Considerations for the Construction of the Solvation Correlation Function and Implications for the Interpretation of Dielectric Relaxation in Proteins

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Abstract
The dielectric response of proteins is conveniently measured by monitoring the time-dependent Stokes shift of an associated chromophore. The interpretation of these experiments depends critically upon the construction of the solvation correlation function, $C(t)$, which describes the time-dependence of the Stokes shift and hence the dielectric response of the medium to a change in charge distribution. We provide an analysis of various methods of constructing this function and review selected examples from the literature. The naturally occurring amino acid, tryptophan, has been frequently used as a probe of solvation dynamics in proteins. Its nonexponential fluorescence decay has stimulated the generation of an alternative method of constructing $C(t)$. In order to evaluate this method, we have studied a system mimicking tryptophan. The system is comprised of two coumarins (C153 and C152) having different fluorescence lifetimes but similar solvation times. The coumarins are combined in different proportions in methanol to make binary probe mixtures. We use fluorescence upconversion spectroscopy to obtain wavelength-resolved kinetics of the individual coumarins in methanol as well as the binary mixtures of 75:25, 50:50, and 25:75 of C153:C152. The solvation correlation functions are constructed for these systems using different methods and are compared.

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Considerations for the Construction of the Solvation Correlation Function and Implications for the Interpretation of Dielectric Relaxation in Proteins

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The dielectric response of proteins is conveniently measured by monitoring the time-dependent Stokes shift of an associated chromophore. The interpretation of these experiments depends critically upon the construction of the solvation correlation function, \( C(t) \), which describes the time-dependence of the Stokes shift and hence the dielectric response of the medium to a change in charge distribution. We provide an analysis of various methods of constructing this function and review selected examples from the literature. The naturally occurring fluorescent amino acid, tryptophan, has been frequently used as a probe of solvation dynamics in proteins. Its nonexponential fluorescence decay has stimulated the generation of an alternative method of constructing \( C(t) \). In order to evaluate this method, we have studied a system mimicking tryptophan. The system is comprised of two coumarins (C153 and C152) having different fluorescence lifetimes but similar solvation times. The coumarins are combined in different proportions in methanol to make binary probe mixtures. We use fluorescence upconversion spectroscopy to obtain wavelength-resolved kinetics of the individual coumarins in methanol as well as the binary mixtures of 75:25, 50:50, and 25:75 of C153:C152. The solvation correlation functions are constructed for these systems using different methods and are compared.

Introduction

It has been well established by numerous experimental and theoretical studies that solvation dynamics in polar solvents can be described by linear response theory.\(^1\)\(^{13}\) In general, the full frequency dependent dielectric function of the polar solvent (and, perhaps, even of ionic solvents\(^4\)) gives a good description of the solvation dynamics from the ultrafast regime to that of diffusive relaxation. Some direct and successful comparisons between theory and experiments have been established.\(^11\)\(^{12}\)\(^{14}\)\(^{15}\)

The reason for such success is largely because the dielectric fluctuations of polar solvents can be described accurately by simple linear response models, such as the dielectric continuum model.\(^16\)\(^{18}\) On the other hand, the dielectric response in proteins is more complicated. There exist many length scales due to the structural constraints created by the carbon backbone. Some studies indicate that a linear response model may be valid from atomistic simulations.\(^19\)\(^{20}\) A simple dielectric continuum description is clearly insufficient, even though such a description has been widely used to correlate experimental data.\(^21\)\(^{24}\)

Studies of the solvation dynamics in proteins offer the best means of investigating the dielectric response and making a comparison with theory. A range of theoretical and experimental work has been performed to study dielectric responses in proteins, but the results have been very disparate. Early studies suggested that slow relaxation on the nanosecond time scale exists in myoglobin\(^25\)\(^{26}\) in contrast to polar solvents. This may not be unexpected owing to structural constraints, but the role of a protein’s interior motions in its dielectric relaxation is presently unclear from various experimental studies.\(^27\)\(^{30}\) Recently, Boxer and co-workers\(^31\)\(^{32}\) have incorporated a synthetic fluorescent amino acid, Aladan, into seven different sites of the B1 domain of the S6-amino acid protein, streptococcal protein G, GB1, to measure the time-dependent Stokes shifts from the femtosecond to nanosecond time scales. The seven sites range from buried within the protein core to fully solvent-exposed on the protein surface. Their results clearly offer another demonstration that the protein dielectric response is highly inhomogeneous, which is also demonstrated from Golosov and Karplus’ molecular dynamics simulations for the same system.\(^33\)

Experimental and theoretical studies of lysozyme suggest that significant contributions of the observed dynamical fluctuations come from the surrounding water solvent and the water molecules attached on the protein surface.\(^28\)

As another example, Zewail and co-workers used the intrinsic single tryptophan as a probe to study solvation dynamics in proteins\(^29\)\(^{34}\)\(^{39}\) and have reported slow relaxation from which they inferred the presence of “biological water”; water molecules in the immediate vicinity of a surface believed to have different properties from those of bulk water.\(^1\)\(^{40}\)\(^{43}\) For example, they report that the dynamics are significantly slower for the surface tryptophan residues in Subtilisin Carlsberg\(^43\) and in monellin\(^16\) than for that of tryptophan in bulk water, and they argue that the slow relaxation arises from the water molecules constrained on the protein surface.\(^29\)

We have previously discussed the solvation dynamics of the complexes of coumarin 153 (C153, Figure 1) with the monomeric hemeproteins, apomyoglobin, and apoleghemoglobin in water.\(^44\) There are four main considerations for our choice of
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this system. First, coumarin 153 (C153) is a well-characterized and widely used chromophore for solvation dynamics studies. Second, binding studies and molecular dynamics simulations indicate that coumarin indeed is in the hemepocket. We have experimentally obtained a binding constant of ~6 µM for coumarin 153 and apomyoglobin and have characterized the complex. In fact, one of our motivations for using coumarin to probe the hemepocket was the existence of an NMR structure of the dye ANS in the hemepocket of apomyoglobin. Third, while myoglobin and leghemoglobin share a common globin fold, they have differences in their hemepockets, the region to be probed by the coumarin. Fourth, we can produce a broad range of mutant proteins in which one or several amino acids are strategically replaced, so as to test how specific substitutions can affect solvation dynamics.

Prasun Mukherjee received his B.Sc. in Chemistry from Alipurduar College, North Bengal University (2000), M.Sc. (2003) in Physical Chemistry from the Indian Institute of Technology, Bombay, and Ph.D. in Physical Chemistry from Iowa State University (2008) with Jacob W. Petrich. His doctoral work mainly focused on solvation dynamics in room temperature ionic liquids and in monomeric hemeproteins, the photophysics of the antiviral agent hypericin in complex with proteins, and the elucidation of the structure of a novel hypericin-like pigment from a marine ciliate *Maristentor dinoferus*. He has recently joined the University of Pittsburgh as postdoctoral fellow in the Department of Chemistry.
We found that
1. Almost 60% of the solvation is complete in both apoMb and apoLba within the time resolution of our instrument (300 fs).
2. The initial faster solvation is followed by a slower response, which is slower in apoLba than in apoMb by about a factor of 4 (Figure 2).
3. There is excellent agreement between the \(C(t)\) from fluorescence upconversion experiments and those obtained from molecular dynamics simulations.

The rapidity of the solvation in both the proteins studied here suggests that water plays a dominant role, which is consistent with the report by Fleming and co-workers\(^\text{28}\) who studied solvation in the lysozyme/eosin system. (Solvation in bulk water is characterized largely by an \(\sim 30\) fs component and is complete in \(\sim 15\) ps.\(^\text{15,62}\) The remainder of the solvation can be attributed to motions of the protein matrix or coupled protein motions. Of course, the protein’s contribution to solvation should not be neglected. For example, Nilsson and Halle have simulated protein motion (or protein water)\(^\text{35,38,39,65,66}\) and we suggest that the origins of the discrepancies lie in the methods used to compute \(C(t)\). More recently, Zhong and co-workers have studied the solvation of different mutants of apomyoglobin.\(^\text{66}\) All of the solvation correlation functions they report decay much more slowly than those presented in Figure 2. (We note however that the simulations of Singer and co-workers\(^\text{63}\) are consistent with the dynamics reported in Figure 2.) Here, we evaluate various methods of constructing \(C(t)\), present new data on the solvation dynamics of systems containing two different solvation probes in varying ratios, and comment on the consequences of using the \(C(t)\)s thus generated.

**Materials and Methods**

Coumarin 153 (C153) and Coumarin 152 (152) (Exciton Inc., Dayton, OH) were used as received. Methanol (HPLC grade) from Aldrich was used without further purification. Five sets of solutions in methanol were made with C153/C152 mole fraction ratios of 1:0, 0.75:0.25, 0.50:0.50, 0.25:0.75, and 0:1. The total concentration of the probe was fixed at \(8 \times 10^{-6}\) M for all the mixtures for both steady-state and lifetime experiments. Stock solutions of \(1 \times 10^{-3}\) M were prepared for both C153 and C152 and then diluted in methanol to maintain the required mole-fractions of the probes in mixtures.

**Preparation of Micellar Solutions.** \(N\)-acetyl-L-trytophanamide (NATA) and the surfactant, TX-100 (reduced), were obtained from Sigma. For experiments in micelles, the NATA concentration was kept at \(\sim 5 \times 10^{-6}\) M in \(\sim 25 \times 10^{-3}\) M TX-100 (reduced) \(\sim 100\) times CMC). Under these conditions, there is one NATA molecule for every 50 micelles (assuming an aggregation number of 100) to minimize aggregation.

**Steady-State Experiments.** Steady-state absorption spectra were obtained on a Hewlett-Packard 8453 UV–visible spectrophotometer with 1 nm resolution. Steady-state fluorescence spectra were obtained on a Spex Fluoromax-4 with a 3 nm bandpass and corrected for lamp spectral intensity and detector response. For absorption and fluorescence measurements, a 1 cm path-length quartz cuvette was used. Coumarin and NATA samples were excited at 407 and 295 nm, respectively.

**Lifetime Experiments.** The lifetime measurements were acquired using the time-correlated single-photon counting (TCSPC) method, which has been described in detail elsewhere.\(^\text{67}\) Recent modifications in the TCSPC experimental setup include the replacement of NIM-style electronics by the Becker & Hickl photon counting module Model SPC-630. In the CFD channel, our previous ORTEC preamplifier has also been replaced by Becker & Hickl HFAC preamplifier. The data were acquired in 1024 channels with a time window of 12 ns. The instrument response function had a full width at half-maximum (fwhm) of \(\sim 50\) ps. A 1 cm path length quartz cuvette was used for all the time-resolved measurements. Fluorescence decays were collected at the magic angle (polarization of \(54.7^\circ\)) with respect to the vertical excitation light at 407 nm with 65 000 counts at the peak channel.

**Upconversion Experiments.** The apparatus for fluorescence upconversion measurements is described elsewhere.\(^\text{67}\) The instrument response function had a full width at half-maximum (fwhm) of 300 fs. A rotating sample cell was used. To construct the time-resolved spectra from upconversion measurements, a series of decays were collected typically from 480 to 570 nm at 10 nm intervals in a time window of 10 ps. Experiments were also done on a 100 ps time scale to ensure that complete solvent relaxation was observed.

**Methods of Constructing the Solvation Correlation Function, \(C(t)\).** Two methods of constructing the solvation correlation function will be discussed in detail. In the first method, wavelength-resolved fluorescence transients were fit to sums of exponentials (typically 2 or 3, as necessary to fit the data) and time-dependent spectra were reconstructed from these fits by normalizing to the steady-state spectra.
\[ I(\lambda, t) = \frac{\int_0^{\infty} I_0(\lambda) \, \nu^i C(t) \, \nu^i d\nu}{\int_0^{\infty} I_0(\lambda) \, \nu^i C(t) \, \nu^i d\nu} \quad (1) \]

\[ I(\lambda, t) = \sum_i a_i \tau_i + \sum_j b_j \tau_j \quad (4) \]

where the lifetime-associated emission spectra are

\[ p^{\text{popul}}(\lambda, t) = \sum_i a_i \tau_i + \sum_j b_j \tau_j \quad (5) \]

The function, \( \nu(t) \), containing contributions from solvation and population relaxation is obtained from the maxima of log-normal fits to the TRES obtained from eq 4. \( \nu(t) \) is similarly obtained from eq 5 and provides the contributions solely from population relaxation. \( \nu(0) \) and \( \nu(0) \) are extrapolated zero-time points obtained by setting \( t = 0 \) in eqs 4 and 5. The emission maximum \( \nu(t) \) becomes almost equal to \( \nu(t) \) at a time, \( \tau_{sc} \), where the solvation is assumed to be complete, and is defined as \( \nu_{sc} \) (the subscript sc denoting solvation complete). Note that this time is not equivalent to that at which the spectrum attains its steady-state value. Here, the solvation correlation function, \( C(t) \), is

\[ C(t) = \frac{\nu(t) - \nu(0)}{\nu(0) - \nu_{sc}} \quad (6) \]

Finally, by subtracting the contributions from population relaxation, \( \nu(t) \), from \( \nu(t) \), the expression for \( C(t) \) used by Zewail, Zhong, and co-workers is obtained

\[ C(t) = \frac{\nu(t) - \nu(t)}{\nu(t) - \nu(t)} \quad (7) \]

**Results and Discussion**

**Accounting for Experimentally Unresolvable Solvation in Constructing \( C(t) \)**

While obtaining the time-resolved emission profiles is crucial to a determination of the time-dependent Stokes shift and an understanding of the dielectric response of the medium, equally crucial is the construction of the solvation correlation function. Because \( C(t) \) is a normalized function, its computation and interpretation depend critically on the values used in its denominator for the zero-time and steady-state spectra (eq 2). Failure to provide accurate values for these terms can overemphasize slow events and ignore fast events in solvation.

The expressions for \( C(t) \) given by eqs 3–7 were obtained because it was concluded, based on earlier work such as that for subtilisin Carlsbergef{45} and monellin,ef{36} that aqueous solvation of tryptophan in proteins could be significantly slower than (or comparable to the time scales of) population relaxation. In particular, because rapid solvation could be resolved for tryptophan in water, it was proposed that all the solvation in water was resolved, not only for tryptophan but also for its analogs and proteins containing it.ef{35,36} This is a crucial assumption that can severely affect the interpretation of the computed \( C(t) \) if indeed all the solvation dynamics have not been resolved or accounted.

In order to assess how much solvation may have been missed in such an experiment, we provide spectral parameters in Table 1 and spectra in Figure 3 for NATA. As Table 1 indicates, the zero-time spectrum obtained for NATA/TX-100 by Zewail and co-workers is red shifted with respect to that obtained using the Fee and Maroncelli method, resulting in a total spectral shift that is about 20% smaller. We suggest that this 20% comprises solvation events that were unresolvable with their experimental
Szabo and Rayner have shown that the fluorescence decay of tryptophan is well described by a biexponential with two components of \( \sim 600 \) ps and \( \sim 3 \) ns, each corresponding to different spectra whose maxima are at \( 335 \) and \( \sim 350 \) nm, respectively.

Similarly, based upon the assumption that NATA provides a good model for tryptophan in proteins, we suggest that the zero-time spectrum they propose for subtilisin Carlsberg (30710 cm\(^{-1}\) rather than 31160 cm\(^{-1}\) obtained from the Fee and Maroncelli method) is indicative of unresolved solvation events. Subsequent work by Zewail, Zhong, and co-workers on a 10 ps time scale from 480 to 570 nm at 10 nm intervals (Figure 1), which are mixed together in different proportions in methanol. We use fluorescence upconversion spectroscopy to study the solvation dynamics, using the individual coumarins in methanol as well as the binary mixtures of 75:25, 50:50, and 25:75 of C153/C152.

Fluorescence lifetime measurements of five sets of C153/C152 mixtures in methanol are shown in Figure 4. Pure C153 and C152 have single exponential lifetimes of 4.0 and 0.9 ns, respectively, whereas the mixtures have biexponential decays with the same time constants, whose amplitudes were proportional to their ground-state population ratios, as indicated in Table 2. The representative wavelength-resolved traces obtained on a 10 ps time scale from 480 to 570 nm at 10 nm intervals are presented in Figure 5.

Using the approach leading to eq 2 and the Fee and Maroncelli method for obtaining \( \nu("0") \), \( C(t) \) was computed. The spectral positions are compiled in Table 3. The average solvation times obtained for pure C153 and C152 in methanol were 4.35 and 5.00 ps, respectively. That is, as expected for similar solvation probes, the solvation times are nearly identical within experimental error. Furthermore, as expected, despite the difference in the fluorescence lifetimes of the two

<table>
<thead>
<tr>
<th>system</th>
<th>( \nu_{\text{exc}} )</th>
<th>( \nu(&quot;0&quot;) )</th>
<th>( \nu(\infty) )</th>
<th>( \Delta \nu )</th>
<th>( \lambda(&quot;0&quot;) )</th>
<th>( \lambda(\infty) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NATA/hexane</td>
<td>37450</td>
<td>32260</td>
<td>32260</td>
<td>32260</td>
<td>32260</td>
<td>32260</td>
</tr>
<tr>
<td>NATA/H(_2)O</td>
<td>35970</td>
<td>29250</td>
<td>29250</td>
<td>29250</td>
<td>29250</td>
<td>29250</td>
</tr>
<tr>
<td>NATA/CH(_3)CN</td>
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<td>29880</td>
<td>29880</td>
</tr>
<tr>
<td>NATA/TX-100</td>
<td>35970</td>
<td>30310</td>
<td>30310</td>
<td>30310</td>
<td>30310</td>
<td>30310</td>
</tr>
<tr>
<td>C153/hexane</td>
<td>25770</td>
<td>22370</td>
<td>22370</td>
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<td>22370</td>
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<tr>
<td>C153/CH(_3)CN</td>
<td>23980</td>
<td>19160</td>
<td>19160</td>
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<tr>
<td>apoMb/C153</td>
<td>22940</td>
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<td>18730</td>
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<td>18730</td>
<td>18730</td>
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<tr>
<td>apoLba/C153</td>
<td>23200</td>
<td>19060</td>
<td>19060</td>
<td>19060</td>
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<td>19060</td>
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<tr>
<td>subtilisin Carlsberg</td>
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<td>29270</td>
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**Figure 3.** Normalized excitation and emission spectra of NATA in 10 mM potassium phosphate buffer of pH 7 (solid line), acetonitrile (dashed line), and TX-100 micelle (dotted line). Corresponding time zero spectra in acetonitrile and TX-100 micelle are also included. The construction of the zero-time spectrum is discussed elsewhere.

**Figure 4.** Fluorescence decay traces (\( \lambda_{\text{exc}} = 407 \) nm, \( \lambda_{\text{em}} \geq 425 \) nm) of five different mixtures of C153 and C152 in methanol. The five traces shown are with 100:0, 75:25, 50:50, 25:75, and 0:100% C153/C152 mixtures. The labels given in the figure are the percentages of C153 in the mixtures. The lifetime decreased consistently from 4 to 0.9 ns with increasing percentage of C152. The components of the individual lifetimes reflected the corresponding percentages of the coumarins in the excited state. Because the optical densities of the pure coumarins at 407 nm differ only slightly, the excited state percentages for C153 were found to be 79.5, 53.0, and 26.5, nearly the same as the corresponding molar percentages of 75, 50, and 25 in the ground state.
mixtures. The data for coumarin mixtures were also subjected to the approach leading to eq 7. Figures 6a–c provide a comparison of the C(t)s obtained from eqs 2 and 7 for three coumarin mixtures. In each case, eq 7 suggests that the amplitude of slow solvation is significantly greater than that provided by eq 2. This discrepancy is clearly a result of the difference in the values of zero-time (Table 3) used in the respective equations. In fact, if the Fee and Maroncelli zero-time is inserted into eq 7, much better agreement of the correlation functions is obtained.

As a control experiment, our C(t) for C153 in methanol obtained using eq 2 is compared with that obtained by Maroncelli and co-workers69 in Figure 6d. The agreement is very good, even though the time resolution of the systems used is different. Ours is ∼300 fs while theirs is ∼110 fs. The C(t) for solvation in methanol has been well documented. Gustavsson et al.66 and Jarzeba et al.77 have obtained similar results. Equation 7 clearly exaggerates the slow component of solvation in methanol.

Approximate Methods. On the basis of the comparison above and work we have presented elsewhere,44,70,78 we suggest that the method of Fee and Maroncelli for obtaining the zero-time spectrum is the soundest available. Its details are clearly described in their paper,69 briefly alluded to above, and provide the basis for the construction of an entire emission spectrum. In this paper, Fee and Maroncelli also provide a simple equation for approximating the maximum of the zero-time spectrum

\[ \nu_{em}^P(0) = \nu_{abs}^P - (\nu_{abs}^P - \nu_{em}^P) \]  

where P and nP refer to the position of the emission or absorption spectra in polar or nonpolar solvents, respectively. In our experience, this approximation usually deviates from that obtained by the full method by at least a few hundred wavenumbers. Comparisons are provided in Table 3. Bhattacharya and co-workers use eq 10 as a quick way of estimating the position of the zero-time spectrum,43,79 but we propose that it is no substitute for using the full method—especially when quantitative interpretations of C(t) are required.

Table 2: Solvation and Lifetime Parameters for Different C153/C152 Mixtures in Methanol

<table>
<thead>
<tr>
<th>System</th>
<th>Lifetime Parameters</th>
<th>Solvation Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a(1)</td>
<td>r(1) (ns)</td>
</tr>
<tr>
<td>C153 (%)</td>
<td>C152 (%)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
<td>0.25</td>
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<tr>
<td>50</td>
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<tr>
<td>25</td>
<td>75</td>
<td>0.72</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The average lifetimes, <τ>, are associated with an error bar of ±0.2 ns based on the average of three measurements. Solvation time <τ> was calculated using the traditional method of C(t) calculation according to eq 2, using the zero-time peak maxima from Fee-Maroncelli’s method69.

Table 3: Comparison of Zero-Time Spectral Positions (cm⁻¹) Using Different Methods

<table>
<thead>
<tr>
<th>System</th>
<th>Time Zero</th>
<th>Full Method</th>
<th>Approx Method</th>
<th>Difference of Midpoint</th>
<th>Difference of Peak-Maxima</th>
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<tr>
<td>C153 (%)</td>
<td>C152 (%)</td>
<td>ν(0)a</td>
<td>midpointb</td>
<td>peak maximae</td>
<td>midpointb</td>
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<td>0</td>
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<tr>
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<td>100</td>
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<td>21670</td>
<td>21790</td>
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</table>

a ν(0), obtained from eq 7, is the extrapolated zero-time point obtained by setting t = 0 in eq 4.37–39,66 b Calculated using full Fee and Maroncelli’s method69; where the entire zero-time spectrum is constructed. Approximation using νemption(0) = νemP - (νemP - νemP).43,79 d Midpoint frequencies are calculated as νmid = (νc + νn)/2, where νc and νn are the midpoint frequencies at the blue and red edges of the spectrum, respectively. e Peak maxima of the zero-time spectrum obtained from log-normal fitting using the full Fee and Maroncelli procedure and are used in eq 2 for the construction of C(t).43,79 Differences are calculated as (full method) − (approximate method).

Figure 5. Representative normalized fluorescence upconversion traces for the 75:25–C153/C152 mixture obtained at wavelengths from 480 to 570 nm at 10 nm intervals. These traces were fit in two ways, one with a sum of 2–3 exponentials, and also with four exponentials, using \( Σ a_i \exp(-tτ_i) + Σ b_j \exp(-tτ_j) \), keeping two of the longer components, \( τ_j \), fixed at the two lifetimes of the coumarins in binary mixtures.

Figure 6a–c provide a comparison of the C(t)s obtained from eqs 2 and 7 for three coumarin mixtures. In each case, eq 7 suggests that the amplitude of slow solvation is significantly greater than that provided by eq 2. This discrepancy is clearly a result of the difference in the values of zero-time (Table 3) used in the respective equations. In fact, if the Fee and Maroncelli zero-time is inserted into eq 7, much better agreement of the correlation functions is obtained.

As a control experiment, our C(t) for C153 in methanol obtained using eq 2 is compared with that obtained by Maroncelli and co-workers69 in Figure 6d. The agreement is very good, even though the time resolution of the systems used is different. Ours is ∼300 fs while theirs is ∼110 fs. The C(t) for solvation in methanol has been well documented. Gustavsson et al.66 and Jarzeba et al.77 have obtained similar results. Equation 7 clearly exaggerates the slow component of solvation in methanol.

Approximate Methods. On the basis of the comparison above and work we have presented elsewhere,44,70,78 we suggest that the method of Fee and Maroncelli for obtaining the zero-time spectrum is the soundest available. Its details are clearly described in their paper,69 briefly alluded to above, and provide the basis for the construction of an entire emission spectrum. In this paper, Fee and Maroncelli also provide a simple equation for approximating the maximum of the zero-time spectrum

\[ \nu_{em}^P(0) = \nu_{abs}^P - (\nu_{abs}^P - \nu_{em}^P) \]  

where P and nP refer to the position of the emission or absorption spectra in polar or nonpolar solvents, respectively. In our experience, this approximation usually deviates from that obtained by the full method by at least a few hundred wavenumbers. Comparisons are provided in Table 3. Bhattacharya and co-workers use eq 10 as a quick way of estimating the position of the zero-time spectrum,43,79 but we propose that it is no substitute for using the full method—especially when quantitative interpretations of C(t) are required.
slower solvation phenomenon that may be attributed to “biological water”, water–protein interactions, or the protein itself.

We stress that $C(t)$ is a normalized function whose form and interpretation depend critically upon the terms in its denominator, namely the positions of the zero-time and steady-state spectra, the former of which we argue is most accurately provided by the full method of Fee and Maroncelli. Finally, we note the excellent agreement between experiment and theory that is emerging in the study of solvation dynamics of proteins, for example, our earlier study of monomeric hemeproteins, and recent work by Boxer and co-workers and Golosov and Karplus.

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References and Notes
