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Tryptophan and ATTO 590: Mutual Fluorescence Quenching and Exciplex Formation

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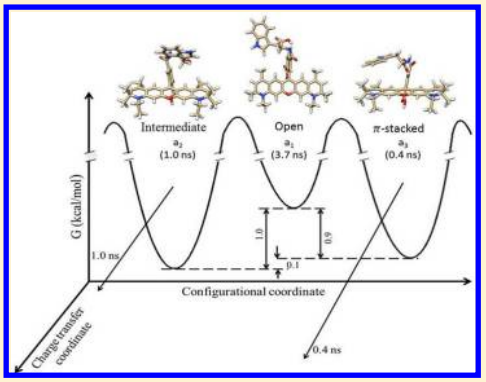
Supporting Information

ABSTRACT: Investigation of fluorescence quenching of probes, such as ATTO dyes, is becoming an increasingly important topic owing to the use of these dyes in super-resolution microscopies and in single-molecule studies. Photoinduced electron transfer is their most important nonradiative pathway. Because of the increasing frequency of the use of ATTO and related dyes to investigate biological systems, studies are presented for inter- and intramolecular quenching of ATTO 590 with tryptophan. In order to examine intramolecular quenching, an ATTO 590−tryptophan conjugate was synthesized. It was determined that tryptophan is efficiently quenching ATTO 590 fluorescence by excited-state charge transfer and two charge transfer complexes are forming. In addition, it was discovered that an exciplex (whose lifetime is 5.6 ns) can be formed between tryptophan and ATTO 590, and it is suggested that the possibility of such exciplex formation should be taken into account when protein fluorescence is monitored in a system tagged with ATTO dyes.



INTRODUCTION

Because of their experimentally desirable features of, for example, high quantum yield and photochemical stability, ATTO dyes such as ATTO 590 (Figure 1) are finding ever growing applications as probes for biological studies using fluorescence-based techniques[1−3] in, for example, super-resolution microscopies[4−6] and single-molecule measurements.[7−9] It is important, however, to understand how possible interactions with the environment can affect the ATTO photophysics. Marmé et al. observed fluorescence quenching of red absorbing ATTO dyes in the presence of amino acids.[10] They noted that tryptophan proved to be the most efficient quencher of ATTO fluorescence. They attributed the quenching to the production of a ground-state ATTO−tryptophan complex, which they concluded was nonfluorescent. This conclusion was based upon their observation that static quenching is dominant. Zhu et al. carried out transient absorption experiments of ATTO 655 in the presence of tryptophan in aqueous solution.[11] Assuming that the standard redox potential of the oxazine dye (MR 121) is similar to that of ATTO 655, they searched for photoinduced electron transfer from tryptophan to ATTO 655. They, however, were unable to find any evidence of a charge-separated species, thus concluding that it decayed faster than the time resolution of their instrument, 150 fs. Using a similar system, Yu and co-workers reported two charge separation rates, which they interpreted in terms of two different charge-separated complexes.[12]

Stimulated by these studies, we performed further experiments with ATTO 590 in order to elucidate inter- and intramolecular quenching processes with tryptophan. Our results do not support the existence of nonfluorescent ground-state complexes in the concentration range investigated, but they are consistent with excited-state electron transfer being the predominant mechanism for fluorescence quenching. Unfortunately, the time resolution of our transient absorption spectrometer was not sufficient to observe the charge-separated species.

In the course of these studies, we also investigated the quenching of *tryptophan fluorescence* by ATTO 590, which quenches fluorescence very efficiently by means of formation of an exciplex in an ATTO−tryptophan conjugate. This is consistent with the work of Rivarola et al., which reported exciplex formation of excited-state indole derivatives and monosubstituted benzenes in cyclohexane.[13] (In the case of benzonitrile and chlorobenzene, a charge transfer interaction with indole is, however, suggested to be more likely.) In a similar study by Previtali and co-workers, indole derivatives including tryptophan were found to form exciplexes in solutions of reverse micelle solutions made with the cationic surfactant benzylhexadecyldimethylammonium chloride in benzene, particularly at low molar ratio water/surfactant.[14] There is also evidence of exciplex formation with tryptophan and alcoholic solvents.[15−17] To our knowledge, our report is the first of tryptophan exciplex formation with a commonly used fluorescent label, ATTO 590, in aqueous solution. This finding is particularly relevant for a thorough and correct interpretation of fluorescence data in biological systems tagged with such dyes.

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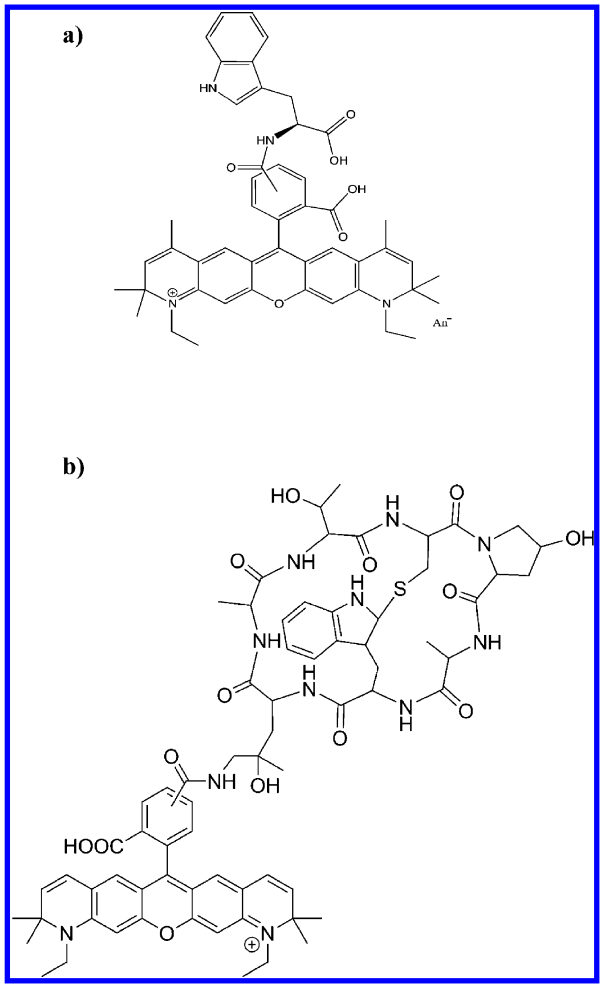

Figure 1. (a) Structure of the conjugate of ATTO 590 and L-tryptophan. (b) Structure of ATTO 590−phalloidin.

EXPERIMENTAL SECTION

Materials. ATTO 590 (free carboxy acid) was purchased from ATTO-TEC GmbH, Siegen, Germany, and L-tryptophan was purchased from Sigma-Aldrich. They were used as received. All the experiments were done in 10 mM phosphate buffer at pH 7.

Preparation of the Conjugate of ATTO 590 and Tryptophan. A 0.3 mg (0.0015 mmol) portion of L-tryptophan was dissolved in 1 mL of 1 M $NaHCO_3$ solution. A 0.5 mg (0.00063 mmol) portion of ATTO 590 NHS ester, dissolved in 50 μL of DMF, was added to the reaction vessel. After 2.5 h of stirring at ambient temperature in the dark, the solvent was removed *in vacuo*. The product was purified on a preparatory TLC plate (1000 μm, Analtech Silica) in the dark in 1:1 MeOH:$CHCl_3$. The bottom fluorescent spot (R_f = 0.44) was isolated. EI/MS, formula $C_{48}H_{49}N_4O_6$ requires a mass of 777.3652 (observed, 777.3646).

Steady-State Measurements. Absorbance was recorded with an Agilent 8453 UV−vis spectrometer with 1 nm resolution. Steady-state fluorescence measurements were done with a Spex Fluoromax-4 with a 3 or 4 nm bandpass and corrected for lamp spectral intensity and detector response. To avoid reabsorption, reemission effects, or dye aggregation, concentrations were kept near 1 μM in all measurements.

Lifetime Measurements. Measurements of excited state lifetimes were carried out with the time-correlated single-photon counting (TCSPC) technique. The apparatus for time-correlated single-photon counting is described elsewhere.[18] The fundamental from a homemade mode-locked Ti-sapphire oscillator was modulated by a Pockels cell (model 350-160, Conoptics Inc.) to reduce the repetition rate to 8.8 MHz. An excitation wavelength of 415 or 266 nm was used. The second and third harmonics were obtained from the 830 nm fundamental by means of a U-Oplaz Technologies (model TP-2000B) doubler/tripler. Recently, a Becker & Hickl photon counting module (model SPC-630) has been employed. With the system described, the full width at half-maximum of the instrument response (IRF) is ∼40−50 ps. Generally, for lifetime experiments, 65 000 counts were taken in the peak channel unless otherwise mentioned. A cuvette of 3 mm or 1 cm path length was used for the time-resolved measurement, depending upon the sample. Different filters were also chosen to see fluorescence decay of a particular band. The decay parameters were determined by fitting to a sum of exponentials after deconvolution of the instrument response function.

Calculations. DFT computations (B3LYP/STO-3G) were used to compute the energies of the ATTO−Trp conjugate. A PCM water solvation model was employed. At this low level of theory, the energies may be underestimated due to DFT having difficulties calculating dispersion forces. The computed lowest energies for the 5′ and 6′ open and stacked forms of the conjugate are shown (Supporting Information), along with their energies (in kcal/mol) and energy differences between the open and stacked forms. The energy differences of the open and stacked forms of both isomers lie within 5 kcal/mol, which is within the measurable error of Gaussian. Because of this, it is difficult to draw quantitative conclusions about the conformations. On the other hand, the calculations are consistent with the notion that the ATTO−Trp conjugate can exist in many conformations, including those that are stretched and stacked. We invoke such geometries in our discussion of lifetime data below. All DFT computations were done with Gaussian 09. The hybrid B3LYP functional was used, which consists of the Becke three-parameter exchange functional[19] with the correlation functional of Lee, Yang, and Parr.[20]

RESULTS AND DISCUSSION

Steady-State Measurements. Figure 2 presents the absorption spectra of ATTO 590 in pH 7 buffer in the presence and absence of tryptophan. Marmé et al. proposed formation of a nonfluorescent ground-state complex responsible for static quenching of tryptophan and ATTO 590.[10] They calculated the association constant, *K*, to be 50, which implies that, in a mixture of 50 mM tryptophan and a micromolar solution of ATTO dye, about half of the ATTO 590 should exist in complexed form. As it is very unlikely that the ATTO monomer and an ATTO−Trp complex would have the same absorption spectra, we would expect a noticeable change in the absorption spectra in the presence of 50 mM tryptophan. This, however, was not the case. The absorption spectra of ATTO 590 are essentially the same regardless of the concentration of tryptophan in pH 7 buffer, from which we conclude that there is no significant formation of a ground-state complex. Furthermore, even for the ATTO−tryptophan conjugate, where ground-state complex formation would have been more facile, the ATTO 590 monomer absorption spectrum is conserved.

The situation of ATTO 590 and tryptophan in this work stands in clear contrast to, for example, the documented example of oxazine and tryptophan, examined by Sauer and co-workers,[21] which do form a ground-state complex, as evidenced by about a 10 nm red shift in the absorption of oxazine as the concentration

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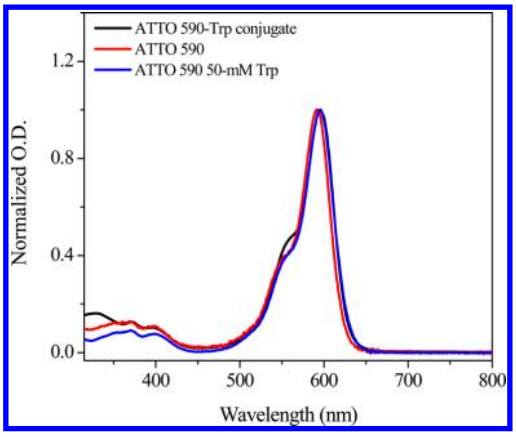

Figure 2. Normalized absorption spectra of (black) ATTO 590–tryptophan conjugate, (red) ATTO 590, and (blue) ATTO 590 in the presence of 50 mM tryptophan in pH 7 buffer. The concentration of ATTO 590 was kept constant at 1 μM. The blue spectrum was collected by using 50 mM tryptophan as a blank. The spectra were normalized with respect to that of ATTO 590–tryptophan conjugate. The maximum O.D. values were 0.119 and 0.118 for the red and blue spectra, respectively. That the optical density of the ATTO, or its spectrum, does not change as a function of tryptophan concentration is important for eliminating the possibility of a ground-state complex between the two chromophores.

of tryptophan is increased from 0 to 50 mM. Over this range of tryptophan concentration, the absorption spectrum of oxazine also changes in shape and intensity. On the other hand, the spectra of ATTO 590 are unchanged both in shape and intensity within the resolution of our absorption spectrometer (±1 nm) over the entire range of tryptophan concentration and in the ATTO 590–tryptophan conjugate (Figure 2).

On the other hand, the possibility of excited-state charge transfer is possible when the two chromophores are sufficiently near. Namely, the fluorescence spectra of the conjugate and of ATTO 590 in the presence or absence of tryptophan show one emission maximum at 610 nm for excitation at any wavelength corresponding to absorption by the ATTO 590 chromophore. When, however, the conjugate is excited (at λ_{ex} = 266 nm), a new fluorescence band with a maximum at 455 nm (Figure 3)

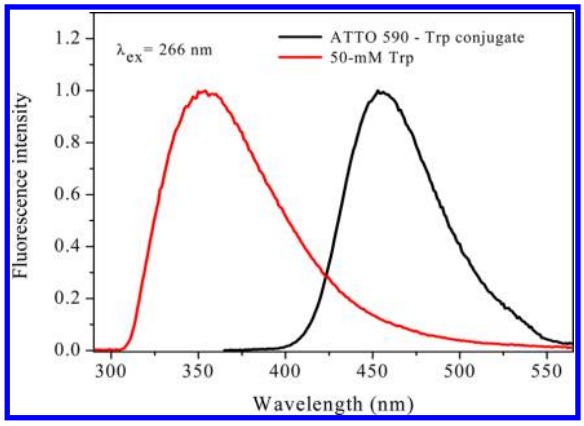

Figure 3. Exciplex fluorescence of the ATTO 590–tryptophan conjugate, λ_{ex} = 266 nm. The control experiment performed in the absence of tryptophan demonstrates that the band at 455 nm is not a result of an excimer of tryptophan.

is observed; simultaneously, the "normal" fluorescence maximum of tryptophan, ~350 nm, is strongly quenched. We did not observe the same band with ATTO 590 dissolved in 50 mM tryptophan solution in pH 7 buffer, which is not surprising, as the average distance between ATTO 590 and Trp is greater (~60 Å) than that in the conjugate (~10 Å); in other words, 50 mM is not concentrated enough compared to the "effective concentration" of tryptophan in the conjugate, which is estimated to be ~110 mM by inspection of the Stern–Volmer plot (Figure 5).

The excitation spectra of the conjugate are given in Figure 4. When emission is collected at 630 nm, the excitation spectrum

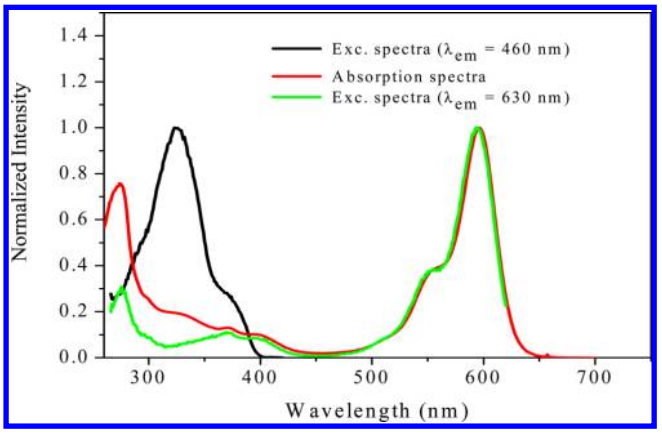

Figure 4. Excitation spectra of the ATTO 590–tryptophan conjugate: (black) emission collected at 460 nm, corresponding to the exciplex fluorescence; (green) emission collected at 630 nm, corresponding to ATTO 590 fluorescence; and (red) absorption spectra of the conjugate. Note the superposition of the green and red spectra.

agrees very well with the ATTO 590 absorption spectrum. However, when the emission is collected at 460 nm, the excitation spectrum differs completely from the absorption spectrum. This indicates that an exciplex is forming between tryptophan and ATTO 590. This band is not a result of excimer formation between two tryptophans, as is demonstrated by its absence in a solution of 50 mM tryptophan containing no ATTO 590 (Figure 3).

Di Mascio and co-workers[22] have observed spectra similar to the spectrum displayed in Figure 3 for photo-oxidized tryptophan. It is highly unlikely that our spectrum arises from such a product. The experimental conditions employed in this work are considerably different than ours. Di Mascio and co-workers irradiate their sample with 500 W for 3 h. They also maintain their sample in an environment that is very rich in oxygen. On the other hand, we excite our sample with only a 3 or 4 nm bandpass of very low intensity light in a fluorometer, and the spectrum is acquired in a few minutes. In addition, we obtain the *same* spectrum when the sample is deoxygenated (Figure 5).

Time-Resolved Experiments. Table 1 summarizes excited-state lifetime data for ATTO 590 as a function of tryptophan concentration and makes comparisons with the ATTO–Trp conjugate and ATTO–phalloidin (Figure 1b), a commonly used probe in biological studies.[6] The weights for the shortest and intermediate lifetimes of ATTO 590 increase with increasing tryptophan concentration. A Stern–Volmer plot was constructed from the data in Table 1 quantifying the quenching efficiency of tryptophan. A quenching constant of 0.03 ± 0.01 M^{-1} was obtained (Figure 6a). The ATTO–Trp conjugate and the ATTO–phalloidin are also included on this plot, for completeness, to provide an indication of the "effective concentration" of

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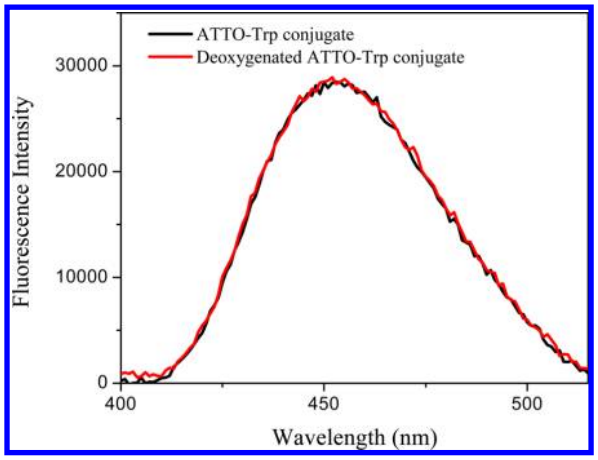

Figure 5. Fluorescence spectra of the ATTO 590−tryptophan conjugate in pH 7 buffer, λ_{ex} = 266 nm: (black) with ambient oxygen concentration; (red) after deoxygenation. Deoxygenation was carried out by bubbling Ar gas through the solution in an airtight cuvette for 30 min.

Table 1. Fluorescence Lifetimes of ATTO 590[a]

species	τ_1 (ns)	a_1	τ_2 (ns)	a_2	τ_3 (ns)	a_3	$\langle\tau\rangle$ (ns)
ATTO 590	3.7	1					
ATTO 590, 1.5 mM Trp	3.7	0.87	2.6	0.06	0.4	0.07	3.4
ATTO 590, 10 mM Trp	3.7	0.53	2.7	0.28	0.4	0.19	2.8
ATTO 590, 30 mM Trp	3.7	0.25	2.1	0.48	0.4	0.27	2.0
ATTO 590, 50 mM Trp	3.7	0.15	1.9	0.51	0.4	0.34	1.7
ATTO 590−Trp conjugate	3.7	0.08	1.0	0.53	0.4	0.39	1.0

[a]Emission was collected at λ_{em} > 550 nm. The ATTO 590 concentration was maintained at 1 μM. Lifetime values have a ±5% error. $\langle\tau\rangle$ is proportional to the fluorescence quantum yield and is given by $\langle\tau\rangle = \sum a_i\tau_i$.

tryptophan in the two complexes. (The same Stern−Volmer plot was constructed from the steady-state data. As the steady-state data are simply the time integrals of the time-resolved data and as the quenching constant obtained from the time-resolved data is obtained from the *average lifetime*, which is identical to the fluorescence quantum yield, it is not surprising that the steady-state Stern−Volmer plot yields exactly the same result (Figure 6b).)

Tables 2 and 3 present the lifetimes of the ATTO−50 mM Trp mixture and ATTO−Trp conjugate, respectively, as a function of temperature. In pH 7 buffer, uncomplexed ATTO has a single-exponential and *temperature independent* lifetime of 3.7 ns. Addition of tryptophan to the ATTO solution shortens the ATTO lifetime.

In order to simplify the analysis, it was convenient and instructive to use the temperature dependent lifetimes of the ATTO−50 mM Trp mixture as a model. The data were always well fit to the same three lifetime components with varying weights. Namely, there is always a 400 ps component in the presence of tryptophan; unquenched ATTO provides a 3.7 ns component, as stated above; and there is a component of intermediate duration, which is well described by 1.9 ns. These lifetime components are all well reproduced by the ATTO−Trp conjugate, which serves as a self-consistency check of the

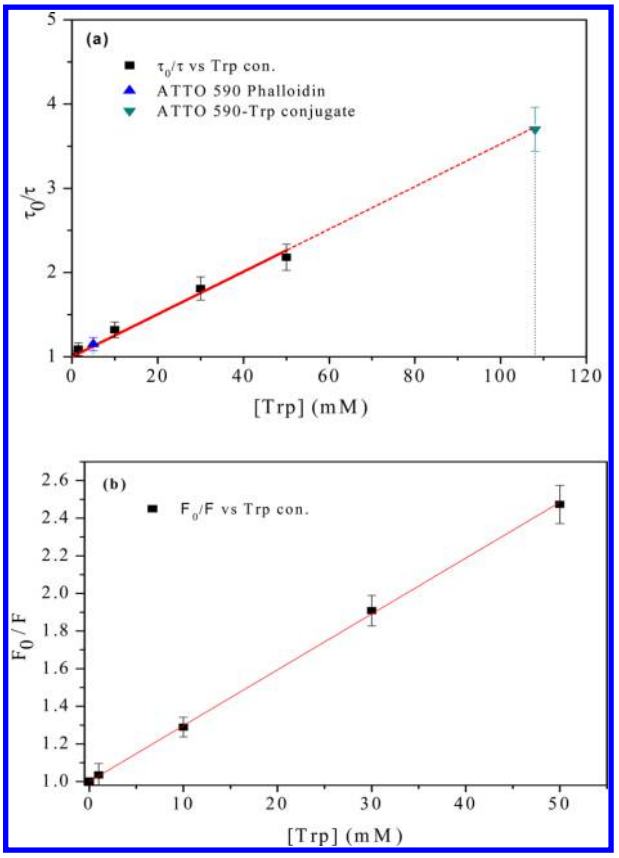

Figure 6. (a) Stern−Volmer plot of the average lifetime of ATTO 590 as a function of tryptophan concentration. The plot shows the "effective concentration" of tryptophan in the ATTO 590−Trp conjugate and in ATTO 590−phalloidin; i.e., these two species are positioned on the straight line obtained to construct the Stern−Volmer plot according to their average fluorescence lifetimes. (b) Stern−Volmer plot of the same species as in part a but based upon the steady-state data. Both methods yield the same quenching constant of 0.03 ± 0.01 M^{-1}.

Table 2. Temperature Dependence of ATTO 590 Lifetimes in the Presence of 50 mM Tryptophan

temp (°C)	τ_1 (ns)	a_1	τ_2 (ns)	a_2	τ_3 (ns)	a_3	$\langle\tau\rangle$ (ns)
5	3.7	0.23	1.9	0.44	0.4	0.33	1.8
22	3.7	0.15	1.9	0.51	0.4	0.34	1.7
45	3.7	0.08	1.9	0.6	0.4	0.32	1.6
65	3.7	0.04	1.9	0.66	0.4	0.30	1.5
85	3.7	0	1.9	0.69	0.4	0.31	1.4

Table 3. Temperature Dependence of ATTO 590−Trp Conjugate Lifetimes

temp (°C)	τ_1 (ns)	a_1	τ_2 (ns)	a_2	τ_3 (ns)	a_3	$\langle\tau\rangle$ (ns)
5	3.7	0.11	1.0	0.64	0.4	0.25	1.2
22	3.7	0.08	1.0	0.53	0.4	0.39	1.0
45	3.7	0.07	1.0	0.29	0.4	0.64	0.8
65	3.7	0.06	1.0	0.16	0.4	0.78	0.7
85	3.7	0.05	1.0	0.10	0.4	0.86	0.6

analysis, and only the intermediate lifetime component was 1.0 ns for conjugate. The transient data along with the absorption and excitation spectra referred to earlier are inconsistent with the claim[10] of the formation of a *nonfluorescent* ground-state complex, whose presence would be expected to generate a

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single-exponential fluorescence decay whose intensity, but not lifetime, decreased with increasing tryptophan concentration.

We interpret the weights of the lifetimes, a_1, a_2, and a_3, to be directly proportional to three types of ground-state configurations between ATTO and Trp: a_1, to configurations in which charge transfer does not occur at all; a_3, to a configuration in which charge transfer is as rapid as it can be (400 ps); and a_2, to intermediate configurations in which charge transfer is possible but is not as efficient as in a_3. Consequently, plots of $\ln(a_i/a_j)$ vs $1/T$ can be used to obtain the standard free energy differences among these three populations (Figure 7). Table 4 summarizes the values of standard thermodynamic quantities obtained from the fits.

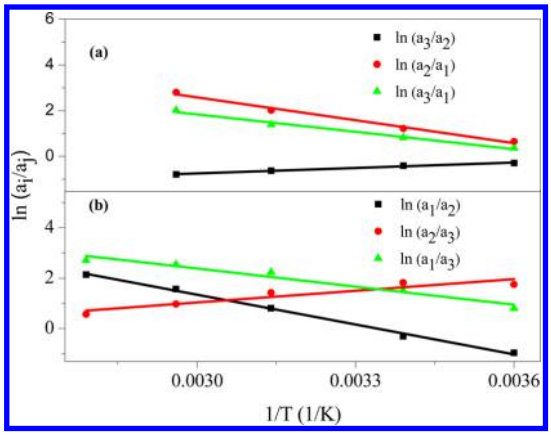

Figure 7. (a) Plot of the natural logarithm of the ratio of the amplitudes as a function of temperature in the ATTO 590−50 mM Trp mixture. (b) Plot of the natural logarithm of the ratio of the amplitudes as a function of temperature in the ATTO 590−Trp conjugate.

Table 4. ΔG^0 Obtained from the Fit to Plots of $\ln(a_i/a_j)$ vs $1/T$ at 298 K[a]

	ATTO−Trp Conjugate		
	ΔH^0 (kcal/mol)	ΔS^0 (kcal/(mol K))	ΔG^0 (kcal/mol)
$a_3 \leftrightarrow a_2$	8.0	0.027	0.006
$a_2 \leftrightarrow a_1$	−3.1	−0.007	−0.094
$a_3 \leftrightarrow a_1$	4.8	0.019	−0.090
	ATTO−50 mM Trp Mixture		
	ΔH^0 (kcal/mol)	ΔS^0 (kcal/(mol K))	ΔG^0 (kcal/mol)
$a_2 \leftrightarrow a_3$	−1.8	−0.007	0.029
$a_1 \leftrightarrow a_2$	7.6	0.028	−0.074
$a_1 \leftrightarrow a_3$	5.5	0.020	−0.049

[a] a_1 corresponds to the species with the shortest lifetime, which is fixed in the fitting procedure to 0.4 ns; a_2, to a species with an intermediate lifetime, which is fixed to 1.9 or 1.0 ns; and a_3, to the unquenched lifetime of ATTO, which was determined to be 3.7 ns (see Tables 1−3).

We performed lifetime measurement of the ATTO 590−Trp conjugate using λ_{ex} = 415 and 266 nm (Figure 8a). No excitation-wavelength dependence was observed when only fluorescence from ATTO 590 was monitored. However, when λ_{ex} = 266 nm and λ_{em} = 400−530 nm, a lifetime of 5.6 ns was observed (Figure 8b). (In this case, 20 000 counts were obtained in the peak channel for the lifetime owing to the lower fluorescence intensity.) A growth (or induction) time should be observed for the transient fluorescence of an exciplex, corresponding to its formation, but we only observe an exponential decay, indicating that the exciplex is formed too rapidly to be detected with

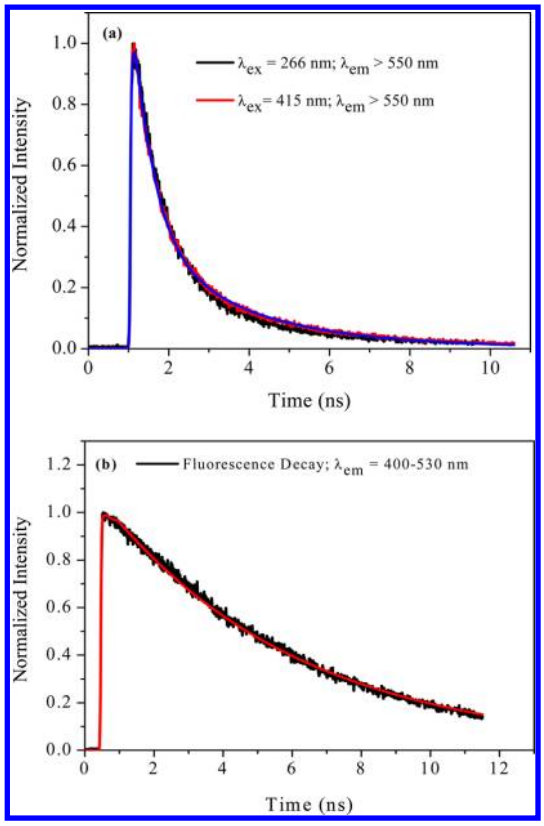

Figure 8. (a) Lifetime decay of the excited state of the conjugate. Emission was collected using a 550 nm long-pass filter to monitor only ATTO fluorescence. λ_{ex} = 266 nm (black); λ_{ex} = 415 nm (red), τ_1 = 3.7, a_1 = 0.08; τ_2 = 1.0, a_2 = 0.53; τ_3 = 0.4, a_3 = 0.39; $\langle\tau\rangle$ = 1.0 ns. Visual inspection of the two decay curves indicates that there is no significant excitation wavelength dependence when only ATTO fluorescence is considered. (b) Exciplex lifetime decay (black); fit to the decay, τ = 5.6 ns (red). λ_{ex} = 266 nm. The fluorescence was collected with a filter combination permitting transmission in the window from 400 to 530 nm. 20 000 were collected in the peak channel.

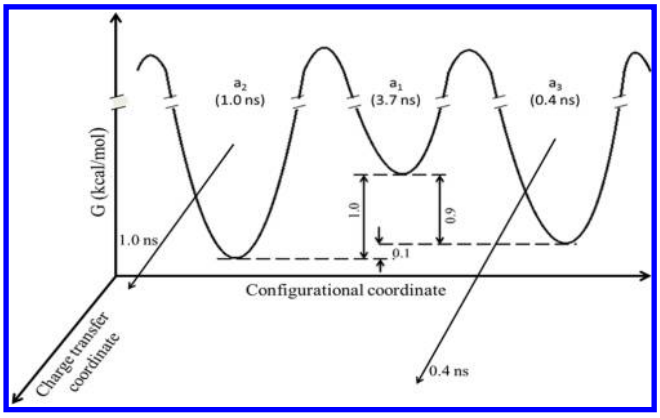

Figure 9. Schematic diagram for the excited-state potential energy surface of ATTO 590−Trp conjugate as a function of a configurational coordinate and a charge transfer coordinate. The three wells arise from the preexisting ground-state conformations (see the Supporting Information). We have categorized these conformations into three different groups, yielding lifetimes of 3.7 ns (a_1), 1.0 ns (a_2), and 0.4 ns (a_3), based upon the efficiency of charge transfer from ATTO to Trp in each conformation. The size of the arrows along the charge transfer coordinate qualitatively indicates the efficiency of charge transfer. The magnitudes of the barriers are unknown, and hence, the break marks are provided.

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our instrumentation. With 266 nm excitation, either tryptophan or ATTO 590 is excited. When tryptophan is excited, it forms an exciplex with ATTO 590, and when ATTO is excited, it follows the same decay path as obtained with 415 nm excitation.

Finally, as there is large overlap between the absorption spectrum of ATTO 590 and the fluorescence spectrum of tryptophan, there is also a possibility of fluorescence resonance energy transfer (FRET). However, with the time resolution of our instrument, we could not find any growth time in ATTO 590 fluorescence exciting at 266 nm.

The data from Tables 1−4 are summarized schematically by the potential energy surface given in Figure 9.

CONCLUSIONS

Stimulated by the work of Marmé et al., we monitored the quenching of ATTO 590 by tryptophan. Seeing no change in absorption spectra in the presence of tryptophan, we can conclude that there is not any ground state complex formation. We saw three-exponential decays in the presence of tryptophan. The fast component of the three-exponential decays is attributed to a conformation which forms the most effective charge-separated species among the other conformations. We found out that, when tryptophan is excited, it forms an exciplex in the presence of ATTO dyes and the lifetime of the exciplex is 5.6 ns; this is potentially responsible for quenching of tryptophan fluorescence by ATTO 590. This exciplex fluorescence should be taken into account for monitoring tryptophan fluorescence in the presence of tagging ATTO dyes. This information can also be utilized to detect the proximity of tryptophan to the ATTO labels in biological systems, though a detailed study would be required to conclude something quantitatively.

ASSOCIATED CONTENT

Supporting Information

Tables showing the coordinates and energy, Δenergy, and distance of the 5′ and 6′ isomers in different conformations and intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Lehmann, A. D.; Parak, W. J.; Zhang, F.; Ali, Z.; Roecker, C.; Nienhaus, G. U.; Gehr, P.; Rothen-Rutishauser, B. Fluorescent−Magnetic Hybrid Nanoparticles Induce a Dose-Dependent Increase in Proinflammatory Response in Lung Cells in Vitro Correlated with Intracellular Localization. *Small* **2010**, *6*, 753−762.

(2) Salerno, D.; Daban, J.-R. Comparative Study of Different Fluorescent Dyes for the Detection of Proteins on Membranes Using the Peroxyoxalate Chemiluminescent Reaction. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2003**, *793*, 75−81.

(3) Freeman, R.; Finder, T.; Gill, R.; Willner, I. Probing Protein Kinase (CK2) and Alkaline Phosphatase with CdSe/ZnS Quantum Dots. *Nano Lett.* **2010**, *10*, 2192−2196.

(4) Rittweger, E.; Rankin, B. R.; Westphal, V.; Hell Stefan, W. Fluorescence Depletion Mechanisms in Super-Resolving Sted Microscopy. *Chem. Phys. Lett.* **2007**, *442*, 483−487.

(5) Heilemann, M.; Van de Linde, S.; Mukherjee, A.; Sauer, M. Super-Resolution Imaging with Small Organic Fluorophores. *Angew. Chem., Int. Ed.* **2009**, *48*, 6903−6908.

(6) Lesoine, M. D.; Bose, S.; Petrich Jacob, W.; Smith, E. A. Supercontinuum Stimulated Emission Depletion Fluorescence Lifetime Imaging. *J. Phys. Chem. B* **2012**, *116*, 7821−7826.

(7) In *Biophotonics 2007: Optics in Life Science*, Proceedings of SPIE-The International Society for Optical Engineering; Popp, J., Bally, G. v., Eds.; 2007; pp 66331Z/1−66331Z/12.

(8) Ross, J.; Buschkamp, P.; Fetting, D.; Donnermeyer, A.; Roth, C. M.; Tinnefeld, P. Multicolor Single-Molecule Spectroscopy with Alternating Laser Excitation for the Investigation of Interactions and Dynamics. *J. Phys. Chem. B* **2007**, *111*, 321−326.

(9) Miller, A. E.; Fischer, A. J.; Laurence, T.; Hollars, C. W.; Saykally, R. J.; Lagarias, J. C.; Huser, T. Single-Molecule Dynamics of Phytochrome-Bound Fluorophores Probed by Fluorescence Correlation Spectroscopy. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 11136−11141.

(10) Marmé, N.; Knemeyer, J.-P.; Sauer, M.; Wolfrum, J. Inter- and Intramolecular Fluorescence Quenching of Organic Dyes by Tryptophan. *Bioconjugate Chem.* **2003**, *14*, 1133−1139.

(11) Zhu, R.; Li, X.; Zhao, X. S.; Yu, A. Photophysical Properties of Atto655 Dye in the Presence of Guanosine and Tryptophan in Aqueous Solution. *J. Phys. Chem. B* **2011**, *115*, 5001−5007.

(12) Sun, Q.; Lu, R.; Yu, A. Structural Heterogeneity in the Collision Complex between Organic Dyes and Tryptophan in Aqueous Solution. *J. Phys. Chem. B* **2012**, *116*, 660−666.

(13) Rivarola, C.; Bertolotti, S. G.; Borsarelli, C. D.; Cosa, J. J.; Previtali, C. M.; Neumann, M. G. Hydrogen Bonding and Charge Transfer Interactions in Exciplexes Formed by Excited Indole and Monosubstituted Benzenes in Cyclohexane. *Chem. Phys. Lett.* **1996**, *262*, 131−136.

(14) Borsarelli, C. D.; Bertolotti, S. G.; Previtali, C. M. Exciplex-Type Behavior and Partition of 3-Substituted Indole Derivatives in Reverse Micelles Made with Benzylhexadecyldimethylammonium Chloride, Water and Benzene. *Photochem. Photobiol.* **2001**, *73*, 97−104.

(15) Vekshin, N.; Vincent, M.; Gallay, J. Excited-State Lifetime Distributions of Tryptophan Fluorescence in Polar Solutions. Evidence for Solvent Exciplex Formation. *Chem. Phys. Lett.* **1992**, *199*, 459−464.

(16) Hershberger, M. V.; Lumry, R. W. The Photophysics of 5-Methoxyindole. A Non-Exciplex Forming Indole: Additional Evidence for Two Classes of Exciplexes. *Photochem. Photobiol.* **1976**, *23*, 391−397.

(17) Hershberger, M. V.; Lumry, R. W.; Verall, R. The 3-Methylindole/N-Butanol Exciplexes: Evidence for Two Exciplex Sites in Indole Compounds. *Photochem. Photobiol.* **1981**, *33*, 609−617.

(18) Chowdhury, P. K.; Halder, M.; Sanders, L.; Calhoun, T.; Anderson, J. L.; Armstrong, D. W.; Song, X.; Petrich, J. W. Dynamic Solvation in Room-Temperature Ionic Liquids. *J. Phys. Chem. B* **2004**, *108*, 10245−10255.

(19) Becke, A. D. Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A* **1988**, *38*, 3098−3100.

(20) Lee, C. T.; Ynag, W. T.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B* **1988**, *37*, 785−789.

(21) Doose, S.; Neuweiler, H.; Sauer, M. A Close Look at Fluorescence Quenching of Organic Dyes by Tryptophan. *ChemPhysChem* **2005**, *6*, 2277−2285.

(22) Ronsein, G. E.; Oliveira, M. C. B.; Miyamoto, S.; Medeiros, M. H. G.; Mascio, P. D. Tryptophan Oxidation by Singlet Molecular Oxygen [O_2 ($^1\Delta_g$)]: Mechanistic Studies Using ^{18}O-Labeled Hydroperoxides, Mass Spectrometry, and Light Emission Measurements. *Chem. Res. Toxicol.* **2008**, *21*, 1271−1283.