Solvation Dynamics in Dimyristoyl-Phosphatidylcholine Entrapped Inside a Sol−Gel Matrix

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Solvation dynamics of coumarin 480 (C480) is studied in a sol−gel matrix containing dimyristoyl-phosphatidylcholine (DMPC). In the presence of DMPC, the average solvation time ($\langle\tau_s\rangle$) in the sol−gel glass is found to be 500 ps. This indicates that in the sol−gel matrix solvation dynamics in the presence of DMPC is slower than that in its absence. The solvation dynamics of C480 in the DMPC entrapped TEOS sol−gel matrix is much faster than that in DMPC liposomes in bulk water. This suggests that the DMPC entrapped TEOS sol−gel matrix is very different from DMPC liposomes in bulk water. The rotational relaxation of C480 is found to be very fast giving rise to a very small initial anisotropy value ($r_0 = 0.08$). This shows that even after incorporation of lipids inside the TEOS sol−gel matrix, the probe C480 remains highly mobile.

1. Introduction

The nanoporous inorganic sol−gel matrix, obtained from the hydrolysis of tetraalkyl orthosilicate, has been demonstrated to be an excellent host for many biological macromolecules.[1−6] In such a host, the biological molecules remain in their active form for a long time. Many enzymes such as glucose oxidase[2a] and urease[2b] and nonenzymes such as human serum albumin[5a,6] and phospholipid liposomes[3] have been trapped inside the sol−gel matrixes. Such a sol−gel glass, doped with organic and biological molecules is called an ormosil. They have potential applications as chemical and biosensors and in photonics.[1−4]

Recently, many groups studied dynamics of liquids confined in sol−gel glasses. Awschalom et al. studied reorientational dynamics of nitrobenzene in a sol−gel glass of pore size 22 Å and detected a component of 126 ps, which is 3 times slower than that in bulk nitrobenzene (39 ps).[7] Fourkas and co-workers studied reorientational dynamics of methyl iodide and acetonitrile in sol−gel glasses of different pore sizes.[8] They observed that for both liquids, the decay of OKE signal is multiexponential with a major bulk-like component and an additional component, which is about 4 times slower. Solvation dynamics of water molecules inside the pores of the TEOS sol−gel matrix is found to be much slower than that in bulk water.[9] In bulk water, the longest component of solvation dynamics is about 1 ps.[10] However, for water molecules confined in the TEOS sol−gel matrix, the average solvation time is 150 ± 30 ps.[9] Baumann et al. studied solvation dynamics of ethanol in a sol−gel matrix.[11] In bulk ethanol, the average solvation time is 12.5 ps. They found that the average solvation time of a probe, nile blue, inside a sol−gel matrix increases from 18. 6 ps in 75 Å pores to 39 ps in 50 Å pores[11a] and that of another probe, coumarin 153, inside a sol−gel glass matrix increases from 28.6 ps in 50 Å pores to 36.9 ps in 25 Å pores.[11b]

Apart from simple liquids, there are several reports on the dynamics in sol−gel matrixes containing biological macromolecules. Bright et al. studied solvation dynamics of acrylodan-labeled human serum albumin (HSA) in a sol−gel matrix and detected a nanosecond component.[6] In bulk water the same system (acrylodan labeled HSA) exhibits a solvation dynamics with a very fast component of 3 ps.[12]

Very recently, Brennan et al.[3] incorporated several liposomes (DPPC and egg PC) in sol−gel glasses. They observed that the liposomes do not exhibit phase transition when entrapped in TEOS glasses. This suggests that encapsulation results in rupture of the liposomes.[3b] The fluorescence decay of DPH in DPPC liposomes in a sol−gel glass is similar to that in DPPC liposomes in bulk.[3b] However, the time-resolved anisotropy exhibits significant differences. In bulk water, DPH displays fast decay in DPPC liposomes with a large residual anisotropy (0.16). However, when DPPC is entrapped in the TEOS sol−gel glass the residual anisotropy is found to be very small (0.05).[3b] This also indicates that the structure and dynamics of the liposomes inside the sol−gel matrix is different from that in bulk water.

Solvation dynamics in several liposomes in bulk water has been studied by several groups.[13,14] However, the effect of dimyristoyl-phosphatidylcholine (DMPC) on the solvation dynamics of water molecules trapped inside the sol−gel matrix has not yet been studied. In the present work, we focus on the study of solvation dynamics of a probe coumarin 480 (C480) in the TEOS sol−gel matrix containing entrapped DMPC molecules.

2. Experimental Section

C480 (laser grade, Exciton), tetraethyl orthosilicate (TEOS) (Fluka, Purum grade), and DMPC (Sigma) were used as received. TEOS sol was prepared by vortexing a mixture of 4.5 mL of TEOS, 1.4 mL of double distilled, deionized water, and 100 μL of 0.1 N high-purity HCl. The pH of the mixture was about 4.5. The unilamellar vesicle was prepared separately by the ethanol injection method. Briefly a 93-μL solution of DMPC (3 mg/100 μL) in ethanol was injected by microliter

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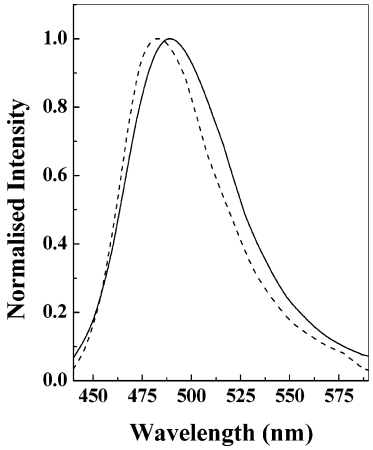

Figure 1. Steady-state emission spectra of (λ_{ex} = 405 nm) C480 in water (—) and in DMPC entrapped TEOS sol−gel matrix (- - -).

syringe in a 4-mL solution of C480 in tris buffer of pH 7.4. The concentration of DMPC in tris buffer was 1 mM. The DMPC buffer solution containing 50 μM C480 was vortexed and kept at 30 °C (i.e. above the gel transition temperature of 23 °C) for an hour. TEOS sol (1 mL) and a 1-mL solution of DMPC-tris buffer containing C480 were mixed in a quartz tube and exposed to air for aging. In the final mixture, the probe: DMPC ratio is 1:20. The fluorescence experiments were done with a substantially aged semitransparent sol−gel composite after 25 days.

The steady-state absorption and emission spectra were recorded in a JASCO 7850 spectrophotometer and a Perkin-Elmer 44B spectrofluorimeter, respectively. For lifetime measurements, the samples were excited at 405 nm with a picosecond diode laser (IBH Nanoled-07). The emission was collected at a magic angle polarization with a Hamamatsu MCP photomultiplier (2809U). The time correlated single photon counting (TCSPC) setup consists of a Ortec 935 QUAD CFD and a Tennelec TC 863 TAC. The data are collected with a PCA3 card (Oxford) as a multichannel analyzer. The typical fwhm of the system response is about 80 ps. The exciting laser beam (at 405 nm) scattered by the sol−gel sample was blocked with a Melles Griot (03FCG061/GG435) filter.

To study fluorescence anisotropy decay, the analyzer was rotated at regular intervals to get perpendicular ($I_\perp$) and parallel ($I_{||}$) components (λ_{em} = 450 nm). Then $r(t)$ was calculated using the formula

$$r(t) = \frac{I_{||} - GI_\perp}{I_{||} + 2GI_\perp} \quad (1)$$

The G value of the setup was determined by using a probe whose rotational relaxation is very fast, e.g., nile red in methanol, and the G value was found to be 1.

3. Results

3.1. Steady-State Results. The emission maximum of C480 in the DMPC entrapped TEOS sol−gel matrix (483 nm) is slightly blue shifted from that of C480 in water (490 nm) (Figure 1). The very small blue shift in the emission maximum of C480 inside the DMPC entrapped TEOS sol−gel indicates that the system is highly polar and the polarity inside the pore is close to that in bulk water. The emission maximum of C480 (483 nm) in the TEOS sol−gel glass containing DMPC is close to that in the TEOS sol−gel glass (480 nm)[9a] and in the DMPC vesicle in the bulk water (480 nm).[13a]

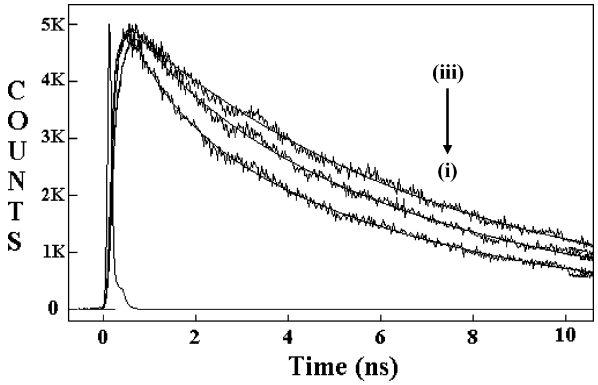

Figure 2. Fluorescence decays of C480 in DMPC entrapped TEOS sol−gel matrix at (i) 440, (ii) 470, and (iii) 580 nm.

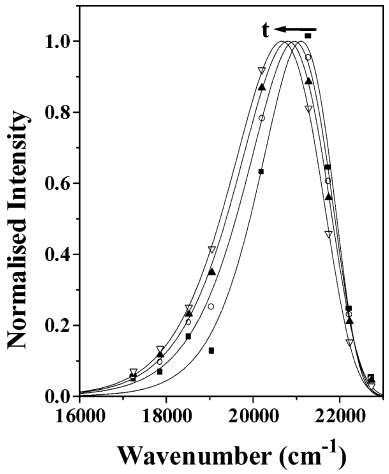

Figure 3. Time-resolved emission spectra of C480 in DMPC entrapped TEOS sol−gel matrix at 0 (■), 50 (○), 150 (▲), and 7000 ps (▽).

3.2. Time-Resolved Studies: Solvation Dynamics. In DMPC entrapped TEOS sol−gel, C480 exhibits wavelength-dependent emission decays with a growth at the red end and a decay at the blue end. This is typical of a probe displaying solvation dynamics. In this case, at the blue end (440 nm) the decay is fitted to a biexponential with two components of 960 ps (30%) and 5.6 ns (70%) (Figure 2). However, at the red end (e.g. at 580 nm), C480 in the DMPC entrapped TEOS sol−gel matrix exhibits a distinct rise of 120 ps followed by a decay component of 6.5 ns (Figure 2). Following the procedure given by Fleming and Maroncelli,[19a] the time-resolved emission spectra (TRES) were constructed by using the parameters of best fit to the fluorescence decays and the steady-state emission spectrum. The TRES show a time-dependent Stokes shift (Figure 3). The solvation dynamics is described by the decay of the solvent correlation function $C(t)$, defined as

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \quad (2)$$

where $\nu(0)$, $\nu(t)$, and $\nu(\infty)$ are the peak frequencies at time 0, t, and ∞, respectively. The decay of $C(t)$ is shown in Figure 4 and the decay parameters are summarized in the Table 1. The decay of $C(t)$ for C480 in the DMPC entrapped TEOS sol−gel matrix is found to be biexponential with one component of 90 ps (80%) and another of 2150 ps (20%), with an average solvation time, $\langle\tau_s\rangle$ = 500 ps (Table 1). The total Stokes shift is observed to be 450 ± 45 cm^{-1}.

3.3. Fluorescence Anisotropy Decay. The anisotropy decay of C480 in a sol−gel matrix containing DMPC is found to be

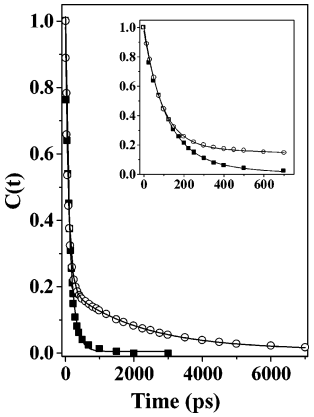

Figure 4. Decay of response function, $C(t)$, of C480 in the DMPC entrapped TEOS sol−gel matrix. The points denote the actual values of $C(t)$ and the solid line denotes the best fit to a biexponential decay. Decay of response function, $C(t)$, of C480 in the DMPC entrapped TEOS sol−gel matrix (○) and in TEOS sol−gel matrix without DMPC (■). The initial parts of the decays of $C(t)$ are shown in the inset.

TABLE 1: Decay Parameters of $C(t)$ for C480 in the DMPC Entrapped TEOS Sol−Gel Matrix

system	$\Delta\nu^a$ (cm^{-1})	a_1	$\tau_1{}^a$ (ps)	a_2	$\tau_2{}^a$ (ps)	$\langle\tau\rangle^{a,b}$ (ps)
C480 in DMPC entrapped TEOS sol−gel matrix	450	0.80	90	0.20	2150	500

[a] ±10%. [b] $\langle\tau\rangle = a_1\tau_1 + a_2\tau_2$.

too fast to be resolved in our setup (response time ~80 ps) with an initial anisotropy (r_0) of 0.08. Thus the probe C480 molecule remains highly mobile inside the sol−gel matrix even in the presence of DMPC.

4. Discussions

This work shows that the solvation dynamics inside the TEOS derived sol−gel is affected on incorporation of the DMPC lipids. It is evident that the probe C480 experiences a very different microenvironment inside the DMPC derived TEOS sol−gel matrix, which resembles neither the TEOS sol−gel matrix[9] nor the DMPC vesicle in bulk water.[13−14]

The time constants of solvation dynamics of C480 in the DMPC entrapped TEOS sol−gel matrix ($\langle\tau_s\rangle = 500$ ps) is about 14 times faster than that in DMPC liposome in bulk water. We have earlier reported that solvation dynamics of C480 in DMPC liposomes in bulk water is described by two components: 600 (40%) and 11000 ps (60%) with $\langle\tau_s\rangle = 6800$ ps.[13a] This demonstrates that the water molecules trapped inside the lipid vesicles in bulk water are much slower than the water molecules inside the biogel in the presence of DMPC. This result clearly indicates that the DMPC entrapped TEOS sol−gel matrix is different from DMPC liposomes in bulk water. The faster dynamics in the case of the DMPC entrapped sol−gel glass compared to the DMPC vesicle in bulk water may be attributed to the fact that the DMPC vesicle is ruptured in TEOS sol−gel as reported by Brennan et al.[3b]

In the TEOS sol−gel matrix, the solvation dynamics of C480 displays an average solvation time, $\langle\tau_s\rangle = 150 \pm 30$ ps.[9] In the presence of DMPC in the TEOS sol−gel matrix, the solvation dynamics of C480 displays two components of 90 (80%) and 2150 ps (20%). For comparison, decay of $C(t)$ in the TEOS sol−gel glass without DMPC is also shown in Figure 4. In the presence of DMPC $\langle\tau_s\rangle$ increases to 500 ps in the sol−gel matrix. The very long component of 2150 ps indicates that the DMPC

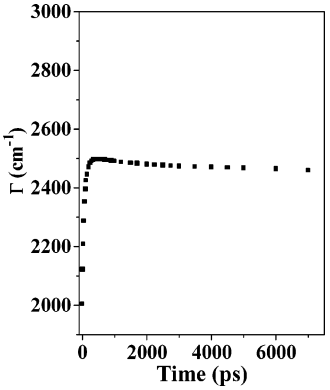

Figure 5. The plot of full width at half-maximum (fwhm, Γ) of the TRES against the time of C480 in the DMPC entrapped TEOS sol−gel matrix.

molecules adsorbed to the inner surface of the TEOS sol−gel impose a restriction on the movement of the water molecules.

In general, the slow solvation dynamics observed in many constrained environments arises from the almost completely immobilized water molecules bound to the surface of these assemblies.[16−18] In the case of a sol−gel matrix containing DMPC, the water molecules in the immediate vicinity of the silicate surface remain bound to the oxygen atoms and the polar headgroups of the DMPC. According to the dynamic exchange model,[18b] the slow component of the solvation dynamics originates from the interconversion of the bound state to the free state of the water molecules. The magnitude of the slow component of solvent relaxation depends on the free energy difference (ΔG^0) between the bound and the free state of water molecules. In the limit of very high ΔG^0, the slow component of solvation (τ_{slow}) is given by[17,18]

$$\tau_{slow} \approx k_{bf}{}^{-1} \tag{3}$$

where k_{bf} is the rate constant for bound-to-free interconversion,

$$k_{bf} = \left(\frac{k_B T}{h}\right) \exp\left(\frac{-(\Delta G^0 + \Delta G^*)}{RT}\right) \tag{4}$$

where ΔG^* is the activation energy for the conversion of free-to-bound water molecules. From eqs 3 and 4 and using the average solvation times one may calculate the free energy difference (ΔG^0) between the bound and free water molecules. Thus, for the DMPC entrapped TEOS sol−gel composite, ΔG^0 is found to be −3.8 kcal mol^{-1} (using $\tau_{slow} = \langle\tau_s\rangle = 500$ ps).

The very fast decay of anisotropy and the extremely low residual anisotropy indicate that the fluorescent probe (C480) remains highly mobile inside the DMPC entrapped TEOS sol−gel composite. The high mobility of the probe (C480) is in sharp contrast to the severely retarded dynamics of the water molecules. Many fluorescent probes exhibit very fast rotational relaxation in a sol−gel matrix.[3,6,9] Brennan et al. reported very fast anisotropy decay (time constant 500 ps) and very low residual anisotropy (0.05) of a probe, DPH inside the DPPC entrapped sol−gel matrix.[3b] Bright et al. reported subnanosecond rotational dynamics of an acrylodan labeled protein in such a biogel.[6] Negri et al. showed that for a titania gel although the bulk viscosity increases abruptly, the steady-state fluorescence anisotropy does not change perceptibly.[15] These results indicate that the microviscosity experienced by a fluorophore is very low inside the pores of a sol−gel glass and the probe remains highly mobile.

Finally, we have studied variation of the spectral width of the TRES in the DMPC entrapped TEOS sol−gel matrix. The parameter Γ is defined as the full width at half-maxima of an emission spectrum. It is observed that in the present system, Γ exhibits a very fast initial growth of about 25% after which Γ becomes almost constant (Figure 5). In the case of coumarin 153 (C153) in bulk solvents Maroncelli et al.[19b] reported a fast growth in Γ of about 15% followed by a slight decay by about 5% so that the overall change is about 10%. The change in Γ reflects the change in local solvation environment. However, because of the inherent error in computing Γ and the relatively small amount of change of Γ with time detected in the present work, we refrain from drawing any further conclusion from these data.

5. Conclusion

The present study shows that the solvation dynamics of a probe C480 inside the DMPC entrapped TEOS sol−gel matrix is very different from that in DMPC lipid vesicles in bulk water and that of the same probe in the TEOS sol−gel matrix. The average solvation time in the DMPC entrapped TEOS sol−gel is found to be about 2.3 times longer than that in TEOS sol−gel. However, the solvation dynamics of C480 inside the DMPC entrapped TEOS sol−gel is nearly 14 times faster than that in DMPC vesicles in bulk solution. This is consistent with a previous experiment[3b] that shows liposomes are ruptured inside the TEOS sol−gel. The reorientation dynamics of C480 in the DMPC entrapped sol−gel composite is found to be very fast. This indicates the probe molecule is highly mobile in the DMPC entrapped sol−gel matrix.

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References and Notes

(1) Binker, C. J.; Scherer, G. W. *Sol−Gel Science*: Academic Press: Boston, MA, 1990.

(2) (a) Narang, U.; Prasad, P. N.; Bright, F. V.; Kumar, A.; Kumar, N. D.; Malhotra, B. D.; Kamalasanan, M. N.; Chandra, S. *Anal. Chem.* **1994**, *66*, 3139. (b) Narang, U.; Prasad, P. N.; Bright, F. V.; Kumar, A.; Kumar, N. D.; Malhotra, B. D.; Kamalasanan, M. N.; Chandra, S. *Chem. Mater.* **1994**, *6*, 1596. (c) Narang, U.; Jordan, J. O.; Bright, F. V.; Prasad, P. N. *J. Phys. Chem.* **1994**, *98*, 8101.

(3) (a) Goring, G.; Brennan, J. D. *J. Mater. Chem.* **2002**, *12*, 3400. (b) Besanger, T.; Zhang, Y.; Brennan, J. D. *J. Phys. Chem. B* **2002**, *106*, 10535.

(4) Prasad, P. N.; Williams, D. J. *Introduction to nonlinear optical effects in molecules and polymers*; Willy: New York, 1991.

(5) (a) del Monte, F.; Ferrer, M. L.; Levy, D. *J. Mater. Chem.* **2001**, *11*, 1745. (b) Ferrer, M. L.; del Monte, F.; Levy, D. *J. Phys. Chem. B* **2001**, *105*, 11076.

(6) Jordan, J. D.; Dunbar, R. A.; Bright, F. V. *Anal. Chem.* **1995**, *67*, 2436.

(7) (a) Warnock, J.; Awschalom, D. D.; Shafer, M. W. *Phys. Rev. Lett.* **1986**, *57*, 1753. (b) Warnock, J.; Awschalom, D. D.; Shafer, M. W. *Phys. Rev. B* **1986**, *34*, 475. (c) Warnock, J.; Awschalom, D. D.; Shafer, M. W. *Phys. Rev. B* **1987**, *35*, 1962.

(8) (a) Loughnane, B. J.; Farrer, R. A.; Scodinu, A.; Reilly, T.; Fourkas, J. T. *J. Phys. Chem. B* **2000**, *104*, 5421. (b) Loughanne, B. J.; Farrer, R. A.; Scodinu, A.; Fourkas, J. T. *J. Chem. Phys.* **1999**, *111*, 5116. (c) Loughnane, B. J.; Fourkas, J. T. *J. Phys. Chem. B* **1998**, *102*, 10288.

(9) (a) Pal, S. K.; Sukul, D.; Mandal, D.; Sen, S.; Bhattacharyya, K. *J. Phys. Chem. B* **2000**, *104*, 2613. (b) In a previous work on TEOS sol−gel glass,[9a] we reported $\langle\tau_s\rangle = 220 \pm 30$ ps using $\lambda_{ex} = 300$ nm and an instrument response function of 50 ps. In our present setup with $\lambda_{ex} = 405$ nm and an instrument response function of 80 ps, we found $\langle\tau_s\rangle = 150 \pm 30$ ps.

(10) (a) Jarzeba, W.; Walker, G. C.; Johnson, A. E.; Kahlow, M. A.; Barbara, P. F. *J. Phys. Chem.* **1988**, *92*, 7039. (b) Jimenez, R.; Fleming, G. R.; Kumar, P. V.; Maroncelli, M. *Nature* **1994**, *369*, 471. (c) Nandi, N.; Roy, S.; Bagchi, B. *J. Chem. Phys.* **1995**, *102*, 1390.

(11) (a) Baumann, R.; Ferrante, C.; Deeg, F. W.; Brauchle, C. *J. Chem. Phys.* **2001**, *114*, 5781. (b) Baumann, R.; Ferrante, C.; Kneuper, E.; Deeg, F.-W.; Brauchle, C. *J. Phys. Chem. A* **2003**, *107*, 2422.

(12) Buzady, A.; Savolainen, J.; Erostyak, J.; Myllyperkio, P.; Somogyi, B.; Korppi-Tommola, J. *J. Phys. Chem. B* **2003**, *107*, 1208.

(13) (a) Datta, A.; Pal, S. K.; Mandal, D.; Bhattacharyya, K. *J. Phys. Chem. B* **1998**, *102*, 6114. (b) Pal, S. K.; Sukul, D.; Mandal, D.; Bhattacharyya, K. *J. Phys. Chem. B* **2000**, *104*, 4529.

(14) Sykora, J.; Kapusta, P.; Fidler, V.; Hof, M. *Langmuir* **2002**, *18*, 571.

(15) Claudia-Marchi, M.; Bilmes, S. A.; Negri, R. M. *Langmuir* **1997**, *13*, 3665.

(16) (a) Bhattacharyya, K. *Acc. Chem. Res.* **2003**, *36*, 95. (b) Nandi, N.; Bhattacharyya, K.; Bagchi, B. *Chem. Rev.* **2000**, *100*, 2013.

(17) Pal, S. K.; Peon, J.; Bagchi, B.; Zewail, A. H. *J. Phys. Chem. B* **2002**, *106*, 12376.

(18) (a) Balasubramanian, S.; Pal, S.; Bagchi, B. *Phys. Rev. Lett.* **2002**, *89*, 115505−1. (b) Nandi, N.; Bagchi, B. *J. Phys. Chem. B* **1997**, *101*, 10954.

(19) (a) Maroncelli, M.; Fleming, G. R. *J. Chem. Phys.* **1987**, *86*, 6221. (b) Horng, M. L.; Gardecki, J. A.; Papazayan, A.; Maroncelli, M. *J. Phys. Chem.* **1995**, *99*, 17311.