Excited-State Dynamics of Thioflavin T: Planar Stable Intermediate Revealed by Nuclear Wave Packet Spectroscopies

Published as part of The Journal of Physical Chemistry virtual special issue “Time-Resolved Vibrational Spectroscopy”.

JunWoo Kim,† Dong Eon Kim,‡ and Taiha Joo*†‡

†Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang, 37673, South Korea
‡Physics Department, Center for Attosecond Science and Technology, and Max Planck Center for Attosecond Science, POSTECH, Pohang 37673, South Korea

Supporting Information

ABSTRACT: Time-domain spectroscopies with time resolution shorter than the vibrational periods of interest were employed to reveal the reaction kinetics and molecular dynamics of the intramolecular charge transfer (ICT) reaction of thioflavin T in liquids. Time-resolved fluorescence spectra provided detailed reaction kinetics, and vibrational wave packets observed in the time-resolved fluorescence and transient absorption provided structural information on the reaction intermediate. Upon photoexcitation, the Franck–Condon state undergoes vibrational relaxation and minor conformational change to form a stable planar intermediate followed by the twisting of the central C–C single bond to form the twisted ICT state. The ICT reaction rate is determined by the solvent fluctuation excluding the inertial component in the solvation function.

1. INTRODUCTION

Thioflavin T (ThT) is a molecular probe whose fluorescence quantum yield varies greatly depending on its local environment. The fluorescence quantum yield of ThT increases generally as the environment becomes more viscous or rigid. In particular, it increases greatly when ThT is adsorbed on fibril structures, which enables ThT to be used as a probe of amyloid fibrillation.1–4 ThT is now a convenient diagnostic tool for the identification of amyloid fibrils in tissues affected by diseases such as Alzheimer’s disease, Parkinson’s disease and type II diabetes.1,3,5–7

The origin of the fluorescence sensitivity of ThT toward local environment has been the subject of extensive studies.8 ThT shown in Figure 1 consists of a benzothiazole and N,N-dimethylaniline moieties linked by the C–C single bond. Conformational isomers may exist along the twisting (θT) and bending (θb) of the C–C bond and the twisting (ϕT) of the dimethylanimo group. Most of the previous studies focused on the dynamics along the θb and θT coordinates on the potential energy surface (PES) of the excited state, and pointed out their role on the nonradiative decay of the locally excited (LE) and intramolecular charge transfer (ICT) states.9–17 There is a consensus that the dynamics of θT is responsible for the change of fluorescence quantum yield in different environments.8 Quantum mechanical calculations showed that the potential minima along θT are located at 37° and 90° in the ground and excited states, respectively.9,17–20 Therefore, the initial photo-excited LE state is converted to the 90° twisted form, known as the TICT state, barrierlessly by the full twist along θT. The fluorescence sensitivity arises from the fact that oscillator strength of the LE state is close to 1, whereas that of the TICT state is very small, ∼ 0.01.20 As a result, the weakly emitting TICT state is formed promptly in less viscous solvents.8–11 Because the LE to TICT reaction involves barrierless large amplitude motion, its rate depends on the environment of ThT, which determines the fluorescence quantum yield. In addition, fluorescence quantum yield of ThT is reduced further in a flexible environment, because nonradiative decay rate of the TICT to ground state increases steeply as the solvent becomes less viscous.8 Viscosity dependence on the fluorescence properties of ThT has been investigated extensively by varying solvent,15–17 temperature,14 and pressure,13 and they all support this model adequately. A computational model analysis also supports this account for the fluorescence properties of ThT.11 When ThT binds to amyloid fibrils, the twisting motion along θT is restricted, and therefore results in enhanced fluorescence.

The twisting of the dimethylanimo group (ϕT) was also invoked as the origin of the fluorescence sensitivity. Notably, Mukherjee et al. proposed in a recent report that rotations of
both $\theta_T$ and $\varphi_T$ bonds lead to two different TICT states.\cite{19}
Through quantum mechanical calculations and time-resolved spectroscopies, they argued that TICT-1 is formed in less than 100 fs in methanol and chloroform solutions by the rotation of $\theta_T$, and TICT-2 is formed by the rotation of $\varphi_T$ in 200 fs and 1.2 ps in methanol and chloroform, respectively. Notably, the former is barrierless, whereas the latter has a small barrier of 3 kcal mol$^{-1}$.\cite{9,16}

Binding structures and photophysical properties of ThT bound on amyloid fibrils have also been studied.\cite{5,22,26}
Fluorescence quantum yield and extinction coefficient of ThT increase greatly upon adsorption to amyloid fibrils. Based on quantum mechanical calculations and experiments,\cite{9,11} it was concluded that the increase is caused by the planar geometry of the bound ThT. Interestingly, Babenko et al. reported that ThT bound on $\alpha$-helical and random coil forms of poly-$\ell$-glutamic acid are not fluorescent and claimed that the enhancement of fluorescence quantum yield on amyloid fibril is due to the enforced planarization of ThT upon adsorption rather than its binding selectivity.\cite{27}

This suggests that ThT may provide further information on amyloid fibrillation such as conformation of the secondary structures in fibrils. Therefore, it is important to reveal the details on the relation between the photophysical property and the structure of ThT along with its excited-state dynamics.

Much of the experimental studies have been performed by employing time-resolved fluorescence (TF) with a time-resolution of 250 fs or longer.\cite{5,12,14,19}

Literature values of the ICT reaction rate of ThT in liquids are scattered even for alcohols,\cite{8,16} although faster than 100 fs was also reported in methanol and chloroform.\cite{19}

In general, an ICT rate is strongly affected by the solvent reorganization process, which usually occurs in a time window ranging from less than 100 fs to a few tens of picoseconds. In some cases, the ICT rate is even limited by the solvent fluctuation. Because both solvation dynamics and the ICT reaction cause apparent dynamic Stokes shift of the emission band, solvation dynamics and the ICT reaction may not be discriminated clearly in time-resolved spectroscopies.\cite{28}

Vibrational and vibronic relaxations also occur in a similar time scale and may obscure the ICT dynamics. As the reaction rate is closely related to the nuclear coordinates responsible for the ICT reaction, which include intermolecular as well as intramolecular solvent coordinates, accurate measurement of the ICT rate by full TF spectra (TFS) with high enough time resolution is critical for the investigation.\cite{28}

Moreover, transient absorption (TA) or TF with a time resolution higher than periods of vibrational modes of interest provides an opportunity to observe the nuclear wave packet motions of the relevant chemical species. The nuclear wave packets can also be generated by the impulsive ICT reaction as well as direct photoexcitation to the Franck-Condon state.\cite{29,31}

The vibrational spectra obtained through the wave packet motions provide important information on the molecular dynamics and structure of the excited-state. In particular, vibrational spectrum of excited state via TF (VETF) gives structural information on the species in the excited state exclusively,\cite{32} whereas the vibrational spectrum via TA (VSTA) comprises both ground and excited states.

In this work, we have investigated the excited-state dynamics of ThT by employing TF and TA with high time-resolutions 55 and 20 fs, respectively, to reveal the dynamics in the excited state and molecular details by VETF and VSTA. Because all relevant chemical species emit, albeit by varying intensities, TFS with high enough time resolution should identify any intermediates (conformers) and the TICT state concerning the excited-state dynamics of ThT and provide precise chemical kinetics. Through experiments and quantum mechanical calculations, we established that the photoexcited ThT undergoes minor conformational relaxation to become an intermediate state, which emits strongly, followed by the twisting along $\theta_T$ to reach the TICT state. VETF (VSTA) of ThT suggests that structure of the intermediate is nearly planar along $\theta_T$ but slightly bent ($\theta_B \sim 17^\circ$), which should help to explain the fluorescence characteristics of ThT adsorbed on amyloid fibrils.

2. EXPERIMENT

Thioflavin T (UltraPure grade) was purchased from AnaSpec Inc., and used without further purification. All sample solutions were prepared to have an absorbance of around 0.3 in a 100 $\mu$m path length fused-silica flow cell. Because ThT is known to be photodegradable,\cite{33} the sample solution was pumped through a $\beta$-barium borate (BBO)
crystal, and the residual fundamental served as the gate pulse in the fluorescence up-conversion by sum frequency generation (SFG) between the gate pulse and fluorescence. Details of the TF apparatus employing noncollinear SFG has been described in detail elsewhere. A 100 μm thick BBO crystal was used for the TF measurement, and a 300 μm thick BBO crystal was used for the TFS measurement to attain better spectral resolution with a compromise of time resolution. Time resolutions of the TF and TFS experiments estimated by the SFG between the gate and solvent Raman scattering were 55 and 85 fs (full width at half-maximum, fwhm), respectively. Instrument response functions (IRFs) for the TF, TFS, and TA are shown in Figure S1. Intensity of TFS was calibrated by quinine sulfate and 3-aminophthalimide as references. A TFS at a fixed time delay was acquired, without resorting to the conventional spectral reconstruction, by scanning the SFG detection wavelength, phase matching angle of the SFG crystal, and the delay stage, which is required to compensate the group velocity dispersion (GVD) of the sample solution, flow cell window (~1.2 mm), and the BBO crystal. Type I phase matching was used for the SFG, and polarization of the pump pulse was rotated for the fluorescence anisotropy experiment and the magic angle (54.7°) detection of TF.

The light source for the TA measurement and the TA apparatus have been described in detail elsewhere. In brief, output of an amplified Ti:sapphire laser (Femtopower, Femtolaser Inc.) operating at 3 kHz repetition rate was focused into a neon gas filled hollow core fiber to generate high power white light continuum followed by pulse compression to ca. 5 fs. The pump pulse at 420 nm was obtained by the SHG of the continuum in a 10 μm thick BBO crystal. The continuum reflected off a window was used as the probe pulse. Time resolution of the TA apparatus was 20 fs (fwhm) as shown in Figure S1. Probe wavelength was spectrally selected by a monochromator (SR 830, Stanford Research).

Gaussian 09 package was used for the quantum chemical calculations. Density functional theory (DFT) and time-dependent DFT (TDDFT) calculations were performed using the range-corrected hybrid exchange–correlation functional CAM-B3LYP and 6-31G(d,p) basis, although several functionals including B3LYP and B88LYP were examined for comparison. Solvation was treated by the continuous polarizable continuum model (CPCM).

3. RESULTS AND DISCUSSION

3.1. Absorption and Fluorescence Spectra. The absorption maxima of ThT in various solvents are located around 410–420 nm, and they hardly depend on solvent dependence (Figure S2a). If the Franck–Condon state has a strong charge transfer (CT) character, the absorption spectrum should exhibit a bathochromic shift with respect to the solvent polarity, because dipole moment of the ground state is calculated to be small (2–3 D). The independence of absorption maxima on solvent polarity indicates that the Franck–Condon state does not carry a strong CT character. Peak positions of the fluorescence spectra in various solvents are also nearly constant implying that dipole moment of the emitting state is not much different from that of the ground state either. Because fluorescence lifetime of ThT is short, properties of the excited state is only partially reflected on the fluorescence spectrum. Instead, dynamics of the excited state is manifested in the fluorescence intensity. For example, the fluorescence quantum yield of ThT shows linear correlation with the solvent viscosity (Figure S2b), which implies that the excited ThT undergoes a large-amplitude structural change toward a non emissive state.

3.2. TF and TFS of ThT. Dynamics of a photoinduced chemical reaction involving CT in solution phase is not straightforward to measure because of the solvation dynamics, which usually occurs in time scales ranging from less than 100 fs to tens of picoseconds. In order to separate the reaction dynamics from the spectral relaxation due to the solvation dynamics, TFS over the full spectral region were measured in various solvents. Figure 2 shows the TFS and time-resolved area-normalized emission spectra (TRANES) of ThT in 1-butanol. Those in H2O, methanol, acetonitrile, benzyl alcohol, and tert-butanol are shown in Figure S3. TFS in Figure 2 shows that fluorescence spectrum of ThT in 1-butanol undergoes ultrafast Stokes shift in 200 fs to the wavelength (~495 nm) where stationary fluorescence is observed. Moreover, a much red-shifted emission band at around 560 nm is also observed with extremely weak intensity at times longer than 20 ps, which continues to redshift to even longer wavelengths. This band should be assigned to the emission of the TICT state that was presumed to be a dark state.

Multiple emitting states can be clearly recognized in TRANES by identifying isosbestic points in an analogy to the isosbestic point in absorption spectra. Although a clear isosbestic point is not observable because of the dynamic Stokes shifts originated by solvation and other processes such as vibrational relaxation and conformational relaxation that cause spectral relaxation, there seems to be two quasi-isosbestic points in the TRANES at around 480 nm at early times (<200 fs) and at 525 nm at later times. These two quasi-isosbestic points are visible to a greater or lesser degree in other solvents as well (Figure S3). The two quasi-isosbestic points indicate that TFS may consist of three bands labeled as $S_{1A}$, $S_{1B}$, and $S_{1C}$. The peak around 460 nm ($S_{1A}$) decays in a few hundred femtoseconds, and the decay time is similar in all other solvents (Figure S3). The second peak around 500 nm

![Figure 2](image-url)
(S1B) rises concurrently with the decay of S1A. The S1B band displays the general features of a fluorescence spectrum undergoing solvation dynamics by showing the redshift and broadening according to the solvation time.36,37 According to the TFS, the steady-state fluorescence spectrum of ThT should be determined mostly by the S1B band, because its lifetime is much longer than that of S1A and its oscillator strength is much larger than that of S1C. The S1C band centered around 560 nm exhibits extremely weak intensity compared to the other bands. Although the isoemissive point between S1B and S1C is rather unclear, the fluorescence band shape nevertheless changes extensively. Consequently, the conversion from S1B to S1C cannot be assigned to the solvation dynamics, but should be assigned to a chemical reaction.

Decomposition of the TFS into the three peaks was not feasible as the changes of the frequency and shape of each band are extensive. Instead, integrated fluorescence intensities vs time delay, I(t), were obtained from the TFS, and they are shown in Figure 3. The traces corresponding to methanol, water, and acetonitrile do not extend to longer times, because the fluorescence intensities of ThT in less viscous solvents decay much faster than in more viscous solvents, as can be seen in Figure 3a. I(t) may represent the dynamics of the excited state, because oscillator strengths are different for different electronic states. For instance, oscillator strength of the TICT state is much smaller than that of the LE state for ThT. I(t) in several solvents are fitted to a biexponential function, and the results are listed in Table 1. First, it should be noted that the conversion from S1A to S1B is not accompanied by the change of I(t), that is, oscillator strengths are the same for the two bands, indicating that they have the same electronic origin, which should be the LE state. Ghosh et al. assigned the fast fluorescence decay around 500 nm to vibrational relaxation.17 In order to clarify the origin of S1A, TFS were measured by excitation with small excess energy. Absence of the S1A band in the TFS excited at 425 nm (Figure S4) verifies that S1A band is the emission from the vibrationally hot LE state. Alternatively, S1A and S1B originate from the same electronic state but they are connected by a minor conformational relaxation. Second, the average decay time of I(t) vs solvent viscosity in Table 1 shows a linear relationship, although water shows deviation. Stsiapura et al. found that the recovery of the ground state bleaching in aqueous solution is slow (>30 ps) and that the recovery was slower in more viscous solvents.16 These observations are consistent with the rise of the S1C (TICT) emission band at the rate determined by the solvation dynamics. Indeed, the solvation functions and I(t) are in excellent agreement, as shown in Figure 4, indicating that the ICT may be driven by the solvent fluctuation. However, the ultrafast inertial component in solvation dynamics occurring around 100 fs is not present in I(t),36,38 even though our time-resolution is high enough to resolve it, which indicates that the inertial component may not be operational for the ICT reaction.

The average and standard deviation of the transition frequency (ω(t)) and Δω(t), respectively, can be useful measures of the excited state dynamics as well as solvation dynamics.36,37 ⟨ω(t)⟩ and Δω(t) of TFS in various solvents are also presented in Figure 3. In contrast to I(t), ⟨ω(t)⟩ and Δω(t) exhibit the ultrafast (<200 fs) component. While ⟨ω(t)⟩ mostly follows the solvation function as expected, Δω(t) shows an abnormal feature at later times. When only the solvation dynamics is operative, the width initially increases and should stay mostly constant.37 However, Δω(t) increases initially and then decreases appreciably as evident in Figures 3c,d. The initial decay of Δω(t) in water is due to the S1A peak that is broader in water than in other solvents. Time scale of the narrowing in each solvent is comparable to that of the slow component in (ω(t)). The broadening and the narrowing at later times

### Table 1. Nonlinear Least Square Fit Results of the TFS Area to a Biexponential Function

<table>
<thead>
<tr>
<th>solvent</th>
<th>A1</th>
<th>t1f (ps)</th>
<th>A2</th>
<th>t2f (ps)</th>
<th>τa (ps)</th>
<th>viscosity (10^-3 Pa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetonitrile</td>
<td>0.74</td>
<td>0.53</td>
<td>0.26</td>
<td>1.6</td>
<td>0.81</td>
<td>0.34</td>
</tr>
<tr>
<td>methanol</td>
<td>1</td>
<td>1.5</td>
<td>0.21</td>
<td>0.65</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>H2O</td>
<td>0.59</td>
<td>0.47</td>
<td>0.41</td>
<td>1.8</td>
<td>0.81</td>
<td>0.54</td>
</tr>
<tr>
<td>1-butanol</td>
<td>0.34</td>
<td>0.21</td>
<td>0.66</td>
<td>14</td>
<td>1.0</td>
<td>2.59</td>
</tr>
<tr>
<td>tert-butanol</td>
<td>0.35</td>
<td>2.8</td>
<td>0.65</td>
<td>23</td>
<td>15.9</td>
<td>1.0</td>
</tr>
<tr>
<td>benzyl alcohol</td>
<td>0.21</td>
<td>2</td>
<td>0.79</td>
<td>25</td>
<td>20.2</td>
<td>5.47</td>
</tr>
</tbody>
</table>

*Average lifetime.

---

**Figure 3.** (a) Integrated fluorescence intensity (TFS area), (b) the average frequency (first moment) of TFS, and (c,d) standard deviation of TFS in benzyl alcohol (navy blue left triangle), tert-butanol (green diamond), 1-butanol (magenta down triangle), water (blue up triangle), methanol (red circle), and acetonitrile (black square).

**Figure 4.** Symbols are the TFS area versus time for ThT in water, methanol (MeOH), acetonitrile (ACN), and 1-butanol (BuOH). The lines represent the solvation function from ref 36.
represent the conversion from $S_{1B}$ to $S_{1C}$, thereby the width decreases at long times where only $S_{1C}$ emits. Therefore, $S_{1B}$ and $S_{1C}$ can be distinguished by the isoemissive point at 525 nm in the TRANES.

3.3. Quantum Mechanical Calculations. To elucidate the structures of ThT responsible for the $S_{1B}$ and $S_{1C}$ bands, quantum mechanical calculations were performed by DFT/ TDDFT methods and CAM-B3LYP functional, and the results are summarized in Table 2. Although B3LYP functional been used extensively in previous reports, range-separated hybrid functional such as CAM-B3LYP is known to accurately describe the charge separation and transitions with poor overlap character. For isolated ThT, the optimized ground state is partially twisted ($\mathbf{p}_{T}$) but not bent with $\theta_{T} = 38^\circ$ and $\theta_{B} = 0^\circ$, whereas the optimized excited state is fully twisted ($\mathbf{T}^{\ast}$) and slightly bent with $\theta_{T} = 90^\circ$ and $\theta_{B} = 24^\circ$, which agrees with the previous reports. For ThT in solution, the effect of solvent was incorporated by the conductor-like polarizable continuum model (CPCM) with dielectric constants of water ($\varepsilon = 78.3553$) and 1-pentene ($\varepsilon = 1.9905$). Oscillator strength to the Franck–Condon state.

<table>
<thead>
<tr>
<th>state</th>
<th>$\theta_{A}$ (deg)</th>
<th>$\theta_{B}$ (deg)</th>
<th>oscillator strength</th>
<th>transition energy (nm)</th>
<th>$\mu$ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ground</td>
<td>38</td>
<td>0</td>
<td>1.09</td>
<td>368</td>
<td>2.4</td>
</tr>
<tr>
<td>$\mathbf{P}^{\ast}$</td>
<td>14</td>
<td>17</td>
<td>1.11</td>
<td>418</td>
<td>6.1</td>
</tr>
<tr>
<td>$\mathbf{T}^{\ast}$</td>
<td>90</td>
<td>27</td>
<td>0.01</td>
<td>587</td>
<td>15.6</td>
</tr>
</tbody>
</table>

“CAM-B3LYP functional and 6-31g(d,p) basis were used. Solvent was included by the CPCM method with dielectric constant of 1-pentene ($\varepsilon = 1.9905$). Oscillator strength to the Franck–Condon state.

used extensively in previous reports, range-separated hybrid functional such as CAM-B3LYP is known to accurately describe the charge separation and transitions with poor overlap character. For isolated ThT, the optimized ground state is partially twisted ($\mathbf{p}_{T}$) but not bent with $\theta_{T} = 38^\circ$ and $\theta_{B} = 0^\circ$, whereas the optimized excited state is fully twisted ($\mathbf{T}^{\ast}$) and slightly bent with $\theta_{T} = 90^\circ$ and $\theta_{B} = 24^\circ$, which agrees with the previous reports. For ThT in solution, the effect of solvent was incorporated by the conductor-like polarizable continuum model (CPCM) with dielectric constants of water ($\varepsilon = 78.3553$) and 1-pentene ($\varepsilon = 1.9905$) to contrast polar and nonpolar environments. Remarkably, a stable planar geometry ($\mathbf{P}^{\ast}$) having $\theta_{T} = 14^\circ$ and $\theta_{B} = 17^\circ$ was obtained in both solvents. Additional TDDFT calculations employing BH/HLYP and oB97XD functionals, which are known to incorporate long-range interaction adequately, also produced a stable $\mathbf{P}^{\ast}$ as well. In contrast, TDDFT calculations employing B3LYP functional give $\mathbf{T}^{\ast}$ as the only minimum energy structure as reported previously. Because the two fluorescence bands $S_{1B}$ and $S_{1C}$ are present, there should be two energy minima. In fact, two minima, $\mathbf{T}^{\ast}$ and $\mathbf{P}^{\ast}$, were reported in a similar calculation employing CAM-B3LYP and analysis of the PES of the solvated excited state using the corrected linear response scheme. The oscillator strength of the $\mathbf{T}^{\ast}$ emission is also very small as reported previously. The oscillator strengths of the photoexcitation to FC state and $\mathbf{P}^{\ast}$ emission are the same as suggested from the TFS where $S_{1A}$ and $S_{1B}$ show the same oscillator strengths. Furthermore, $\mathbf{T}^{\ast}$ has a dipole moment of 15.6 D, which is large enough to be identified as a CT state.

The fact that there are two minima in the excited PES implies that there may be two pathways to reach $\mathbf{T}^{\ast}$, direct twisting and a passage through $\mathbf{P}^{\ast}$, which may be called an intermediate, although the presence of the two fluorescence bands $S_{1B}$ and $S_{1C}$ points to the latter. In the two-step model through $\mathbf{P}^{\ast}$, the initial Franck–Condon state undergoes structural relaxation by planarization and bending followed by the full twist of $\theta_{T}$, which may be triggered by the further bending along $\theta_{B}$.

3.4. Vibrational Spectrum of the Intermediate by TF and TA. Validation of the two-step model and the structural information on the intermediate, which is implied to be the $\mathbf{P}^{\ast}$ from the quantum mechanical calculations, can be obtained from the time-resolved vibrational spectrum. Vibrational spectrum of the excited state can be obtained by TA and TF methods through the wave packets created by the impulsive excitation of vibrations. Details on the method of acquiring VETF have been reported previously. Amplitudes of the peaks in a VETF is proportional to the vibrational reorganization energy $\lambda_{\text{IRF}} = S_{\text{IRF}}$, where $S$ is the Huang–Rhys factor (dimensionless displacement) and $\omega$ is the vibrational frequency. Huang–Rhys factors can be calculated as described previously by the projection of the structure difference vector between the two relevant states onto the normal modes of the final state.

We measured TF and TA of ThT with time resolutions of 55 and 20 fs, respectively, to record the vibrational wave packet motions. Figure 5 shows the TFs of ThT measured at several wavelengths. On top of the population dynamics illustrating the characteristics of the dynamic Stokes shift due to solvation and ICT, oscillation of the signals due to the wave packet motions are apparent. First, it should be noted that amplitudes of the oscillations in the TF at 485 nm are negligibly small. Furthermore, the phases of the oscillations measured at the short and long wavelength sides of the 485 nm are 180° out-of-phase. These two observations denote that the modulation of the TF signals arises from the oscillation of the fluorescence center frequency as a function of time and that the fluorescence spectrum responsible for the oscillation is centered around 485 nm, which should be the $S_{1B}$ band in the TFS.

VETF of ThT was obtained by the Fourier transform of the oscillatory part (560 nm) in Figure 5. VSTA was also obtained, as TA can record higher frequency mode because of the higher time resolution than TF, and they are shown in Figure 6. Amplitudes of the peaks in the VETF and VSTA are attenuated approximately by the factor, $\exp(-\sigma_{\text{IRF}}\omega^2/2)$, where $\sigma_{\text{IRF}}$ is the standard deviation of the IRF, because of the finite time resolutions of TF and TA measurements. For instance, a peak at 268 (736) cm$^{-1}$ in the VETF (VSTA) is attenuated by a factor of 2 because of the finite time resolution of 55 (20) fs.
Therefore, the peak at 502 cm$^{-1}$ in VETF should be attenuated by more than a factor of 10, whereas it is hardly attenuated in VSTA. Moreover, because Fourier power transform was calculated, the peak at 502 cm$^{-1}$ in VETF appears more than 100 times smaller than it actually should be. A VSTA generally consists of vibrations of both the ground and excited states through the ground state bleach (GSB) and excited state absorption components in TA, respectively. The probe wavelength was tuned to 560 nm, away from the ground state absorption components in TA, respectively. The probe wavelength was tuned to 560 nm, away from the ground state absorption, to preclude the contribution of GSB. In the Raman spectrum, strong peaks at 534 and 798 cm$^{-1}$ are apparent in VSTA, VETF and VSTA are similar below 500 cm$^{-1}$ spectral window. Similarity of the two spectra indicates that ground state contributions are excluded in the VSTA. Interestingly, the spectra are significantly different from the ordinary Raman spectrum of ThT in water in both their frequencies and amplitudes, which indicates that the structure of the intermediate is different from that of the ground state. In the Raman spectrum, strong peaks at 534 and 798 cm$^{-1}$ and weak peaks at 418, 501, 618, and 742 cm$^{-1}$ are observed.

Structural information on the intermediate can be inferred from the amplitudes of the peaks in the VETF (VSTA). The selection rule for the VETF (VSTA) is the displacement between two relevant electronic states. Within the Condon approximation, the amplitudes are proportional to the vibrational reorganization energies, which can be obtained directly from the modulation of the TF intensity by the relation:

$$\frac{\Delta I}{I_0} \approx \frac{2 \sqrt{2} \ln 2}{\sigma_b \hbar} e^{-\omega/\sigma_b}$$

(1)

where $\sigma_b$ is the standard deviation of the fluorescence band. To compare the experimental results with the calculated VETF (vide infra), amplitudes of the oscillations in TF and TA were obtained by the linear prediction singular decomposition (LPSVD) method, and divided by the attenuation factors to give the calibrated experimental VETF and VSTA shown in Figure 6.

![Figure 6](image)

Figure 6. Fourier power spectra of the oscillation component of the TF (red) and TA (blue) signals of ThT in tert-butanol. The center spectrum is an expanded view of the top spectrum.

Figure 7a,b, respectively. $\sigma_b = 1740$ cm$^{-1}$ (0.328 rad·fs$^{-1}$) was used to reproduce the experimental spectra, and they match well except the high frequency peaks that are missing in the VETF because of the lower time resolution.

The structures of the ground and $P^*$ and their normal modes were calculated by DFT and TDDFT methods and CAM-B3LYP functional. Vibrational reorganization energies between the ground and $P^*$ were calculated by the normal-mode projection method. Vibrational reorganization energies between the ground and $T^*$ gave unrealistic values as it should be, because their structural difference is too big. The vibrational spectrum of $P^*$ calculated in this way is shown in Figure 7c, and it compares well with the experiments. In particular, the vibrational modes with appreciable amplitudes in both spectra match very well, although agreement of the amplitudes is not so great. It is known that Huang–Rhys factors and therefore the vibrational reorganization energies are very sensitive to the structural difference of the two relevant states, and structure of the excited state calculated by TDDFT method may not be accurate enough to give a good match. Nevertheless, the agreement between the experimental and calculated spectra is good enough to conclude that the intermediate is indeed $P^*$, which has $\theta_1$ and $\theta_2$ of 14° and 17°, respectively. If the nuclear wave packets of the excited ThT propagate toward $T^*$ within the time window of the VETF (VSTA), which is a few picoseconds, VETF (VSTA) must be very different from the calculated one, because $T^*$ and $P^*$ have large geometrical difference including the bending and twisting angles with nearly 50 normal modes below 1000 cm$^{-1}$. Consequently, $S_{P^*}$ can be assigned to the emission from $P^*$ and the excited of ThT becomes planarized before it undergoes twisting.
Both planar and twisted binding structures of ThT on fibril have been reported, indicating that ThT has no binding-site selectivity. The PES along the twisting angle in the ground state may be shallow, and the twisting angle is flexible enough to be determined by its environment, that is, the adsorbed structure may depend on the adsorption site of the fibril. In this case, the fluorescence intensity of ThT adsorbed in fibril, where structural change upon photoexcitation is prohibited, is determined by the structure of the adsorption site. Hence, when the adsorbed structure is planar, P* emits strongly, whereas the adsorbed structure is twisted, photoexcitation creates T* that has small oscillator strength and gives weak fluorescence. Both fluorescent and nonfluorescent ThT were reported previously. Fluorescent ThT is bound to β-sheet having planar structure, whereas the nonfluorescent ThT is bound to α-helix having twisted structure, which agrees well with our conclusion.

4. CONCLUSION

We investigated the excited-state dynamics of ThT by TF and TA methods having high time resolution. TF spectra over the full spectral range revealed detailed reaction kinetics. Initial spectral relaxation by the vibrational relaxation and conformational change followed by the twisting of the central C–C single bond to generate the TICT state emission band is observed clearly. The ICT kinetics follows the solvation function of the solvent excluding the ultrafast inertial component. Quantum mechanical calculations employing DFT/TDDFT and CAM-B3LYP functional yield stable planar structure in the excited state having twisting and bending angles of 14° and 17°, respectively, as well as the well-known TICT state. That is, the ICT reaction from the planar to the TICT states has a barrier, and solvent fluctuation drives the ICT reaction. TF and TA reveal nuclear wave packet motions, which render vibrational spectrum of the intermediate planar state. The amplitude of each peak is weighted by the displacement between the ground and the excited planar state giving the structural information. Calculated and experimental VETF and VSTA agree well to conclude that the structure of the intermediate emitting state is indeed planar. This demonstrates that VETF can be a powerful method to elucidate the structures of the molecules in the excited state undergoing chemical reaction.

■ ASSOCIATED CONTENT

1 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpca.7b11951. IRFs of time-resolved experiments, Absorption maximum wavelengths and fluorescence maximum intensities of ThT in various solvents, TFS and TRANES in various solvents, and TFS in 1-butanol with the excitation wavelength at 425 nm. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: thjoo@postech.ac.kr.

ORCID

Taeha Joo: 0000-0003-2690-7789

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Global Research Laboratory Program (2009-00439) and in part by the Max Planck POSTECH/KOREA Research Initiative Program [2016K1A4A401922028] through the National Research Foundation of Korea (NRF).

■ REFERENCES


