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ABSTRACT

Two-photon excitation (TPE) is an attractive means for controlling chemistry in both space and time. Since isoenergetic one- and two-photon excitations (OPE and TPE) in non-centrosymmetric molecules are allowed to reach the same excited state, it is usually assumed that they produce similar excited-state reactivity. We compare the solvent-to-solute excited-state proton transfer of the super photobase FR0-SB following isoenergetic OPE and TPE. We find up to 62% increased reactivity following TPE compared to OPE. From steady-state spectroscopy, we rule out the involvement of different excited states and find that OPE and TPE spectra are identical in non-polar solvents but not in polar ones. We propose that differences in the matrix elements that contribute to the two-photon absorption cross sections lead to the observed enhanced isoenergetic reactivity, consistent with the predictions of our high-level coupled-cluster-based computational protocol. We find that polar solvent configurations favor greater dipole moment change between ground and excited states, which enters the probability for TPE as the absolute value squared. This, in turn, causes a difference in the Franck–Condon region reached via TPE compared to OPE. We conclude that a new method has been found for controlling chemical reactivity via the matrix elements that affect two-photon cross sections, which may be of great utility for spatial and temporal precision chemistry.

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I. INTRODUCTION

Two-photon excitation (TPE) is an attractive means of chemical activation because it allows one to control chemical processes in space and time with resolution limited only by the laser pulse used, typically sub-micrometer spatial resolution and sub-picosecond temporal resolution. The high spatial resolution achieved via TPE led to the development of multi-photon microscopy, which is capable of providing sub-micrometer resolution through scattering biological tissues. 1-7 These advantages are particularly important when imaging strongly absorbing samples, such as blood, or highly sensitive tissues, e.g., the retina. 1,2 Similarly, TPE has been adopted as a valuable method for sub-micrometer photolithography. 8-14 As part of an effort to develop the tools required for precision chemistry, where chemical reactions can be activated and deactivated with high temporal and spatial control, we have evaluated if strong photobases 15 can be made better photoreagents through the use of TPE.

Precision chemistry of light-induced acid–base reactions requires controlling the underlying excited-state proton transfer (ESPT) processes. 16-20 This broad category of chemical reactions can generally be divided into reversible and irreversible and intramolecular and intermolecular. Here, we focus on reversible intermolecular processes that may be amenable to precision chemistry. From the
point of view of the photo-activated reagent, there are numerous proton-donating species, called photoacids, essentially hydroxylated aromatic compounds, while proton-abstracting molecules, i.e., photobases, are less common. The present work examines the super photobase FR0-SB [7-((butylimino)methyl)-N,N-diethyl-9,9-dimethyl-9H-fluoren-2-amine], a non-centrosymmetric fluorene Schiff base shown in Fig. 1(a), capable of abstracting protons from alcohols ranging from methanol to n-octanol. While other compounds, primarily quinoline derivatives, have been found to undergo ESPT in methanol, with 5-methoxyquinoline reaching an excited-state pK$_a$ value of 15.5, our work has focused on FR0-SB because of its stronger photobasicity (pK$_a^\ast$ = 21). Reversible photobases are relatively scarce because their reactivity depends on having a high excited-state pK$_a$ and the ability to abstract a proton from the solvent within the lifetime of the excitation and the relevant solvent reorganization time. In particular, solvation of the resulting ions has been found to require two or more solvent molecules in a specific configuration.

The primary focus of this work is to explore the reactivity of FR0-SB upon two-photon photo-activation. FR0-SB lacks a center of inversion, so Laporte’s symmetry rule preventing one- and two-photon transitions to the same excited state does not apply. Therefore, both one-photon excitation (OPE) and TPE to the first excited singlet (S$_1$) state of FR0-SB are allowed. While the excitation efficiencies for OPE and TPE are quite different, one might reasonably expect similar reactivity following isoenergetic (in the case of this work, ω$_{OPE} = 2\omega_{TPE}$) excitation. For FR0-SB in alcohol solvents, however, we find the extent of solvent-to-solute ESPT following TPE to be as much as 62% greater than that following OPE. We present direct evidence for this surprising finding through steady-state and time-resolved spectroscopic data. We discuss several hypotheses and support or refute them based on experimental findings and theoretical calculations. Finally, we conclude that the molecular properties governing TPE, which we estimate from the spectroscopic data as well as using high-level quantum chemistry computations, lead to the formation of an excited-state wave packet at a different Franck–Condon region compared to OPE, thus changing the entry point onto the excited-state potential energy surface and, consequently, giving rise to a different trajectory along the reaction coordinate (see Fig. 1).

II. EXPERIMENTAL METHODS

For the OPE and TPE fluorescence measurements, tunable ∼50 fs pulses centered at 800 nm were obtained from a noncollinear optical parametric amplifier (Orpheus-N-3H, Light Conversion) pumped by the third-harmonic of a Pharos Yb:KGW laser producing 50 µJ of pulse energy at a repetition rate of 200 kHz centered at 1030 nm. For OPE, the output was frequency doubled by a β-BBO crystal to produce excitation light centered at 400 nm focused by a 10 cm focal length convex lens onto a 1 cm cuvette. The sample solutions had a ∼3 µM concentration, corresponding to an optical density of less than 0.2. For TPE, the output was focused by a 20 cm focal length convex lens to the same sample. The fluorescence was captured with an optical fiber with detection using an Ocean Optics QE spectrometer.

For time-resolved fluorescence measurements, a Ti:sapphire oscillator (Coherent Vitara-S) producing pulses at 80 MHz centered at 800 nm was used for laser excitation. For OPE, the frequency doubling of the laser output was achieved with a β-BBO crystal, and the sample was excited with linearly polarized (vertical) laser pulses. For TPE, the laser pulses were polarization-rotated by 90° with a half-wave plate to maintain the same linear (vertical) polarization between TPE and OPE experiments. The FR0-SB sample with an optical density of 0.2 or below was contained in a 1 cm cuvette. Fluorescence emitted at right angles was acquired at parallel and perpendicular polarizations with respect to the vertically polarized excitation pulse using a analyzer followed by detection with a 16-photonmultiplier time-correlated single-photon counting (TCSPC) system (SPC-830 TCSPC, Becker-Hickl, GmBH). The reported time constants were obtained after extracting the isotropic component from the fluorescence decays and fitting with a convolute-and-compare routine to account for the instrument response function. Details of the fitting procedure can be found in the supplementary material. Measurements were repeated at least 5 times for each solvent to quantify uncertainties.
The TPE spectra of FR0-SB in methanol, acetonitrile, and cyclohexane were measured in the same optical setup as the TPE fluorescence, except for a glass slide, which was introduced in the path of the excitation beam before the converging lens, to reflect part of the beam to be scattered on a diffuser and detected with a compact Ocean Optics spectrometer. The scattered integrated spectrum was used as a reference laser intensity to normalize the TPE spectra. The spectra were recorded for excitation wavelengths between 650 nm and 860 nm with data acquired every 10 nm. The setup was calibrated against a coumarin 540 (Exciton) solution, which exhibits similar TPE and OPE spectra. The laser power dependence for TPE is given in the supplementary material. The TPE absorption cross section for FR0-SB in acetonitrile is estimated to be 6 GM at 800 nm and 24 GM at its maximum at ~770 nm.

III. COMPUTATIONAL DETAILS

In order to provide further insights into the enhancement of ESPT between FR0-SB and alcohol solvent observed in the case of TPE vs OPE, we augmented our experimental effort by quantum chemistry computations examining the electronic structure of the solvated FR0-SB system in the ground (S0) and first excited singlet S1 states involved in the ESPT process.21 We focused on analyzing the role of solution effects on the S0-S1 vertical and adiabatic transition energies and vertical transition dipole moments, along with the electronic dipoles characterizing the individual S0 and S1 states of FR0-SB, which are key quantities in comparing the one- and two-photon absorption of the FR0-SB species and the corresponding dipoles in the S0 and S1 states, which are all needed to model the one- and two-photon cross sections for each of the alcohol solvents considered in our calculations, we performed the following series of single-point CC and EOMCC computations at the aforementioned CAM-B3LYP/6-31+G*//SMD optimized geometries. First, we determined the S0-S1 electronic transition energies,

\[ \omega_{\text{ESPT}}^{(\text{EOMCC})} = \omega_{\text{ESPT}}^{(\text{CCSD})} - \omega_{\text{ESPT}}^{(\text{CC})}, \]

(1)
corresponding to the [FR0-SB ... H0] complex in the absence of the SMD continuum solvation, where the total electronic energies of the S0 and S1 states entering Eq. (1) were computed as

\[ E_{\text{S0}}^{(\text{CC})} = E_{\text{CCSD}}^{(\text{CCSD} / 6-31+G^*)} + \left[ E_{\text{CR-CC(2,3)}}^{(\text{CR-CC(2,3) / 6-31G})} - E_{\text{CCSD}}^{(\text{CCSD / 6-31G})} \right] \]

(2)
for the ground state and

\[ \left[ E_{\text{S1}}^{(\text{EOMCCSD / 6-31+G*})} = E_{\text{S1}}^{(\text{EOMCCSD / 6-31+G*})} + \left[ E_{\text{EOMCCSD / 6-31+G*}}^{(\text{EOMCCSD / 6-31+G*})} - E_{\text{EOMCCSD / 6-31+G*}}^{(\text{EOMCCSD / 6-31+G*})} \right] \]

(3)
for the first excited singlet state. The first term on the right-hand side of Eq. (2) denotes the total electronic energy of the S0 state computed at the CCSD55 level utilizing the largest basis set considered in this study, namely, 6-31+G*. The term in the square brackets on the right-hand side of Eq. (2) corrects the CCSD/6-31+G* energy for the many-electron correlation effects due to triply excited clusters obtained in the CR-CC(2,3)41,52,53 calculations employing the smaller and more affordable 6-31G basis.49 Similarly, the first term on the right-hand side of Eq. (3) designates the EOMCCSD/6-31+G* energy of the S1 state and the expression in the square brackets represents the triples correction to EOMCCSD obtained in the δ-CR-EOMCC(2,3)/6-31G calculations. Ideally, one would like to use basis sets larger than 6-31+G* and, in particular, incorporate polarization and diffuse functions on hydrogen atoms, but such calculations at the CC and EOMCC levels used in this work turned out to be prohibitively expensive. Nevertheless, we tested the significance of the polarization and diffuse functions on hydrogen atoms by performing the CAM-B3LYP/6-31++G*//SMD calculations for the [FR0-SB ... H0] complexes that show neither the excitation
energies nor the dipole and transition dipole moment values change by more than 1% compared to the CAM-B3LYP/6-31+G*/SMD results.

Before describing the remaining elements of our computational protocol, it is important to emphasize that the composite approach defined by Eqs. (1)–(3) is more general than the analogous expressions shown in Ref. 21 where we focused on the vertical protocol, it is important to emphasize that the composite results.

Indeed, if $E_{S_0}$ and $E_{S_1}^{(EOMCC)}$ are calculated at the minimum on the $S_0$ potential energy surface, $\omega_{10}^{(EOMCC)}$ given by Eqs. (1)–(3) becomes the vertical excitation energy $\omega_{10}^{(EOMCC)}$ (abs.) characterizing the $S_0 \rightarrow S_1$ absorption defined by Eq. (1) of Ref. 21. If $E_{S_0}^{(CC)}$ and $E_{S_1}^{(EOMCC)}$ are determined at the minimum characterizing the [FR0-SB⋯HOR] complex in the $S_0$ state, we obtain the vertical transition energy $\omega_{10}^{(EOMCC)}$ (em.) corresponding to the $S_1 \rightarrow S_0$ emission. The $\omega_{10}^{(EOMCC)}$ energy defined by Eq. (1) becomes the adiabatic transition energy, abbreviated as $\omega_{10}^{(EOMCC)}$ (ad.), when $E_{S_0}^{(CC)}$ and $E_{S_1}^{(EOMCC)}$ are computed at their respective minima. As far as the transition dipole moments characterizing the vertical absorption and emission processes involving the solvated FR0-SB species are concerned, they were calculated from the one-electron transition density matrices obtained at the EOMCCSD level of theory employing the 6-31+G* basis set. Similarly, we used the CCSD/6-31+G* and EOMCCSD/6-31+G* one-electron reduced density matrices to determine the dipole moments of the $S_0$ and $S_1$ states at each of the two potential minima.

Given the large computational costs associated with the EOM-CCSD and δ-CR-EOMCC(2,3) calculations for the [FR0-SB⋯HOR] system, which consists of three alcohol molecules bound to the FR0-SB chromophore and requires correlating as many as 216 electrons and 758 molecular orbitals in the case of the $n$- or $i$-propanol solvents when the 6-31+G* basis set is employed, we replaced the three explicit alcohol molecules with the corresponding effective fragment potentials (EFPs).55 We were able to do this because, based on our CAM-B3LYP/6-31+G*/SMD calculations for the [FR0-SB⋯HOR] complexes, the $S_0$–$S_1$ electronic transition does not involve charge transfer between the photobase and its solvent environment. Indeed, the $S_0$–$S_1$ transition in the bare3 and solvated FR0-SB species has a predominantly $\pi \pi^*$ character with the $\pi$ and $\pi^*$ orbitals localized on the FR0-SB chromophore, i.e., the alcohol solvent molecules are mere spectators to this excitation process (see the supplementary material for further details). The use of EFPs to represent the cluster of three alcohol molecules bonded to FR0-SB in our CC/EOMCC computations allowed us to reduce the system size to that of the bare FR0-SB species embedded in the external potential providing a highly accurate description of the intermolecular interactions between FR0-SB and solvent molecules in the [FR0-SB⋯HOR] complex, including electrostatic, polarization, dispersion, and exchange repulsion effects.55

Once the electronic transition energies and the corresponding one-electron properties of the [FR0-SB⋯HOR] complex were determined, we proceeded to the second stage of our modeling protocol, which was the incorporation of the remaining bulk solvation effects that turned out to be non-negligible as well. As in the case of the aforementioned geometry optimizations, the bulk solvation effects were calculated with the help of the implicit solvation SMD approach. Due to the limitations of the computer codes available to us, we could not perform the CC/EOMCC computations in conjunction with the SMD model, so we estimated the SMD effects using the $a posteriori$ corrections $\delta^{(SMD)}_X$ to the various CC/EOMCC properties $X$ of the [FR0-SB⋯HOR] complex, such as transition energies and dipole moments, using DFT and TD-DFT. These corrections were constructed in the following way. First, for each of the four alcohol solvents considered in our calculations, we performed single-point DFT/TD-DFT calculations for the [FR0-SB⋯HOR] complex at the previously optimized $S_0$ and $S_1$ geometries accounting for the bulk solvation effects using SMD. As in the case of the geometry optimizations, we used the CAM-B3LYP functional and the 6-31+G* basis set and, in analogy to the CC/EOMCC computations, replaced the cluster of three explicit alcohol solvent molecules bound to FR0-SB by the corresponding EFPs. We then repeated the analogous calculations without SMD. This allowed us to determine the desired $\delta^{(SMD)}_X$ corrections using the formula

$$\delta^{(SMD)}_X = X^{(CAM-B3LYP/6-31+G*/SMD)} - X^{(CAM-B3LYP/6-31+G*)},$$

where the first and second terms on the right-hand side of Eq. (4) designate property $X$ obtained in the CAM-B3LYP/6-31+G* calculations with and without SMD, respectively. The final SMD-corrected EOMCC electronic transition energies were computed as

$$\omega_{10} = \omega_{10}^{(EOMCC)} + \delta^{(SMD)}_\omega,$$

where $\omega_{10}^{(EOMCC)}$ is the transition energy for the [FR0-SB⋯HOR] complex defined by Eqs. (1)–(3), whereas the SMD-corrected one-electron properties were determined using the formula

$$X = X^{(EOMCCSD/6-31+G*)} + \delta^{(SMD)}_X,$$

with $X^{(EOMCCSD/6-31+G*)}$ denoting the value of property $X$ calculated at the (EOM)CCSD/6-31+G* level. If the property of interest was a vector, such as dipole or transition dipole moment, we used Eq. (6) for each of the Cartesian components of the vector.

Finally, to gauge the effects of solvation on the various calculated properties, including transition energies and dipole and transition dipole moments, we also performed single-point CC/EOMCC calculations for the bare super photobase, i.e., FR0-SB without the presence of explicit solvent molecules or equivalent EFPs and SMD implicit solvation, at the gas-phase geometry of the $S_0$ state optimized using CAM-B3LYP/6-31+G*. In the case of the $S_0$ minimum-energy structure, we relied on our previous gas-phase CC/EOMCC results reported in Ref. 21.

All of the electronic structure calculations reported in this work, including the CAM-B3LYP geometry optimizations with and without the SMD continuum solvation, the CC/EOMCC single-point calculations without implicit SMD solvation, and the CAM-B3LYP single-point calculations with and without SMD, needed to estimate the SMD corrections to CC/EOMCC properties, were performed using the GAMESS package6,57 (we used the 2019 R2 version of GAMESS). In the case of the $S_0 \rightarrow S_1$ absorption process,
whenever the SMD implicit solvation model was utilized, we incorporated the nonequilibrium solvation effects associated with the solvent relaxation delay, as implemented in GAMESS.\textsuperscript{58} The relevant CCSD, CR-CC(2,3), EOMCCSD, and δ-CR-EOMCC(2,3) computations using the restricted Hartree–Fock (RHF) determinant as a reference and the corresponding left-eigenstate CCSD and EOM-CCSD calculations, which were needed to determine the triples corrections of CR-CC(2,3) and δ-CR-EOMCC(2,3) and the one-electron properties of interest, including the dipole and transition dipole moments, were carried out using the CC/EOMCC routines developed by the Piecuch group,\textsuperscript{41,42,53,54,59–61} which form part of the GAMESS code as well. In all of our CC/EOMCC calculations, the core orbitals associated with the 1s shells of C and N atoms of \textit{FR0-SB} were kept frozen. The EFPs that were used to replace the cluster of three explicit alcohol solvent molecules bound to \textit{FR0-SB} in the CC/EOMCC single-point calculations and the CAM-B3LYP computations aimed at determining the SMD solvation effects were generated using the RHF approach and the 6-31+G* basis set. Thanks to the use of EFPs, our frozen-core CC/EOMCC calculations for the \textit{FR0-SB⋯HOR} complex correlated only 138 electrons of the \textit{FR0-SB} system. In all of the calculations employing the 6-31+G* basis set, we used spherical components of d orbitals. The Cartesian coordinates of all structures used in the quantum chemistry calculations reported in this work, along with the resulting \(S_0\) and \(S_1\) dipole moment vectors, can be found in the supplementary material. Visual representations of the structures and their \(S_0\) and \(S_1\) dipoles are provided in the supplementary material as well.

IV. RESULTS

Steady-state fluorescence spectra following isoenergetic OPE (400 nm) and TPE (2 × 800 nm) for a number of alcohols are presented in Fig. 2. These spectra have been divided by the frequency cubed according to the transition dipole moment representation, which makes fluorescence intensity proportional to population according to the Einstein coefficient of spontaneous emission.\textsuperscript{62} Upon excitation, \textit{FR0-SB} reaches the first excited singlet state (denoted \textit{FR0-SB}^*), which emits at \(\sim 21,000 \text{ cm}^{-1}\). Following proton transfer, the \textit{FR0-HSB}^* excited protonated state is reached, which emits at \(\sim 15,000 \text{ cm}^{-1}\). The probability of proton transfer is observed to decrease as the alkane chain of the linear alcohols.

![FIG. 2. OPE and TPE steady-state fluorescence spectra obtained for \textit{FR0-SB} in (a) methanol, (b) ethanol, (c) \(n\)-propanol, and (d) \(i\)-propanol. In each of the panels, OPE (blue line) is compared with TPE (red line). The fluorescence spectra are normalized to the non-protonated emission intensity. The ratio between the areas for \textit{FR0-HSB}^* (\(\sim 15,000 \text{ cm}^{-1}\)) and \textit{FR0-SB}^* (\(\sim 21,000 \text{ cm}^{-1}\)) emission following OPE and TPE is determined by fits to log-normal functions (thin black lines).](image-url)
increases. This observation was found to correlate with the relative −OH concentration. Interestingly, for i-propanol, significantly less proton transfer takes place, an aspect related to steric hindrance in the formation of an appropriate solvent configuration for proton transfer that has been addressed elsewhere.

Of particular interest in this work is how the probability for proton transfer, i.e., the reactivity of the Schiff base, is affected by the excitation process. For this purpose, we quantify the extent of proton transfer as the \( \frac{[\text{FR0-SB}^+]/[\text{FR0-SB}^-]}{[\text{OPE-SB}^+]/[\text{OPE-SB}^-]} \) ratio for linear and nonlinear excitation by fitting the fluorescence areas to log-normal functions, as shown in Fig. 2, and then correcting the results for differences in the fluorescence quantum yield of the protonated and non-protonated excited-state species in the different solvents. The results, summarized in Table I, are presented in Fig. 3 for both OPE (blue) and TPE (red); note that the vertical axis on the right is a logarithmic scale so differences between the two modes of excitation seem less prominent than they actually are. While both OPE and TPE proton-transfer rates change proportionally for the different solvents, we consistently observe greater proton transfer following TPE compared to OPE. The ratio between the two modes of observed excitation, i.e., the ratio of ratios, is shown as gray bars in Fig. 3. We find that for methanol TPE leads to 62% ± 20% greater reactivity than OPE. Greater reactivity following TPE vs OPE is also observed for ethanol (42% ± 13%), n-propanol (36% ± 4%), and n-hexanol (24% ± 3%), although the percent enhancement decreases with the alcohol aliphatic chain length. The large error bars, especially for methanol, result from the difficulty in measuring the very small FR0-SB* signal, which amounts to one part in 58. In the case of i-propanol, no excess reactivity is found within the uncertainty of the measurements.

The enhanced reactivity following isoenergetic TPE is unexpected. Therefore, we explore the possible involvement of an additional dark state that lies within the absorption band associated with \( S_1 \) or a higher excited singlet state \( S_n \) that is reached via three-photon excitation (3 × 800 nm). These possible contributions are addressed as follows. Excitation–emission matrix (EEM) spectra were obtained for FR0-SB in methanol and ethanol and are shown in Fig. 4. The absorption spectrum is shown as a black solid line. From the spectra in Fig. 4, we observe no evidence for the existence of an additional state within the 325 nm–450 nm \( S_1 \) region. However, we do see evidence for absorption to \( S_n \) with \( n > 1 \) in the 225 nm–290 nm region, which is also associated with FR0-SB** and FR0-SB* emission following \( S_0 \) to \( S_1 \) internal conversion. We measured the excitation intensity dependence of the TPE integrated fluorescence for the different solvents (see Fig. S1 of the supplementary material). The exponent associated with the laser intensity dependence indicates the number of photons associated with the excitation process. The exponent measured was ~1.9, indicating that three-photon excitation, if it occurs, contributes minimally. Thus, to summarize, we exclude the participation of a dark state near \( S_1 \) based on the EEM spectra. Furthermore, given the near quadratic laser power dependence combined with the observation that the probability for proton transfer following 266 nm excitation is similar to that following 400 nm excitation, contributions to the observed ESPT enhancement from three-photon excitation processes seem unlikely. While we cannot rule out the involvement of excited-state absorption in which two-photon \( S_0 \) \( \rightarrow S_1 \) transition is followed by a one-photon \( S_1 \) \( \rightarrow S_0 \) excitation in the observed reactivity enhancement, we note that direct excitation at 266 nm does not lead to enhanced reactivity and, when normalizing for proton transfer emission, we find that maximum proton transfer for OPE is observed at 400 nm excitation (see Fig. S3 of the supplementary material).

The striking solvent dependence observed for the enhanced two-photon reactivity implies that the underlying process depends on the dynamics of proton transfer and solvation. To explore this dependence, we can first rely on our previous time-resolved TCSPC results for the solvents studied here. In Fig. 5, we plot the TPE/OPE enhancement as a function of the measured FR0-SB* lifetime. We observe an inverse correlation, shorter FR0-SB* lifetimes correlate with greater enhancement. When the FR0-SB* lifetime is longer, the observed enhancement decreases. We interpret this finding as follows. Although FR0-SB has a high pKa*, the ability of FR0-SB* to abstract a proton depends on the solvent configuration. We know from our quantum chemistry calculations reported in Ref. 37 that the minimum of three solvent molecules, with one of them directly hydrogen-bonded to FR0-SB, are needed to enable the ESPT process. Achieving such a

| Solvent | OPE ratio | TPE ratio | TPE/OPE |
|---------|-----------|-----------|---------|
| MeOH    | 36 ± 3    | 58 ± 5    | 1.62 ± 0.20 |
| EtOH    | 7.6 ± 0.5 | 10.9 ± 0.7 | 1.42 ± 0.13 |
| n-ProOH | 3.85 ± 0.06 | 5.2 ± 0.1 | 1.36 ± 0.04 |
| n-HxOH  | 1.72 ± 0.02 | 2.13 ± 0.04 | 1.24 ± 0.03 |
| i-ProOH | 0.37 ± 0.01 | 0.35 ± 0.02 | 0.94 ± 0.06 |

*Abbreviations: MeOH = methanol, EtOH = ethanol, n-ProOH = n-propanol, n-HxOH = n-hexanol, and i-ProOH = i-propanol.

FIG. 3. Extent of proton transfer determined following OPE and TPE for FR0-SB in a number of alcohols. The TPE over OPE ratio is given by the gray bars (left y-axis). In all the solvents, but i-propanol, enhanced proton transfer is observed following TPE. The ratios between the fluorescence bands [FR0-SB**]/[FR0-SB*], corrected for fluorescence quantum yield, are plotted as bars (blue) and TPE (red); each ratio is indicated on a logarithmic scale (right y-axis). Abbreviations: MeOH = methanol, EtOH = ethanol, n-ProOH = n-propanol, n-HxOH = n-hexanol, and i-ProOH = i-propanol.
configuration is easiest for small molecules, such as methanol, and much less probable for secondary alcohols, e.g., i-propanol and cyclopentanol. Thus, we postulate that TPE prepares the molecule at a point on the excited-state reaction coordinate that enhances reactivity, but such propensity is lost within a few hundred picoseconds or less.

From the previous observations, it appears that TPE leads to a more reactive species than OPE. We performed fluorescence lifetime measurements following isoenergetic OPE and TPE detecting at both the FR0-SB* and FR0-HSB** wavelength regions. Results from these measurements are summarized in Table II. In the case of aprotic solvents, such as acetonitrile, the average excited-state lifetime $\bar{\tau}_{SB}$ is $\sim 2$ ns. The much shorter values for $\bar{\tau}_{SB}$ in alcohols are associated with the formation of the [FR0-SB*⋯HOR] complex where the proton is already shared by FR0-SB* and ROH, which precedes the separation and solvation of the protonated FR0-HSB** and the deprotonated solvent RO$^-$ species characterized by the rise time $\tau_X$. The latter process has a timescale that is much less dependent on the method of excitation. We find that the first step in protonation, namely, the loss of population in FR0-SB* and the rise of the FR0-HSB** emission are approximately two times faster for TPE than for OPE. This observation is consistent with the enhanced reactivity and with the conclusion that TPE leads to a more reactive species. Measurements carried out in acetonitrile in the FR0-SB* and FR0-HSB** wavelength regions showed no OPE vs TPE difference, indicating that the enhancement depends on the hydrogen-bonding capabilities of protic solvents.

Having ruled out the involvement of an additional excited state, or excitation to a higher $S_n$ excited state with $n > 1$, we now turn to the possibility of reaching a more reactive species via TPE. We begin by comparing the expressions for the absorption cross sections associated with OPE and TPE arising from the first- and second-order time-dependent perturbation theory, respectively (see, e.g., Ref. 63).

$$\sigma^{(1)}_0(\omega) = A|\mu_0|^2 g_M(\omega),$$ (7)

where $\omega$ is the frequency of the exciting photon (in our case, the frequency of a 400 nm laser), $A$ is a constant, $|\mu_0|^2$ denotes the magnitude of the transition dipole moment between the ground and excited electronic states, and $g_M(\omega)$ is the OPE distribution function or linewidth associated with the molecular system of interest. In presenting Eq. (7), we have assumed an isotropic averaging over the directions of the transition dipole moment vector $\mu_0$. To arrive at an expression for the absorption cross section associated with the isoenergetic one-color TPE, where the laser frequency is half of its OPE counterpart, we take advantage of the fact that no resonance at 800 nm is observed in our experiments, in agreement with our
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Table II. Fluorescence lifetime measurements following one- and two-photon isoenenergetic excitation of FR0-SB in methanol, ethanol, and acetonitrile. The initial state, FR0-SB\(^*\), decays with fast \(\tau_{SB1}\) and slow \(\tau_{SB2}\) biexponential lifetimes. The numbers in parentheses indicate the amplitude of the fast decay component \((\alpha_i)\). The protonated state FR0-HSB\(^\#\) shows a fast rise time \(\tau_f\) and a slow decay time \(\tau_{HSB}\). In acetonitrile, no proton transfer takes place; thus, one observes only a single exponential decay of the FR0-SB\(^*\) state that is identical for OPE and TPE within the measurement errors. All numbers are given in picoseconds.

| Solvent | \(\tau_{SB1}\) | \(\tau_{SB2}\) | \(\tau_{X}\) | \(\tau_{HSB}\) |
|---------|----------------|----------------|--------------|-------------|
| MeOH    | 23 ± 7 (0.99)  | 1259 ± 14      | 35 ± 7       | 1183 ± 2    |
| EtOH    | 59 ± 4 (0.84)  | 220 ± 6        | 85 ± 4       | 103 ± 2     |
| ACN     | ...            | 2121 ± 10      | ...          | 1241 ± 33   |

| Solvent | \(\tau_{SB1}\) | \(\tau_{SB2}\) | \(\tau_{X}\) | \(\tau_{HSB}\) |
|---------|----------------|----------------|--------------|-------------|
| MeOH    | 12 ± 6 (0.99)  | 811 ± 30       | 20 ± 6       | 28 ± 9      |
| EtOH    | 35 ± 3 (0.89)  | 212 ± 20       | 54 ± 5       | 90 ± 4      |
| ACN     | ...            | 2138 ± 8       | ...          | 1232 ± 1    |

\(^{a}\) Abbreviations: MeOH = methanol, EtOH = ethanol, and ACN = acetonitrile.

\(^{b}\) For protic solvents (MeOH and EtOH), \(\tau_{SB} = \alpha_1 \tau_{SB1} + \alpha_2 \tau_{SB2}\), where \(\alpha_1 = 1 - \alpha_2\). In the case of ACN, for which there is no ESPT, \(\tau_{SB} = \tau_{SB2}\).

... (Text continues with more details on electronic structure calculations, absorption cross section, Table II, and discussion on fluorescence lifetime measurements...)

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As illustrated in Eqs. (7) and (10), the absorption cross sections for both one- and two-photon excitation processes depend on the square of the absolute value of the transition dipole moment $\mu_{f0}$ characterizing the $0 \to f$ vertical electronic excitation, which, in our case, is the transition dipole $\mu_{10}$ corresponding to the $S_0 \to S_1$ photoabsorption for the FR0-SB system in various solvents. However, in the case of TPE, the absorption cross section also depends on the difference between the electronic dipole moments of the $f$ and $0$ states, $\Delta \mu_{f0}$, which, in our case, is the difference $\Delta \mu_{10} = \mu_f - \mu_0$ between the dipole moment $\mu_f$ characterizing the first excited singlet $S_1$ state of FR0-SB and its $S_0$ counterpart $\mu_0$. Consequently, $\Delta \mu_{10}$ and its dependence on the solvent environment hold the key to understanding the enhancement of the ESPT reactions between the FR0-SB photobase and alcohol solvents observed in the case of TPE. To provide insights into the effect of solvation on $\Delta \mu_{10}$ and other properties characterizing the $S_0$ and $S_1$ states of the solvated FR0-SB chromophore and transitions between them, we performed electronic structure calculations using the CC/EOMCC-based composite approach described in Sec. III. As mentioned in that section, the alcohol solvents considered in our computations were methanol, ethanol, n-propanol, and i-propanol.

In Table III, we report the vertical transition energies $\omega_{10}$(abs.) and transition dipole moments $\mu_{10}$ characterizing the $S_0 \to S_1$ photoabsorption process, along with the dipoles corresponding to the $S_0$ and $S_1$ states, $\mu_0$ and $\mu_1$, respectively, and their ratios resulting from our calculations for FR0-SB in the gas phase and in the aforementioned four solvents determined at the minima on the respective $S_0$ potential energy surfaces. The analogous information for the $S_1 \to S_0$ emission and the dipole moment values of the $S_0$ and $S_1$ states determined at the $S_1$ minima characterizing the isolated and solvated FR0-SB is presented in Table IV. We begin our discussion of computational results by comparing the vertical absorption and emission energies characterizing the solvated FR0-SB species obtained with the CC/EOMCC-based protocol adopted in this work against their experimental counterparts. The vertical excitation energies for the [FR0-SB⋯HOR] complexes calculated at the respective $S_0$ minima, shown in Table III, are essentially identical to the locations of the peak maxima in the corresponding experimental photoabsorption spectra reported in Ref. 37, which are 3.32 eV, 3.33 eV, 3.34 eV, and 3.37 eV for methanol, ethanol, n-propanol, and i-propanol, respectively. The same accuracies are also seen in the case of the vertical emission energies calculated at the $S_1$ minima of the [FR0-SB⋯HOR] species reported in Table IV, which can hardly be distinguished from the maxima in the experimental emission peaks for FR0-SB in methanol, ethanol, n-propanol, and i-propanol of 2.57 eV, 2.61 eV, 2.62 eV, and 2.65 eV, respectively. These observations corroborate the accuracy of the computational protocol used in this study to model the interactions of the FR0-SB photobase with the various alcohol solvents. The observed good agreement between the theoretical vertical transition energies reported in Tables III and IV and the corresponding experimental data can largely be attributed to the use of high-level ab initio CC/EOMCC approaches in describing the [FR0-SB⋯HOR] complexes. This becomes apparent when one considers the errors relative to the experiment characterizing the vertical transition energies obtained in the single-point CAM-B3LYP/6-31+G*//SMD computations, which are about 0.2 eV–0.3 eV (9%–11%).

Having established the accuracy of our quantum chemistry protocol, we proceed to the discussion of our computational findings regarding the dipole moments of the $S_0$ and $S_1$ states and the transition dipoles between them, which are the key quantities for the one- and two-photon absorption cross sections given by Eqs. (7) and (10), respectively. In the absence of direct experimental information, our computations provide insights into the effects of solvation on these quantities. To begin with, as reported in our earlier work for the bare FR0-SB species,21 and as shown in Table III, there is a large, by a factor of more than 3, increase in the electronic dipole moment following $S_0 \to S_1$ photoabsorption, giving rise to the super-base character of FR0-SB. Upon solvation, both $S_0$ and $S_1$ dipole moments of the FR0-SB chromophore are significantly enhanced, becoming approximately twice as large as their gas-phase counterparts. This can be attributed to the polarization of the electron cloud of the FR0-SB photobase by the alcohol molecules surrounding it. Furthermore, the fact that the electronic dipole moment characterizing the $S_1$ state is much larger than its $S_0$ counterpart translates into a stronger stabilization of the $S_1$ state relative to $S_0$, leading to lower $S_0 \to S_1$ vertical excitation energies in the case of FR0-SB in alcohol solvents when compared to the bare FR0-SB system. The transition dipole moment characterizing the $S_0 \to S_1$ photoabsorption process is amplified by solvation as well (by about 40%),

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**Table III.** The vertical transition energies $\omega_{10}$(abs.) (in eV) and transition dipole moments $\mu_{10}$ (in D) corresponding to the $S_0 \to S_1$ absorption, along with the $\mu_0$ and $\mu_1$ dipoles characterizing the $S_0$ and $S_1$ states (in D) and their ratios for FR0-SB in the gas phase and in selected alcohol solvents calculated at the respective $S_0$ minima following the CC/EOMCC-based protocol described in Sec. III.

| Solvent        | $\omega_{10}$(abs.) | $\mu_0$ | $\mu_1$ | $\mu_1/\mu_0$ |
|----------------|---------------------|---------|---------|---------------|
| None (gas phase)| 3.70                | 6.9     | 2.6     | 8.6           |
| MeOH           | 3.30                | 9.6     | 4.4     | 16.4          |
| EtOH           | 3.32                | 9.5     | 4.3     | 15.9          |
| n-ProOH        | 3.32                | 9.5     | 4.2     | 15.9          |
| i-ProOH        | 3.33                | 9.4     | 4.2     | 15.7          |

*Abbreviations: MeOH = methanol, EtOH = ethanol, n-ProOH = n-propanol, and i-ProOH = i-propanol.

*Taken from our previous gas-phase CC/EOMCC calculations reported in Ref. 21.

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**Table IV.** The vertical transition energies $\omega_{10}$(em.) (in eV) and transition dipole moments $\mu_{10}$ (in D) corresponding to the $S_0 \to S_1$ emission, along with the $\mu_0$ and $\mu_1$ dipoles characterizing the $S_0$ and $S_1$ states (in D) and their ratios for FR0-SB in the gas phase and in selected alcohol solvents calculated at the respective $S_1$ minima following the CC/EOMCC-based protocol described in Sec. III.

| Solvent | $\omega_{10}$(em.) | $\mu_0$ | $\mu_1$ | $\mu_1/\mu_0$ |
|---------|-------------------|---------|---------|---------------|
| None (gas phase) | 3.26                | 8.9     | 3.4     | 10.9          |
| MeOH     | 2.68               | 11.8    | 6.6     | 20.0          |
| EtOH     | 2.69               | 11.8    | 6.5     | 19.7          |
| n-ProOH  | 2.70               | 11.8    | 6.5     | 19.6          |
| i-ProOH  | 2.72               | 11.7    | 6.4     | 19.1          |

*Abbreviations: MeOH = methanol, EtOH = ethanol, n-ProOH = n-propanol, and i-ProOH = i-propanol.
which results in larger OPE and TPE absorption cross sections for the solvated FR0-SB species relative to their gas-phase values. Similar trends are observed when we examine the dipole and transition dipoles shown in Table IV. It is also interesting to note that the dipole moments characterizing the S0 and S1 states and the corresponding transition dipoles increase upon geometrical relaxation from the S0 to S1 minima, with a concomitant red shift in the vertical transition energies. This bathochromic shift is more pronounced in the case of the solvated FR0-SB species as a consequence of μ1 being much larger than μ0, implying a stronger stabilization of the S1 state due to the polar solvent environment compared to the S0 state.

As already alluded to above, the transition dipole moments characterizing the S0–S1 absorption and emission processes and the S0 and S1 dipoles at the respective potential minima could not be determined from our experiments. However, by analyzing the solvatochromic shift of the absorption and fluorescence bands in 16 different solvents as a function of solvent dielectric constant and index of refraction, we could estimate the magnitude of the transition dipole moment μ10 and the change in the dipole moment, Δμ10, associated with the S0 → S1 adiabatic excitation. Based on our analysis, we found Δμ10 of FR0-SB in the alcohol solvents considered in our experiments to be ~15 D, a magnitude usually associated with substantial charge transfer, and μ10 to be about 10 D. The procedure outlining how the experimental values of μ10 and Δμ10 were derived is given in the supplementary material. Having access to the dipole moments characterizing the S0 and S1 states at their respective minimum-energy structures and the vertical transition dipole moments associated with the S0–S1 transitions resulting from our quantum chemistry computations (see Tables III and IV) allowed us to assess the quality of our experimentally derived values of μ10 and Δμ10. As shown in Tables III and IV, the vertical transition dipole moments μ10 characterizing the FR0-SB chromophore in the alcohol solvents included in our calculations range from 9.4 D to 11.8 D, in very good agreement with the experimentally derived value of about 10 D. According to the data collected in Table V, the calculated and experimentally derived changes in the dipole moment associated with the S0 → S1 adiabatic transition, which are about 15 D in both cases, are virtually identical. Given that both theory and experiment point to the large values of μ10 and Δμ10 as a result of solvation and that the dipole pathway defined by the second term in Eq. (9) is anticipated to be the dominant TPE pathway, as discussed above, we can conclude that using Eq. (10) in approximating the TPE absorption cross section of FR0-SB in alcohol solvents is justified.

By forming the ratio of Eqs. (7) and (10), we can obtain a new expression that summarizes the difference between OPE and TPE, in which the change in permanent dipole moment acts as an amplification factor,

$$\frac{\sigma(0,2)}{\sigma(0,3)} = \frac{B'}{A} \frac{|\Delta \mu_f|}{|\mu_f|} \frac{\Delta \mu_2}{\mu_2} = C \frac{|\Delta \mu_f|^2}{\mu_f} \frac{\Delta \mu_2}{\mu_2},$$

where C = B'/A. The difference between OPE and TPE typically arises from differences in the expressions for the line shapes, which in the case of large organic molecules in solution, are the Franck-Condon distribution convolved with the extensive homogeneous and inhomogeneous broadening. We have acquired these spectra for FR0-SB in cyclohexane, acetonitrile, and methanol, as shown in Fig. 6. From these spectra, we can obtain the ratio of the spectral line shapes for OPE and TPE.

For cyclohexane, a non-polar solvent for which the change in permanent dipole moment is smallest, the excitation maxima for OPE and TPE coincide, and the ratio between the line shapes can be fit to a line with a negative slope from the peak to higher energies, indicating a steeper decline of the spectrum as a function of excitation energy for TPE. The steeper decline is consistently observed for acetonitrile and for methanol. However, for acetonitrile and methanol, we also see that the two-photon absorption peaks appear at significantly lower energies than their OPE counterparts.

| Table V. A comparison of the calculated S0–S1 adiabatic transition energies without [ω10(ad.)] and with [ω10(0-0)] zero-point energy (ZPE) vibrational corrections (in eV), along with the differences and ratios of the μ0 and μ1 dipoles characterizing the S0 and S1 states at the respective minima (in D) for FR0-SB in the gas phase and in selected alcohol solvents obtained following the CC/EOMCC-based protocol described in Sec. III with the corresponding experimentally derived data. |
|-----------------|-----------------|-----------------|-----------------|
| Theory | Experiment |
| Solvent | ω10(ad.) | ω10(0-0) | Δμ10 | μ1/μ0 | Solvent | ω10(0-0) | Δμ10 | μ1/μ0 |
| None (gas phase) | 3.42 | 3.33 | 8.3 | 4.2 | c-Hexane | 3.4 | . . . | . . . |
| MeOH | 2.88 | 2.80 | 15.6 | 4.6 | MeOH | 2.9 | 15.2 ± 0.2 | 4.4 ± 0.1 |
| EtOH | 2.89 | 2.80 | 15.4 | 4.6 | EtOH | 3.0 | 15.3 ± 0.3 | 4.6 ± 0.1 |
| n-PrOH | 2.89 | 2.81 | 15.3 | 4.6 | n-PrOH | 3.0 | 15.3 ± 0.3 | 4.6 ± 0.1 |
| i-PrOH | 2.88 | 2.80 | 14.9 | 4.5 | i-PrOH | 3.0 | 15.5 ± 0.5 | 4.7 ± 0.1 |

# Abbreviations: MeOH = methanol, EtOH = ethanol, n-PrOH = n-propanol, i-PrOH = i-propanol, and c-Hexane = cyclohexane.

# Calculated as ω10(ad.) + ΔZPE, where ΔZPE is the difference between the zero-point vibrational energies characterizing the S0 and S1 electronic states of the bare FR0-SB molecule in the gas phase computed at the CAM-B3LYP/6-31+G* level of theory. Our calculations with and without solvent indicate that the effect of solvation on ΔZPE is negligible (less than 0.01 eV).

# Calculated using the μ0 values reported in Table III and the μ1 values reported in Table IV.

# Calculated using the theoretical values of μ0 reported in Table III and the procedure based on the analysis of the experimental solvatochromic shifts described in the supplementary material.
The largest shift of the TPE absorption maximum compared to OPE, of ~1140 cm\(^{-1}\), is observed for methanol. Differences between OPE and TPE have been observed experimentally, in particular as blue shifts in the TPE of the green fluorescent protein, and have been explained as non-Condon contributions to TPE. However, the very large red shift observed in this work appears to be unprecedented.

Having strong evidence that TPE must be reaching parts of the \(S_1\) potential surface that enhance reactivity compared to the isoenergetic OPE and that the initial state wave packet following TPE is different from that following OPE, we turn our attention to the relationship between the ESPT enhancement, the inhomogeneous broadening, and the significant red shift observed in our TPE experiments. It is well-established that polar solvents, in particular those capable of forming hydrogen bonds, are responsible for significant inhomogeneous broadening in absorption spectra.\(^{76,79}\)

The inhomogeneous broadening can further be amplified when the solute has hydrogen-bond acceptor and/or donor functional groups and undergoes large permanent dipole changes upon photoexcitation.\(^{79}\) From Eq. (10), we learn that TPE is greatly enhanced by the \(|\Delta \mu_{0f}|^2\) term, favoring chromophore molecules whose local solvation environment gives rise to larger \(|\Delta \mu_{0f}|^2\) values compared to OPE. The substantial increase in the dipole moment upon photoexcitation should result in the different configurations in which protic solvents can be arranged to solvate FR0-SB, giving rise to inhomogeneous broadening. At the same time, larger \(|\Delta \mu_{0f}|^2\) results in the additional stabilization of the [FR0-SB⋯HOR] complex in the \(S_1\) state relative to the ground state, manifesting itself in the observed red shift in the absorption maximum. While this hypothesis needs additional thorough investigations using, for example, two-dimensional spectroscopy and molecular dynamics simulations of the observed excited-state reactivity, it is conceivable that the dynamical restructuring of the solvent around the chromophore molecules following TPE, promoting larger \(|\Delta \mu_{0f}|^2\) values compared to OPE, results in stronger charge transfer between the amine and imine nitrogens of FR0-SB, the additional accumulation of negative charge on the imine nitrogen and, subsequently, the enhancement of the ESPT process, which is what we attempted to schematically illustrate in Fig. 1(b). This is in contrast to some intramolecular ESPT reactions, such as in diethylaminohydroxyylavone, where solvation is inversely correlated with proton transfer.\(^{77}\) The differences observed in FR0-SB following OPE or TPE are caused by the large change in permanent dipoles influencing the probability for TPE of some molecules. Molecules with a solvent configuration that is more likely to result in proton transfer would then be favored to undergo TPE because of their larger \(|\Delta \mu_{0f}|^2\) values.

We find support for the role of inhomogeneous broadening in protic solvents in the experimental data. We note that there is essentially no shift between the maxima for OPE and TPE for cyclohexane, a non-polar molecule. There is an ~1000 cm\(^{-1}\) shift observed for acetonitrile and an ~1140 cm\(^{-1}\) shift for methanol. These shifts are not predicted simply by the non-Condon vibrational makeup of the excited-state wave packet. The existence of differently solvated species, in particular those exhibiting greater dipole moment changes, can be determined experimentally by comparing the absorption spectra for FR0-SB in solvents that exhibit TPE enhancement (methanol, ethanol, and \(n\)-propanol) with that of FR0-SB in \(i\)-propanol, which does not. The comparison is shown in Fig. 7, where we plot the absorption spectra as well as the difference between the absorption spectrum in each solvent and that in

\[
\Delta \mu = |\mu_f - \mu_0| = |\Delta \mu_{0f}| \quad \text{(10)}
\]

![FIG. 6. OPE and TPE spectra for FR0-SB in cyclohexane (c-Hexane), acetonitrile (ACN), and methanol (MeOH). The solid line shown for the two-photon spectra is the result of 3-point smoothing and is included as a guide to the eyes.](image)

![FIG. 7. Absorption spectra of FR0-SB in methanol (MeOH), ethanol (EtOH), \(n\)-propanol (\(n\)-PrOH), and \(i\)-propanol (\(i\)-PrOH) (solid lines) and difference between these spectra and the absorption in \(i\)-propanol (dotted lines). We find a significant difference between the spectra in the 25 000 cm\(^{-1}\) energy region where the experiment was performed.](image)
We observe a large difference in the absorption spectra, especially at 25 000 cm$^{-1}$, where the experiment was carried out. The largest difference is found for methanol, which exhibits the largest TPE enhancement.

From Fig. 7, we can see significant inhomogeneous broadening toward lower energies. This supports our hypothesis that there is a clear bias toward TPE in the case of molecules primed for proton transfer, as seen in the reduction of the protonation time in methanol and ethanol by a factor of 2 compared to the other alcohols. The effect becomes attenuated for $n$-hexanol, given that the long alkyl chain reduces the chances for two or more hydroxyl moieties to be next to the imine group where proton transfer takes place. As shown in our computations and OPE experiments reported in Ref. 37, the solvent configuration leading to proton transfer in $i$-propanol is much less likely, resulting in very low efficiency of the ESPT process, and, thus, TPE can no longer enhance it, confirming our hypothesis.

V. CONCLUSIONS

In this paper, we have presented the observation of up to 62% enhancement in the reactivity of the super photobase FR0-SB following isooenergetic two-photon excitation. We have reported evidence that excitation is to the first excited singlet state, $S_1$, and that no other excited state contributes to proton transfer via one- or multi-photon excitation. We have found that the magnitude of the enhancement in reactivity correlates with the protonation rate; thus, it is faster for methanol but slows down with longer chain alcohols and is not measurable for $i$-propanol.

We have reached the conclusion that the enhanced solvent-tosolute ESPT reactivity is the result of TPE creating a more reactive species than OPE. This is supported by analysis of the molecules properties that contribute to the one- and two-photon transition probabilities, namely, the transition dipole moment $\mu_{10}$ coupling the $S_0$ and $S_1$ states of FR0-SB and the $\Delta\mu_{10}$ difference between the dipole moments of these two states. According to our mathematical manipulations, the ratio of the TPE and OPE absorption cross sections depends on $\Delta\mu_{10}$ but not on $\mu_{10}$. The $\Delta\mu_{10}$ values resulting from the high-level CC/EOMCC-based computations performed in this study were practically identical to those estimated from our experiments. We learned that the very large change in permanent dipoles between the ground and excited states of FR0-SB amplifies differences in the spectroscopic line shape, leading to non-Condon contributions and different vibrational makeups of the initial excited-state wave packet.

Finally, we have considered inhomogeneous broadening as providing an additional aspect leading to different excited-state species accessed via TPE. The permanent dipole change is highly dependent on the arrangement of polar solvent molecules, especially for protic solvents capable of hydrogen bonding. These added contributions are confirmed by red shift observed in the TPE spectra for acetonitrile and methanol, but not for cyclohexane, and by the greater inhomogeneous broadening toward the low energy region in the OPE spectra of solutions exhibiting TPE enhancement. The findings reported here help explain the observation by Tokumura and Itoh on the intramolecular proton transfer in 7-hydroxyquinoline in methanol solution via two-photon excitation. In that study, two-photon excitation in the equivalent 210 nm–250 nm wavelength region leads to exclusive emission from the proton transfer state. Later measurements, based on step-wise solvation supersonic-jet spectroscopy, identified that bridging methanol structures with three methanol molecules help facilitate the proton transfer. These two separate observations support our conclusion that two-photon excitation favors structures primed for excited-state proton transfer.

In conclusion, we have reported unprecedented 62% enhancement in photochemical reactivity upon isooenergetic two-photon excitation compared to one-photon excitation. The long-term goal of our research is to develop tools for precision chemistry and our findings indicate that two-photon excitation not only provides spatial and temporal control, but it can also bring about enhanced reactivity. Future work will include quantitative determination of the two-photon absorption cross section for FR0-SB in different solvents and of newly developed compounds.

SUPPLEMENTARY MATERIAL

See the supplementary material for the laser power dependence of two-photon excited fluorescence, ratios between FR0-SB $^*$ and FR0-SB $^+$ emissions, fitting of the steady-state fluorescence results, time-correlated single-photon counting fitting, experimental determination of permanent dipole moments, optimized geometries and electronic dipole moments resulting from quantum chemistry computations for the bare and solvated FR0-SB system in the $S_0$ and $S_1$ electronic states along with their visual representations, and dominant orbitals defining the $S_0$–$S_1$ transitions in the [FR0-SB…HOR] complexes.

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DATA AVAILABILITY

The data that support the findings of this study are available within the article and its supplementary material. Further data are available from the corresponding authors upon request.
REFERENCES

1. M. Göppert-Mayer, Ann. Phys. 401, 273 (1931).
2. P. T. C. So, C. Y. Dong, B. R. Masters, and K. M. Berland, Annu. Rev. Biomed. Eng. 2, 399 (2000).
3. K. König, J. Microsc. 200, 83 (2000).
4. W. R. Zipfel, R. M. Williams, and W. W. Webb, Nat. Biotechnol. 21, 1369 (2003).
5. C. Stosiek, O. Garaschuk, K. Holthoff, and A. Konnerth, Proc. Natl. Acad. Sci. U. S. A. 100, 7319 (2003).
6. P. Helmchen and W. Denk, Nat. Methods 2, 932 (2005).
7. H. Tu, Y. Liu, D. Turchinovich, M. Marjanovic, J. K. Lyngsø, J. Lægsgaard, E. Chaney, Y. Zhao, S. You, W. L. Wilson, B. Xu, M. Dantus, and S. A. Boppart, Nat. Photonics 10, 534 (2016).
8. S. Saytashev, R. Glenn, G. A. Murashova, S. Osseiran, D. Spence, C. L. Evans, and M. Dantus, Biomed. Opt. Express 7, 3449 (2016).
9. G. A. Murashova, C. A. Mancuso, J. L. Canfield, S. Sakami, K. Palczewski, G. Palczewski, and M. Dantus, Biomed. Opt. Express 8, 5228 (2017).
10. M. Maruo, O. Nakamura, and S. Kawata, Opt. Lett. 32, 1532 (1997).
11. S. Kawata, H. B. Sun, T. Tanaka, and K. Takada, Nature 412, 697 (2001).
12. W. Haske, V. W. Chen, J. M. Hales, W. Dong, S. Barlow, S. R. Marder, and J. W. Perry, Opt. Express 15, 3426 (2007).
13. K.-H. Lee, J. J. Moon, and J. L. West, Biomaterials 29, 2962 (2008).
14. A. Marino, C. Filippeschi, V. Mattoli, B. Mazzolai, and G. Ciofani, Nanoscale 7, 2841 (2015).
15. W. Sheng, M. Nairat, P. D. Pawlaczyk, E. Mroczka, B. Farris, E. Pines, J. H. Geiger, B. Borhan, and M. Dantus, Angew. Chem., Int. Ed. 57, 14742 (2018).
16. E. Mosover and D. Huppert, Annu. Rev. Phys. Chem. 37, 127 (1986).
17. L. G. Arnaut, S. J. Formosinho and L. G. Arnaut, J. Phys. Chem. A 103, 4247 (1999).
18. P. Hohenberg and W. Kohn, Phys. Rev. B864 (1964).
19. M. E. Casida, in Recent Advances in Density Functional Methods, Part 1, edited by D. P. Chong (World Scientific, Singapore, 1995), pp. 155–192.
20. F. Yanai, D. P. Tew, and N. C. Handy, Chem. Phys. Lett. 393, 51 (2004).
21. W. J. Hehe, R. Ditchfield, and J. A. Pople, J. Chem. Phys. 56, 2257 (1972).
22. P. C. Hariharan and J. A. Pople, Theor. Chim. Acta 28, 213 (1973).
23. T. Clark, J. Chandrasekhar, G. W. Spitznagel, and P. R. Schleyer, J. Comput. Chem. 4, 294 (1983).
24. G. D. Purvis III and R. J. Bartlett, J. Chem. Phys. 76, 1910 (1982).
25. P. Piecuch and M. Wloch, J. Chem. Phys. 123, 224105 (2005).
26. P. Piecuch, M. Wloch, J. R. Gour, and A. Kinal, Chem. Phys. Lett. 418, 467 (2006).
27. M. S. Gordon, L. Slipchenko, H. Li, and J. H. Jensen, in Annual Reports in Computational Chemistry, edited by D. C. Spellmeyer and R. Wheeler (Elsevier, 2007), pp. 177–193.
28. M. S. Gordon and M. W. Schmidt, in Theory and Applications of Computational Chemistry: The First Forty Years, edited by C. E. Dykstra, G. Frenking, K. S. Kim, and E. G. Scuseria (Elsevier, Amsterdam, 2005), pp. 1167–1189.
29. G. M. J. Barca, C. Bertoni, L. Carrington, D. Datta, N. De Silva, J. E. Deustua, D. G. Fedorov, J. R. Gour, A. O. Hunina, E. Guize, T. Harville, S. Irle, J. Ivanic, K. Kowalski, S. S. Leang, H. Li, W. Li, J. J. Lyt, I. Magonus, J. Mato, V. Mironov, H. Nakata, B. Q. Pham, P. Piecuch, D. Poole, S. R. Pruitt, A. P. Rendell, L. B. Roskop, K. Ruedenberg, T. Sattathushchana, M. W. Schmidt, J. Shen, L. Slipchenko, M. Sosonkina, V. Sundriyal, A. Tiwari, J. L. Galvez Vallejo, B. Westheimer, M. Wloch, P. Xu, F. Zahariev, and M. S. Gordon, J. Chem. Phys. 152, 154102 (2020).
30. M. Cossi and V. Barone, J. Chem. Phys. 115, 4708 (2001).
31. P. Piecuch, S. A. Kucharski, K. Kowalski, and M. Musial, Comput. Phys. Commun. 149, 71 (2002).
32. K. Kowalski and P. Piecuch, J. Chem. Phys. 120, 1715 (2004).
33. M. Wloch, J. R. Gour, K. Kowalski, and P. Piecuch, J. Chem. Phys. 122, 214107 (2005).
34. G. Angulo, G. Grampp, and A. Rosspenter, Spectrochim. Acta A 65, 727 (2006).
35. J. J. Sakurai and J. Napolitano, Modern Quantum Mechanics, 2nd ed. (Addison-Wesley, 2011).
36. P. Bräunlich, in Progress in Atomic Spectroscopy, edited by W. Hanle and H. Kleintoppen (Plenum, New York, 1979), pp. 777–827.
37. W. J. Meath and E. A. Power, J. Phys. B: At. Mol. Phys. 17, 763 (1984).
38. B. N. Jagatap and W. J. Meath, Chem. Phys. Lett. 258, 293 (1996).
39. B. Dick and G. Hohnleicher, J. Chem. Phys. 76, 5755 (1982).
40. R. Birge, L. P. Murray, R. Zidovetzki, and H. M. Knapp, J. Am. Chem. Soc. 109, 2090 (1987).
41. P. R. Callis, Annu. Rev. Phys. Chem. 48, 271 (1997).
42. M. Drozbiak, F. Meng, A. Rebane, Y. Stepanenko, E. Nickel, and C. W. Spangler, J. Phys. Chem. B 110, 9802 (2006).
43. M. Drozbiak, N. S. Makarov, S. E. Tillo, T. E. Hughes, and A. Rebane, Nat. Methods 8, 393 (2011).
44. M. Alam, M. Chattopadhyaya, and S. Chakrabarti, J. Chem. Phys. A 116, 11034 (2012).
45. W. J. Meath, AIP Adv. 6, 075202 (2016).
46. C. Reichardt, Chem. Rev. 94, 2319 (1994).
A. Kawski and P. Bojarski, Spectrochim. Acta  A 82, 527 (2011).

H. Hosoi, S. Yamaguchi, H. Mizuno, A. Miyawaki, and T. Tahara, J. Phys. Chem. B  112, 2761 (2008).

E. Kamarchik and A. I. Krylov, J. Phys. Chem. Lett. 2, 488 (2011).

A. M. Moran, D. S. Egolf, M. Blanchard-Desce, and A. M. Kelley, J. Chem. Phys. 116, 2542 (2002).

R. Moca, S. R. Meech, and I. A. Heisler, J. Phys. Chem. B 119, 8623 (2015).

C. A. Rumble, J. Breffke, and M. Maroncelli, J. Phys. Chem. B 121, 630 (2017).

K. Tokumura and M. Itoh, J. Phys. Chem. B 88, 3921 (1984).

L. W. Peng, M. Dantus, A. H. Zewail, K. Kemnitz, J. M. Hicks, and K. B. Eisenthal, J. Phys. Chem. B 91, 6162 (1987).