *J. Chem. Eng. Data* **16**(2), 222 (1971).

temperature dependence is attributed to a decrease in the contribution of non-radiative deactivating pathways.

The lifetime of ANS in pure methanol is single exponential until a temperature of around 200 K is reached, at which point the data is best described by biexponential kinetics, with a long component of 10.5 ns and a short component of 100 ps (Table I). On cooling to 193 K this short component increases to around 300 ps. We attribute this short component to solvent relaxation, and a wavelength dependent study at 200 K shows the pre-exponential factor for the short lifetime component varies from a positive decay at short wavelength to a negative rise time at long wavelength (Table II). The data for the temperature dependence of ANS in methanol is well described by a two-state solvent relaxation model [12,13].

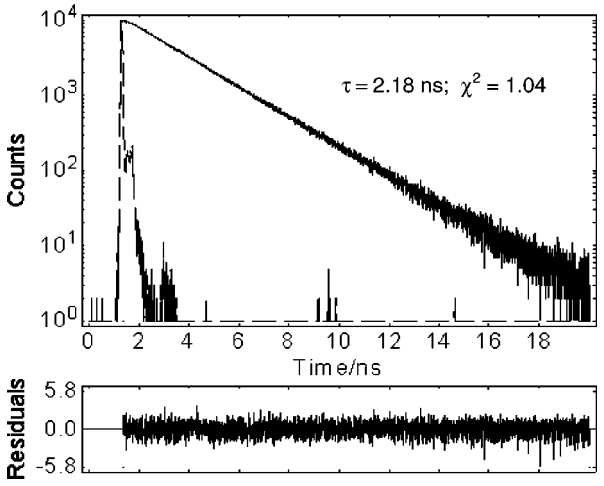

Fig. 1. Fluorescence decay of ANS in methanol-water ($x = 0.5$) at room temperature; $\lambda_{em} = 510$ nm. The IRF, single exponential fit and residuals are also shown. The measured decay and fit are indistinguishable.

The temperature dependence of the ANS fluorescence in the $x = 0.5$ mixture also shows a transition to biexponential kinetics at low temperature, but in this case the change occurs at a much higher temperature (ca. 230 K) and the difference between the two lifetimes is much less, with the shorter lifetime being an order of magnitude greater than for the methanol solution at 200 K. Again, we propose that the onset of biexponential kinetics is due to a slowing of solvent relaxation dynamics upon cooling. A wavelength dependent study of the fluorescence decay at 200 K again agrees with a two-state solvent relaxation model (Table II). The fluorescence decay, biexponential fit and residuals for single and double exponential fits for ANS in methanol-water ($x = 0.5$) at 200 K are shown in Fig. 3.

DISCUSSION

ANS fluorescence has received considerable attention, owing to its extensive use as a biomolecular probe [8]. The photophysics of ANS in pure solvents can be described in terms of a solute-solvent interaction model [14], and in polar solvents is dominated by emission from an

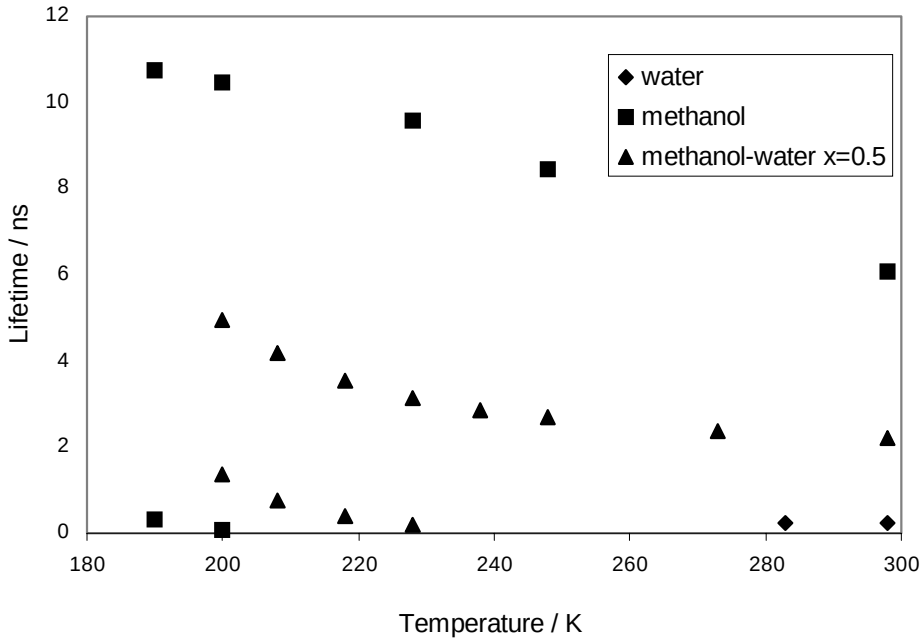

Fig. 2. Temperature dependence of ANS fluorescence decay times in water ($\lambda_{em} = 520$ nm), methanol ($\lambda_{em} = 490$ nm) and water-methanol ($x = 0.5$, $\lambda_{em} = 510$ nm).

intermolecular charge transfer state [15]. ANS is an ideal probe of the structure and dynamics of a water-methanol mixture because of the 25-fold difference in lifetime of ANS in water compared with ANS in methanol. Whilst the effect of solvent on fluorophores has been the subject of considerable investigation [5,16], relatively little of this has focused on mixed solvent systems (e.g. [6,17–21]). In addition, the binary systems studied have not usually been mixtures of two protic polar solvents. A recent notable exception is a study of intramolecular non-radiative decay in rhodamine 3B in water-ethanol mixtures, which produced evidence of solvent clustering [22]. However, the rhodamine 3B probe has essentially identical lifetimes in either of the pure solvents, so the use of ANS is a very different approach to the investigation of molecular segregation.

In general, studies of fluorophores in mixed solvents have shown complex fluorescence decays, in some cases attributed to a distribution of fluorophore environments [5,23], but also to site-specific solvated species [17]. In this context, the first interesting feature of the fluorescence of ANS in the methanol-water mixture is that it displays single exponential kinetics at ambient temperatures (see below).

Table II. Global Analysis of a Two Component Mixture of 1,8-anilinonaphthalene Sulfonate (ANS) in $x = 0.5$ and $x = 1$ Methanol/Water Solution at 200 K Measured at Five Emission Wavelengths

	ANS ($x = 0.5$ methanol/water, $\tau_1 = 4.85$ ns, $\tau_2 = 0.795$ ns)[a]				ANS ($x = 1.0$ methanol, $\tau_1 = 9.85$ ns, $\tau_2 = 0.115$ ns)[a]			
$\lambda_{em.}$ (nm)	α_1[b]	f_1[b]	α_2[b]	f_2[b]	α_1[b]	f_1[b]	α_2[b]	f_2[b]
470	0.233	0.650	0.767	0.350	0.343	0.978	0.657	0.022
490	0.582	0.894	0.418	0.106	0.478	0.987	0.522	0.013
510	0.809	0.963	0.191	0.037	0.644	0.994	0.356	0.006
530	0.961	0.993	−0.039	−0.007	0.823	0.997	0.177	0.003
550	0.811	0.963	−0.189	−0.037	0.913	0.999	−0.087	−0.001

[a]For the ANS $x = 0.5$, fit $\chi^2 = 1.17$; for the ANS $x = 1.0$, fit $\chi^2 = 1.04$.
[b]Where α_1 and α_2 are preexponential factors and f_1 and f_2 are fractional intensities.

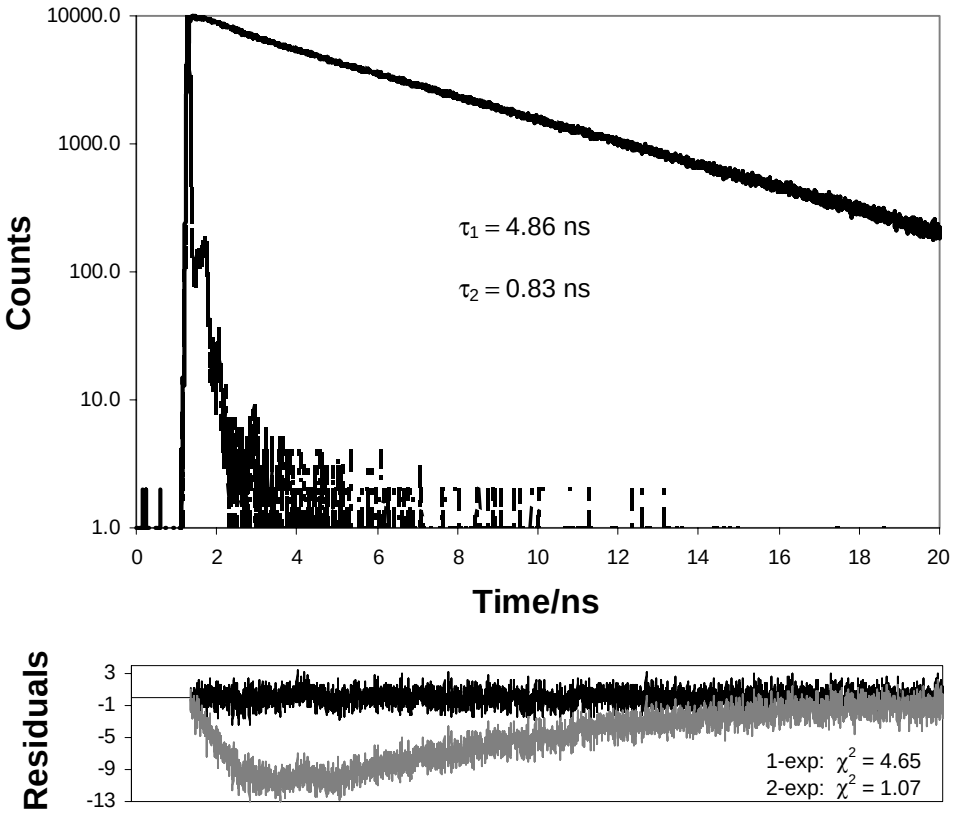

Fig. 3. Fluorescence decay of ANS in methanol-water ($x = 0.5$) at 200 K, the IRF and the double exponential fit are shown in the top panel: the decay and double exponential fit are indistinguishable; $\lambda_{em} = 510$ nm. The lower panel shows residuals for single (grey) and double (black) exponential fits.

At low temperature, the lifetime of ANS in both methanol and the mixed solvent are best described by biexponential kinetics. The wavelength dependent data were globally analysed and were found to fit a two-state solvent relaxation model. It may seem surprising that such a simple kinetic scheme can explain solvent relaxation, which is known to be an extremely complex process [16]. However, it has been demonstrated in a number of independent studies that solvent relaxation in certain systems (notably where charge transfer is involved) is best described by a two-state process [12,13,24,25]. In these systems, an analysis in terms of excited-state reaction kinetics appears to be more appropriate [5,12]. In ANS, the initially locally-excited singlet state relaxes to a twisted intramolecular charge transfer state (TICT) [15]. Solvent relaxation in structurally similar molecules with TICT states has been described previously in terms of a two-state model [24,26,27].

Solvent relaxation in TICT systems can often be correlated with simple properties relating to solvent mobility such as viscosity [24,27]. For most low viscosity solvents under ambient conditions solvent relaxation typically occurs on a picosecond timescale, resulting in fluorescence being almost exclusively observed from the solvent-relaxed state. This has been demonstrated for ANS in pure water or methanol at room temperature [28]. Included in Table I are the viscosities of methanol and the mixed solvent, showing that the order of magnitude increase in relaxation time, and the earlier onset of solvent relaxation for the mixed solvent is broadly consistent with the changes in the bulk viscosity of the solutions with temperature.

On the other hand, it has recently been proposed that the low-temperature dynamics of aqueous solvents, in which the solute inhibits freezing, is dominated by the behaviour of the aqueous component, and that the solutions behave as if the solutions are approaching a glass transition at lower temperatures [3]. The change towards glassy dynamics occurs at around 240 K, which is approximately the temperature at which solvent relaxation becomes prominent in the methanol-water mixture reported herein. Like the Brillouin study, there is a sharp change

at this temperature; in this case it is the lifetime and solvent relaxation time that increase. The time-resolved fluorescence measurements therefore support the explanation that a partial demixing of methanol-water mixtures occurs at the molecular level in these systems.

The mounting evidence of self-association by alcohol molecules in aqueous solutions does not explain the simplicity of the fluorescence intensity decays observed in the present study. Indeed, the temperature and wavelength dependence of the data is consistent with a homogeneous solvent system. An earlier report of the use of a fluorescent molecule as a probe of water-alcohol mixtures also reported single exponential decay kinetics [29]. In that study, the ratio of the intensities of vibronic peaks of pyrene fluorescence was used as an indicator of the degree of pyrene accumulation in self-associating alcohol aggregates. Whilst steady-state fluorescence measurements indicated self-association was occurring, the observation of single exponential lifetimes was interpreted as being due to the alcohol aggregates having a very short lifetime, relative to the timescale of pyrene fluorescence decay. At room temperature, pyrene has a lifetime of ca. 200 ns in water and ca. 340 ns as an aggregate, giving an upper limit on the lifetime of alcohol aggregates of around 1μs.

In the present study, however, the ANS fluorescence decay is approximately 2–3 orders of magnitude faster than for pyrene, which will allow the solvent exchange dynamics in the binary mixture to be probed on a correspondingly shorter timescale. The difference between the lifetime in pure methanol and water is also far greater for the ANS probe. Based on a similar interpretation as used for the pyrene experiment [29], the implication is that solvent rearrangement around the fluorophore occurs on the sub-nanosecond timescale at room temperature (2–3 orders of magnitude faster than the previous upper estimate). A recent study of fast diffusion of small hydrophobic species in water illustrates the feasibility of rearrangement dynamics on these timescales due to rapid hydrogen bond fluctuations [30].

However, for ANS to function as a probe of molecular-scale heterogeneity, it is important that it is small enough to sample different solvent environments. Analysis of neutron diffraction data suggests that at room temperature, the average methanol cluster is made up of around 100 molecules, which can be approximated as a spherical cluster of volume ca. 42,000 $Å^3$ [31]. This is much larger than an ANS molecule, which has a volume of ca. 400 $Å^3$ [32]. However, it is possible that the clusters are not spherical but rather more spread out, which would have the effect of preventing the ANS from being inside a cluster. Another possibility for the simple decay kinetics could involve the ANS having a solvent shell that is independent of the surrounding bulk solvent. Although there is evidence of specific interactions of water with ANS [9], the gradual change in fluorescence properties observed upon altering solvent composition makes this explanation unlikely [10]. Finally, the ANS molecule may reside in the interfacial regions of bulk solvent and clusters, preventing it from sampling distinct environments.

CONCLUSION

In this study, variations in the fluorescence lifetime of a solvent-sensitive probe were used to probe the structure and dynamics of a methanol-water mixture down to low temperatures. The mixture displays simple decay kinetics, with prominent solvent relaxation at low temperatures. The temperature dependence of the fluorescence lifetime is consistent with recent structural studies regarding the self-association of alcohols in water and with Brillouin spectroscopic data showing precursor glassy dynamics. In contrast, a simple two-state model, without the need to invoke solvent clustering, can explain the time-resolved data. In order to gain an understanding of the exact interplay of structure and dynamics in such simple aqueous solutions, we are extending our study to include an investigation over a wider range of temperature and pressures, and also to mixtures of water with other alcohols.

ACKNOWLEDGMENTS

The authors wish to thank Alan K. Soper for helpful discussions.

REFERENCES

1. A. K. Soper (2002). Water and ice. *Science* **297**, 1288.
2. S. Dixit, J. Crain, W. C. K. Poon, J. L. Finney, and A. K. Soper (2002). Molecular segregation observed in a concentrated alcohol-water solution. *Nature* **416**, 829.
3. H. Vass, D. Edington, and J. Crain (2003). Optical spectroscopy of simple aqueous solutions under extreme conditions. *J. Chem. Phys.* **118**(24), 11066.
4. W. S. Price, H. Ide, and Y. Arata (2003). Solution dynamics in aqueous monohydric alcohol systems. *J. Phys. Chem. A* **107**, 4784.
5. J. R. Lakowicz (1999). *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.
6. P. Suppan (1988). Time-resolved luminescence spectra of dipolar excited molecules in liquid and solid mixtures. *Faraday Discuss. Chem. Soc.* **85**, 173.
7. D. V. O'Connor and D. Phillips (1984). *Time-Correlated Single Photon Counting* Academic Press, New York.
8. J. Slavik (1982). Anilinonapthalene sulfonate as a probe of membrane composition and function. *Biochem. Biophys. Acta* **694**, 1
9. T. W. Ebbesen and C. A. Ghiron (1989). Role of specific solvation in the fluorescence sensitivity of 1,8-ANS to Water. *J. Phys. Chem.* **93**, 7139.

10. G. W. Robinson, R. J. Robbins, G. R. Fleming, J. M. Morris, A. E. W. Knight, and R. J. S. Morrison (1978). Picosecond studies of the fluoresence probe molecule anilinonapthalenesulfonic acid. *J. Am. Chem. Soc.* **100**, 7145.
11. H. Nakamura and J. Tanaka (1981). Temperature dependence of fluorescence lifetimes of 8-anilino-1-naphthalene sulfonate and solvent isotope effect. *Chem. Phys. Lett.* **78**, 57.
12. W. R. Laws and L. Brand (1979). Analysis of two-state excited-state reactions. The fluorescence decay of 2-Naphthol. *J. Phys. Chem.* **83**(7), 795.
13. A. S. R. Koti and N. Periasamy (2000). Solvent exchange in excited-state relaxation in mixed solvents. *J. Fluoresc.* **10**(2), 177.
14. S. K. Chakrabarti and W. R. Ware (1971). Nanosecond time-resolved emission spectroscopy of 1-anilino-8-napthalene sulfonate. *J. Chem. Phys.* **55**, 5494.
15. E. M. Kosower (1982). Intramolecular donor-acceptor systems. *Acc. Chem. Res.* **15**, 259.
16. R. M. Stratt and M. Maroncelli (1996). Nonreactive dynamics in solution: The emerging molecular view of solvation dynamics and vibrational relaxation. *J. Phys. Chem.* **100**, 12981 and references therein.
17. T. Molotsky and D. Huppert (2003). Site specific solvation dynamics of coumarin dyes in hexane-methanol mixture. *J. Phys. Chem. A* **107**(16), 2769.
18. N. K. Petrov, A. Wiessner, and H. Staerk (1998). Transient dynamics of solvatochromic shift in binary solvents. *J. Chem. Phys.* **108**(6), 2326.
19. H. Shirota and E. W. Castner Jr. (2000). Solvation in highly nonideal solutions: A study of aqueous 1-propanol using the coumarin 153 probe. *J. Chem. Phys.* **112**, 2367.
20. D. E. Wetzler, C. Chesta, R. Fernández-Prini, and P. F. Aramendía (2002). Dynamic solvation of aminophthalimides in solvent mixtures. *J. Phys. Chem. A.* **106**, 2390.
21. J. Gardecki and M. Maroncelli (1999). Solvation and rotational dynamics in acetonitrile/proplylene carbonate mixtures: A binary system for use in dynamical solvent effect studies. *Chem. Phys. Lett.* **301**, 571.
22. J. A. B. Ferreira and S. M. B. Costa (2003). Non-Markovian effects in the radiationless decay of rhodamine $3B^+$ in water: Ethanol mixtures. *Phys. Chem. Chem. Phys.* **5**, 1064.
23. S. R. Meech and D. Phillips (1987). Time-resolved fluorescence of *p*-dimethylaminobenzonitrile in mixed solvents. *J. Chem. Soc. Faraday Trans.* 2 **83**(11), 1941.
24. D. Huppert, H. Kanety, and E. M. Kosower (1981). Kinetic studies on intramolecuar electron transfer in solution. *Chem. Phys. Lett.* **84**, 48.
25. M. M. G. Krishna (1999). Excited-state kinetics of the hydrophobic probe Nile Red in membranes and micelles. *J. Phys. Chem. A* **103**, 3589.
26. D. Huppert, V. Ittah, and E. M. Kosower (1988). New insights into the mechanism of fast intramolecular electron transfer. *Chem. Phys. Lett.* **144**, 15.
27. Z. R. Grabowski, K. Rotkiewicz, and W. Rettig (2003). Structural changes accompanying intramolecular electron transfer: Focus on twisted intramolecular charge-transfer states and structures. *Chem. Rev.* **103**, 3899 and references therein.
28. S. K. Pal, J. Peon, and A. H. Zewail (2002). Ultrafast surface hydration dynamics and expression of protein functionality: α-chymotrypsin. *Proc. Natl. Acad. Sci. U.S.A.* **99**(24), 15297.
29. R. Zana and M. J. Eliebari (1993). Fluorescence probing investigation of self-association of alcohols in aqueous solutions. *J. Phys. Chem.* **97**, 11134.
30. B. Kirchner, J. Stubbs, and D. Marx (2002). Fast anomalous diffusion of small hydrophobic species in water. *Phys. Rev. Lett.* **89**(21), 215901.
31. Manuscript in preparation.
32. M. Kumbar and V. T. Maddaiah (1977). A conformational study of *N*-phenyl-1-naphthylamine and 1-anilino-8-naphthalene sulfonate by the empirical method. *Biochim. Biophys. Acta* **497**, 707.