# Trifluoroethanol Quenches Indole Fluorescence by Excited-State Proton Transfer

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2,2,2-Trifluoroethanol (TFE) is a structure-promoting cosolvent for polypeptides in aqueous solution.[1] TFE induces α-helical or β-sheet structure in peptides and β-sheet to α-helix transitions in peptides and proteins.[2−4] TFE titration is used as a quantitative probe of helical propensity in small peptides.[5] Although the mechanism by which TFE stabilizes α-helices is not well understood, the weaker basicity of the solvent compared to water is thought to favor intramolecular hydrogen bonding in the peptide backbone.[3,6,7] The trifluoromethyl group confers special properties compared to nonfluorinated alcohols. The high electronegativity of the fluorine atom together with the large field effect of the trifluoromethyl group increases the acidity of TFE relative to ethanol, making the hydroxyl group a better proton donor.[8,9] McClelland and co-workers observed excited-state H−D exchange at aromatic carbons of dimethoxybenzenes in hexafluoro-2-propanol.[10] They also suggested that excited-state H−D exchange occurs at the C4 position of tryptophan in acidic TFE.[11]

The fluorescence properties of proteins are usually dominated by tryptophan residues, whose photophysics are sensitive to local environment.[12] The indole chromophore has multiple nonradiative pathways that quench the fluorescence, several of which are environmentally sensitive. Two isotopically sensitive temperature-dependent nonradiative processes are solvent quenching[13,14] and excited-state proton transfer reactions.[15−18] Excited-state H−D exchange at aromatic carbons on the indole ring occurs under neutral conditions in the presence of a good proton donor, such as ammonium. This paper demonstrates fluorescence quenching by excited-state proton transfer from TFE solvent and discusses the implications for fluorescence studies of protein conformation.

Figure 1 is a Stern−Volmer plot of TFE quenching of 3-methylindole fluorescence. The bimolecular quenching constant $k_q = 3 \times 10^7\ M^{-1}\ s^{-1}$ calculated from the linear portion of the curve (inset) is much slower than the diffusion-controlled rate. Table 1 gives fluorescence quantum yield $\phi$ and lifetime $\tau$ data for neat solvent and for 30% (vol/vol) TFE, which corresponds to 4.1 M. Both quantum yields and lifetimes of indole, 3-methylindole, and *N*-acetyl tryptophanamide (NATA) decrease in TFE compared to water by 2−18-fold. In contrast, the quantum yields of indole and 3-methylindole increase in ethanol (0.32 and 0.39). The absorption and emission spectra of indole and 3-methylindole in TFE are similar to the spectra in water or ethanol with emission maxima in the alcohols about 10 nm to the blue of the maximum in water. The broad structureless emission spectra indicate that emission is from the $L_a$ state. The radiative rates $k_r = \phi/\tau$ of indole and 3-methylindole increase slightly in TFE relative to water, as also

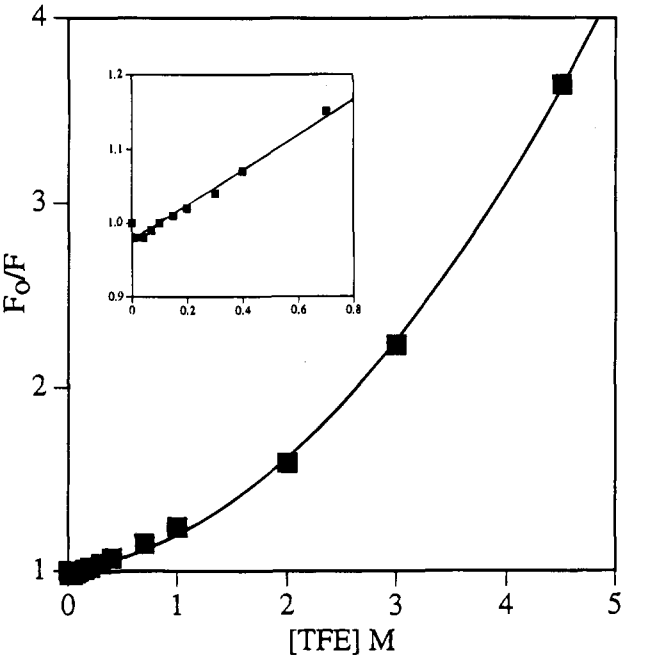


**Figure 1.** Stern−Volmer plot of TFE quenching of 3-methylindole fluorescence. $K_{SV} = k_q\tau_0 = 0.25\ M^{-1}$ is the slope of the linear portion shown in the inset. The subscript 0 denotes fluorescence intensity $F$ or lifetime in the absence of TFE.

**Table 1.** Deuterium Isotope Effects on Fluorescence Quantum Yield and Lifetime

| solvent | $\phi^a$ | $\tau$,[b] ns | $10^{-7}k_r$, $s^{-1}$ | $10^{-7}k_{nr}$, $s^{-1}$ | $E^*$, kcal | $A$, $s^{-1}$ |
|---|---|---|---|---|---|---|
| | | | Indole | | | |
| $H_2O$ | 0.23 | 4.0 | 5.9 | 19 | 12.5[c] | |
| $D_2O$ | 0.36 | 5.8 | 6.2 | 11 | 12.4[c] | |
| $CF_3CH_2OH$ | 0.033 | 0.45 | 7.3 | 210 | 1.3 | $18 \times 10^9$ |
| $CF_3CH_2OD$ | 0.12 | 1.45 | 8.3 | 62 | 1.3 | $4.7 \times 10^9$ |
| 3:7 $CF_3CH_2OH/H_2O$ | 0.18 | | | | | |
| 3:7 $CF_3CH_2OD/D_2O$ | 0.35 | | | | | |
| | | | 3-Methylindole | | | |
| $H_2O$[d] | 0.34 | 8.2 | 4.2 | 8.2 | 12.7 | $7.8 \times 10^{16}$ |
| $D_2O$ | 0.50 | 12.0 | 4.2 | 4.2 | 11.6 | |
| $CF_3CH_2OH$ | 0.022 | 0.45 | 4.9 | 220 | 1.4 | $23 \times 10^9$ |
| $CF_3CH_2OD$ | 0.068 | 1.40 | 4.9 | 71 | 1.4 | $5.6 \times 10^9$ |
| 3:7 $CF_3CH_2OH/H_2O$ | 0.13 | | | | | |
| 3:7 $CF_3CH_2OD/D_2O$ | 0.31 | | | | | |
| | | | NATA | | | |
| $H_2O$ | 0.15 | 2.6 | 5.8 | 29 | 6.6[c] | |
| $D_2O$ | 0.18 | 3.3 | 5.5 | 25 | 5.8[c] | |
| $CF_3CH_2OH$ | 0.029 | 0.65 | 4.5 | 150 | | |
| $CF_3CH_2OD$ | 0.082 | 1.7 | 4.8 | 54 | | |

[a] 280 nm excitation wavelength, 10% error. [b] 288 nm excitation wavelength, 5% error. [c] Reference 28. [d] References 18 and 29.

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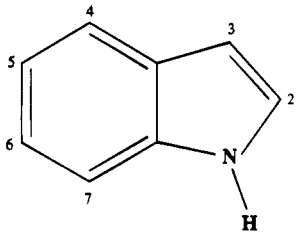
**Table 2.** Photochemical H−D Exchange in TFE-$d_3$

| irradn time, h | exchange, % C2 | C3 | C4 | C7 |
|---|---|---|---|---|
| | Indole[a] | | | |
| 1.5 | 5.0 | 32 | 4.9 | 7.2 |
| 2.5 | 14 | 34 | 7.9 | 8.0 |
| | 3-Methylindole[b] | | | |
| 1.5 | 5.7 | | 17 | 16 |
| 2.5 | 7.8 | | 27 | 27 |

[a] Ground-state H−D exchange was subtracted.[30] At 250 MHz proton resonances for C2, C5, and C6 overlap. Exchange at C2 calculated assuming no exchange at C5 and C6. [b] No ground-state exchange was detected.

observed in methanol;[19] the radiative rates of NATA decrease slightly. The nonradiative rates $k_{nr} = \tau^{-1} - k_r$ of all three compounds increase dramatically with about twice as large a deuterium isotope effect in TFE as in water. This means that TFE introduces another deactivation process. For 3-methylindole, the ratio of $k_{nr}$ in protium and deuterium solvents is 2.0 in water and 3.1 in TFE. The value for TFE is in the range for primary isotope effects indicative of proton transfer reactions.[20] The temperature dependence of the nonradiative rates of indole and 3-methylindole in TFE also differs from that in water. The activation energies $E^*$ are about 10-fold less for TFE than water; the 1 kcal/mol activation energies are reminiscent of the zero value reported for methanol.[19] The frequency factors $A$ are $10^7$-fold less for TFE than water. The about 4-fold isotope effect on the frequency factors in TFE is larger than the 2−3-fold isotope effect observed for solvent quenching.[13,14]




Excited-state proton transfer from TFE was demonstrated by photochemical H−D exchange experiments. Indole and 3-methylindole in TFE-$d_3$ were irradiated under conditions similar to those used before.[18] H−D exchange was monitored on a Bruker AC-250 NMR at ambient temperature. Percentage of exchange was calculated from integrated resonances relative to freshly prepared unirradiated sample. H−D exchange occurred at all aromatic carbon hydrogen positions except C5 and C6 (Table 2). In 3-methylindole the exchange appears to be faster at C4 and C7 than at C2. This differs from the pattern of reactivity for glycine-induced H−D exchange in 50% methanol, where the exchange occurs at similar rates at all three carbons.[18] The photochemical yield for isotope exchange $\Phi_R = 0.5 \pm 0.1$ was determined for 3-methylindole by ferrioxalate actinometry.[21,22] The isotope exchange reaction is proposed to occur in two steps: electrophilic attack of ring carbons by deuterons followed by loss of a proton or a deuteron from the tetrahedral intermediate.[23] The yield for proton transfer $\Phi_P = 0.7$ from TFE-$d_3$ is estimated from $\Phi_R = [k_H/(k_H + k_D)]\Phi_P$, where $k_H$ and $k_D$ are the rate constants for proton and deuteron loss with $k_H/k_D = 2$. Therefore, the apparent proton transfer rate $k_P' = \Phi_P/\tau$ for 3-methylindole in TFE-$d_3$ is about $5 \times 10^8$ s$^{-1}$, which accounts for most of the nonradiative rate $k_{nr}$. This pseudo-first-order rate constant can be converted to a bimolecular rate constant. Assuming that intersystem crossing is the only other nonradiative process and that intersystem crossing rates are about the same in TFE and TFE-$d_3$, $k_P'$ in TFE would be about $2 \times 10^9$ s$^{-1}$.

The hydrogen donation ability of a bulk solvent is measured on the α scale, where a higher α value signifies a better proton donor. The α value is 1.51 for TFE, 1.12 for water, and 0.85 for ethanol.[24] We found a proton transfer yield of 0.7 for 3-methylindole in TFE-$d_3$. Previous attempts failed to detect excited-state H−D exchange in simple indoles in water or 30% methanol.[18] Thus, the excited-state proton transfer reaction occurring in TFE is due to the trifluoromethyl group, which makes the solvent a better hydrogen donor. In 30% TFE, the quantum yields of indole and 3-methylindole are also decreased compared to water with a large deuterium isotope effect (Table 1). The p$K_a$ of TFE in water is 12.37 compared to a p$K_a$ of 16 for ethanol.[25] One trifluoromethyl group usually increases the acidity of a neighboring hydroxyl by 3−4 units. Glycine and other good proton donors also quench indole fluorescence by excited-state proton transfer. Trichloroethanol (TCE) is a highly efficient quencher of indole fluorescence.[26] The bimolecular quenching constant for TCE quenching of 3-methylindole fluorescence is $k_q = 5.4 \times 10^9$ M$^{-1}$ s$^{-1}$. The quantum yields of 3-methylindole in 1:20 $CCl_3CH_2OH/H_2O$ and 1:20 $CCl_3CH_2OD/D_2O$ are 0.0068 and 0.0084, corresponding to nonradiative rates of $6.0 \times 10^9$ and $4.9 \times 10^9$ s$^{-1}$. The isotope effect on $k_{nr}$ is only 1.2, much smaller than in TFE. The p$K_a$ of TCE in water is 12.24, only slightly different from that for TFE.[25] The proton transfer rates in TCE and TFE are expected to be similar on the basis of hydrogen donation ability. Clearly the major contribution to the quenching by TCE is not proton transfer, but may be electron transfer as proposed by Evans et al.[27]

In summary, we have shown that TFE quenches indole fluorescence in neat solvent and aqueous mixtures by excited-state proton transfer. This finding has implications for studies of peptides and proteins. First, TFE can be used to probe solvent accessibility of tryptophan residues. Although alcohols usually denature proteins, some proteins retain their native structure in the presence of moderate concentrations of alcohol. Tryptophans in contact with solvent will undergo H−D exchange, which can be monitored by NMR or mass spectrometry. Second, changes in intrinsic tryptophan fluorescence induced by TFE should be interpreted cautiously. In a TFE titration, increasing the TFE concentration will progressively quench the fluorescence of accessible tryptophans in the absence of a protein conformation change. Dissecting changes in excited-state proton transfer rates from changes in solvent accessibility may be tricky.

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