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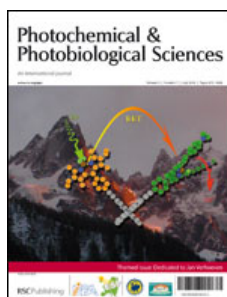


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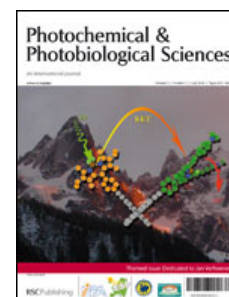


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On the origin of fluorescence quenching of pyridylindoles by hydroxylic solvents†

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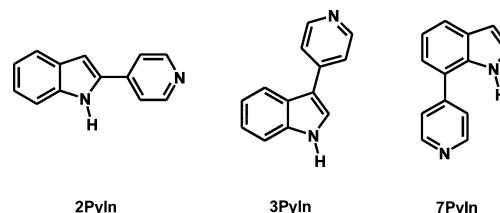
Three isomeric 4'-pyridyl-substituted indoles, with the substituent in positions 2, 3 and 7, reveal strong fluorescence in aprotic solvents, both polar and nonpolar, whereas the emission is strongly quenched in water and alcohol solutions. Both viscosity and alcohol acidity play a role in efficient excited state deactivation. The process becomes faster for more acidic alcohols. It can be slowed down by increasing viscosity, which indicates that the proton movement is accompanied by large amplitude motions in the hydrogen-bonded complex. Quenching is not observed upon formation of solvates in which pyridylindoles act as hydrogen bond donors. The experimental results, combined with calculations can be explained by a model which assumes excited state protonation of the pyridine nitrogen atom, followed by twisting of the pyridyl group, leading to a low-energy structure. An alternative mechanism is also considered, in which the excited state proton transfer is accompanied by electron transfer from water or alcohol into a half-filled orbital of the chromophore, which leads to a conical intersection of the S_1 and S_0 energy surfaces.

1. Introduction

It is well established that the photophysical properties of a molecule can drastically change upon formation of an intermolecular hydrogen bond.^{1–21} In many cases, excited state intermolecular proton transfer has been reported, manifested by the appearance of the emission occurring from the phototautomer. Quite often, no excited state reaction is detected, but the fluorescence yield decreases significantly, indicating the presence of a fast non-radiative channel activated by hydrogen bonding. Our studies of numerous bifunctional azaromatic molecules which contain both a hydrogen bond donor and an acceptor group^{3,22–38} have demonstrated that it is possible to alternately induce these two types of photophysics in the same chromophore. Depending on the structure and stoichiometry of the hydrogen bonding formed with protic partners, the same molecule can reveal either phototautomerization or enhanced S_1 – S_0 internal conversion. The former is observed in cyclic 1:1 solvates, whereas the latter has been attributed to complexes involving two alcohol or water molecules, also bound to an excited chromophore in a cyclic fashion. The origin of the quenching was not established unequivocally. An “aborted” multiple proton transfer³⁹ in excited 1:2 species has been postulated.³ Strong dependence of the quenching of the initial excited state on viscosity²⁴ suggested that this process

is accompanied by solvent rearrangement around the excited molecule.

In this work, we probe the origin of the quenching by studying a series of molecules especially designed for this purpose. The chromophores under study are three pyridyl-substituted indoles: 2-(4'-pyridyl)indole (**2PyIn**), 3-(4'-pyridyl)indole (**3PyIn**), and 7-(4'-pyridyl)indole (**7PyIn**) (Scheme 1).



Scheme 1 2-(4'-Pyridyl)indole (**2PyIn**), 3-(4'-pyridyl)indole (**3PyIn**), and 7-(4'-pyridyl)indole (**7PyIn**).

The topology of these molecules excludes the possibility of forming cyclic 1:1 solvates with water or alcohol, and, therefore, of excited state proton transfer *via* a solvent bridge. The probability of forming cyclic 1:2 species should also be reduced. Finally, while their isomers can exist in two rotameric forms, *syn* and *anti*, it is not the case for the pyridylindoles selected for the present study. This is illustrated in Fig. 1, which shows different modes of specific solvation of three isomeric 2-(pyridyl)indoles by water. It is evident that for **2PyIn**, the proton and acceptor centers form independent hydrogen bonds. Thus, by studying the three (4'-pyridyl)indoles, we check whether the quenching can occur when no solvent bridges exist between the proton donor, NH group, and the acceptor, pyridine nitrogen. The results show that the quenching still occurs, albeit less efficiently than in the case of cyclic 1:2 solvates. Both experiment and calculations demonstrate

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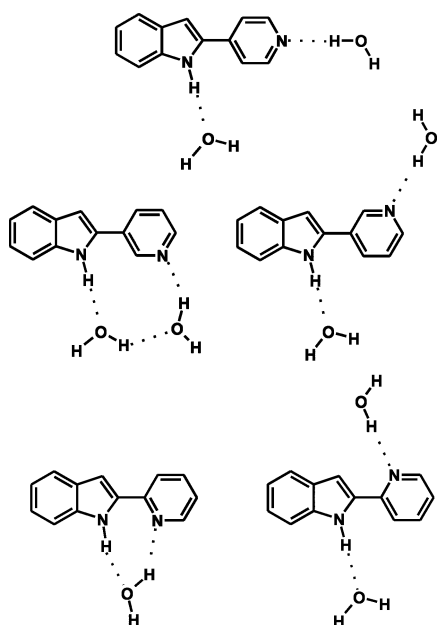
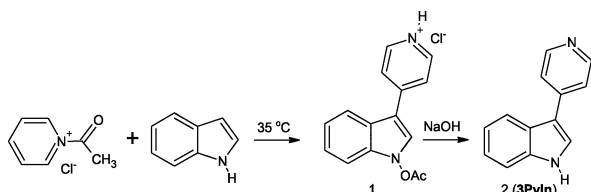


Fig. 1 Top: hydrogen-bonded 2-(4'-pyridyl)indole (**2PyIn**); middle: cyclic and non cyclic 1 : 2 solvates of *syn* and *anti* forms of 2-(3'-pyridyl)indole; bottom: 1 : 1 and 1 : 2 complexes of *syn* and *anti* 2-(2'-pyridyl)indole, respectively.

that the origin of the fluorescence quenching involves a proton shift along the hydrogen bond formed by the pyridine nitrogen as an acceptor and the external water or alcohol molecule as the donor. However, efficient deactivation requires more than one water or alcohol molecule, because no quenching is observed for a complex of 1 : 1 stoichiometry with a strong hydrogen bond donor, 1,1,1,3,3,3-hexafluoro-2-propanol, when the complex is embedded in a nonpolar environment. We propose two alternative models which can account for the solvent-dependent photophysics of pyridylindoles.

2. Experimental and computational details

2PyIn⁴⁰ and **7PyIn**⁴¹ were obtained using previously described procedures. **3PyIn** was prepared according to the method of Bergman⁴² (Scheme 2).



Scheme 2 Synthesis of 3-(4'-pyridyl)indole (**3PyIn**).

4-(*N*-Acetyl-3-indolyl)-pyridinium chloride (**1**)

Pyridine (15.82 g, 0.2 mol) was added at 20–25 °C to acetyl chloride (15.70 g, 0.2 mol) in EtOAc (100 mL). A white solid, *N*-acetylpyridinium chloride, was formed immediately. Indole (11.70 g, 0.1 mol) in EtOAc (20 mL) was added to this mixture. The temperature was maintained at 35 °C for 2 h, after which the mixture was cooled to 10–15 °C. Filtration provided a slightly

yellow solid (**1**, 5.50 g, 20%), which was recrystallized from MeOH–EtOH (1 : 3): mp 276–278 °C (lit.⁴² mp 212–217 °C).

3-(4'-Pyridyl)-indole (**2**, **3PyIn**)

A solution of 4-(*N*-acetyl-3-indolyl)-pyridinium chloride (**1**, 5.30 g, 19.4 mmol) in aqueous 2 M NaOH (50 mL) and EtOH (50 mL) was refluxed for 2 h. After cooling, H₂O (200 mL) was added to provide off-white crystals of **2** (3.15 g, 84%): mp 216–218 °C (lit.⁴² mp 218–219 °C); ¹H NMR (CDCl₃) 8.65 (bs, 1H, NH), 8.62 (d, 2H, *J* = 2.7 Hz, H2' and H6'), 8.00 (d, 1H, *J* = 7.5 Hz, H4), 7.61 (d, 2H, *J* = 4.8 Hz, H3' and H5'), 7.57 (d, 1H, 2.7 Hz, H2), 7.47 (d, 1H, *J* = 7.2 Hz, H7), 7.33–7.23 (m, overlapping, 2H, H6 and H5).

Electronic absorption spectra were recorded on a Shimadzu UV 3100 spectrophotometer equipped with variable-temperature chambers. Stationary fluorescence spectra were obtained using an Edinburgh FS 900 CDT spectrofluorometer. Low-temperature quantum yield measurements were performed on a Jasný spectrofluorometer.⁴³ The solvents used for our studies: n-hexane (HEX), dibutyl ether (BE), diethyl ether (EE), butyl acetate (BA), ethyl acetate (EA), dichloromethane (DCM), 1,2-dichloroethane (DCE), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), acetonitrile (ACN), methanol, ethanol, 1-propanol, 1-butanol, and 1,1,1,3,3,3-hexafluoro-2-propanol were of spectroscopic or fluorescence grade (Aldrich or Merck). Butyronitrile (BN, Merck, for synthesis) was distilled successively over KMnO₄ + K₂CO₃, P₂O₅ and CaH₂. Water was used after four distillations over KMnO₄ in a quartz apparatus. Perchloric acid (Merck, p.a.) was used to obtain cations. All solvents were checked for the presence of fluorescing impurities. The solvents have been selected to cover a wide range of the static permittivity values ϵ (HEX, BE, EE, BA, EA, DCM, DCE, THF, BN, DMSO, ACN),⁴⁴ as well as that of the index α describing the solvent hydrogen bond donor ability of bulk water and alcohols.⁴⁵

For the determination of quantum yields, quinine sulfate in 0.1 N H₂SO₄ was used as a standard ($\phi = 0.51$).⁴⁶

Fluorescence decay lifetimes were measured using time-resolved single photon counting on an Edinburgh FL 900 CDT spectrofluorometer.

Ground state geometries were optimized using density functional theory (DFT), with the B3LYP hybrid functional and 6-31+G(d,p) or TZVP basis sets. Because of well-known problems of time-dependent DFT (TDDFT) calculations with reproducing the excitation energies of charge transfer states,^{47,48} calculations of the electronic transition energies and the excited state geometry optimizations were carried out using both TDDFT and configuration interaction singles (CIS) procedures. For all the stationary points, frequencies have been calculated to check if the geometry corresponds to the energy minimum. Coordinate-driven minimum energy path approach or Synchronous Transit-Guided Quasi-Newton (STQN) method implemented in Gaussian were used to obtain the reaction paths. All calculations were performed using Gaussian 03 and TURBOMOLE 5.7 suites of programs.

3. Results and discussion

Fig. 2–4 show room temperature absorption and fluorescence spectra recorded for three different solvents: nonpolar n-hexane,

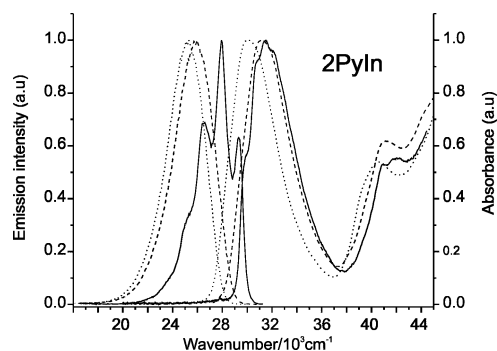


Fig. 2 Normalized room temperature absorption and fluorescence spectra of **2PyIn** in n-hexane, acetonitrile, and 1-propanol (solid, dashed and dotted lines, respectively).

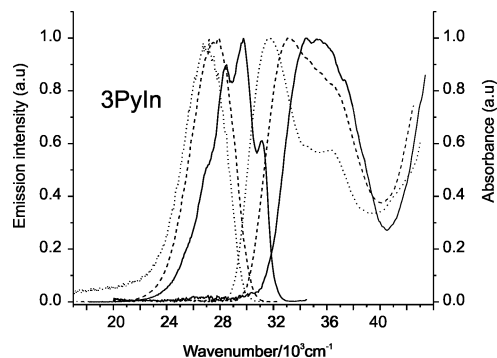


Fig. 3 Normalized room temperature absorption and fluorescence spectra of **3PyIn** in n-hexane, acetonitrile, and 1-propanol (solid, dashed and dotted lines, respectively).

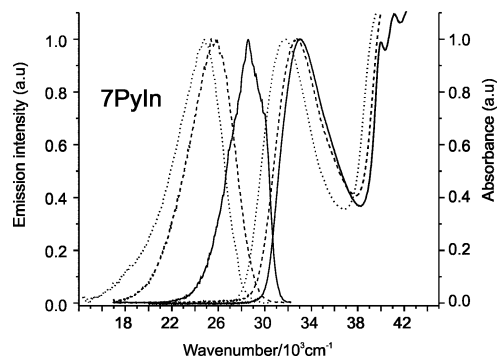


Fig. 4 Normalized room temperature absorption and fluorescence spectra of **7PyIn** in n-hexane, acetonitrile, and 1-propanol (solid, dashed and dotted lines, respectively).

polar aprotic acetonitrile, and protic 1-propanol. Both absorption and emission shift to the red with increasing polarity. Moreover, a red shift is observed upon passing from acetonitrile to a less polar 1-propanol, indicating that specific hydrogen-bonding interactions stabilize the excited state to a larger degree than the ground state. Formation of ground state complexes with alcohol is illustrated in Fig. 5 by the results of titration of hexane solution of pyridylindoles with small amounts of 1-butanol. In contrast to our previous studies of 2-(2'-pyridyl)indole and 2-(3'-pyridyl)indole,^{24,27} where the 1 : 1 or 1 : 2 stoichiometries could readily be determined, the spectral changes accompanying titrations are quite complicated. This is manifested by poorly-defined isosbestic points, present only

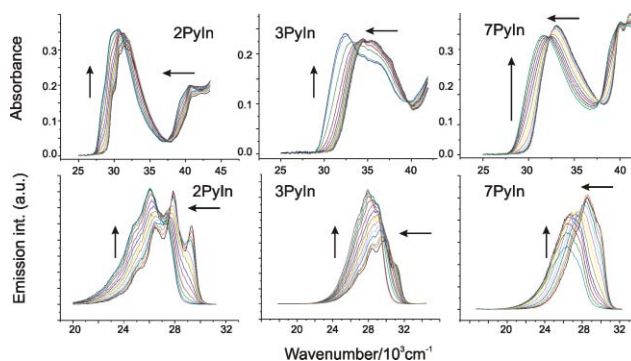


Fig. 5 Changes in absorption and emission observed upon titrating n-hexane solutions of **2PyIn**, **3PyIn**, and **7PyIn** with 1-butanol. The arrows show the spectral evolution with increasing 1-butanol concentration, from 0 to 0.35 M. Because of the lack of clear isosbestic points, fluorescence intensities reflect changes in quantum yield only in an approximate fashion.

in a small concentration range, if at all, and by continuous red shifts and intensity changes in both absorption and emission. This behaviour suggests that various types of complexes are formed, with one or more molecules of alcohol attached either to the NH group or to the pyridine nitrogen atom.

The pattern of relative absorption intensities for the S_0 – S_1 transition in the three compounds is reproduced by calculations and correlates with the twisting of the pyridyl ring with respect to the indole plane. The angles predicted using B3LYP/6-31+G(d,p) are 21, 35 and 46 degrees for **2PyIn**, **3PyIn**, and **7PyIn**, respectively, while the corresponding oscillator strengths are 0.34, 0.24, and 0.13. These results suggest a charge transfer character of the lowest excited singlet state. However, this correlation may be fortuitous, since the calculations performed for the three molecules with the same fixed twisting angle, yield the same ordering of intensities. Still, the calculations predict that the dominant configuration in the S_1 state involves promotion of an electron from an orbital localized on the indole moiety into an orbital localized on the pyridyl unit. Such a charge shift should lead to a simultaneous enhancement of the acidity of the NH group and of the basicity of the pyridyl nitrogen. This behavior is experimentally demonstrated by the changes of pK_a in S_1 with respect to S_0 . For **2PyIn**, a value of $\Delta pK_a = 12.1$ has been reported for the pyridyl nitrogen.²⁷ For **3PyIn**, we obtain $\Delta pK_a = 9.4$. The corresponding value estimated for **7PyIn** is 10.6–11.3; for this compound, a large change of pK_a , –14.0, also has been determined with respect to the deprotonation of the NH group in S_1 .²⁹

The shift of electronic charge from the indole onto the pyridine moiety caused by electronic excitation should also result in the increase of the dipole moment in S_1 . The B3LYP/6-31+G(d,p) calculated ground state values are 2.1, 4.7, and 2.1 D for **2PyIn**, **3PyIn**, and **7PyIn**, respectively. The corresponding values for the excited state (TD-B3LYP/TZVP), calculated for the ground state geometry are 9.0, 11.0, and 10.9 D. Because of the tendency of TDDFT to overestimate the dipole moments of charge transfer states, these values probably provide the upper limits. The solvatochromic plot obtained from the absorption of **3PyIn** in aprotic solvents of varying polarity yielded a value of 8.6 D, assuming the Onsager radius of 3.77 Å.

Table 1 presents the photophysical data obtained for different solvents. Qualitatively similar trends are observed for all three

Table 1 Photophysical characteristics of pyridylindoles measured at 293 K and at selected lower temperatures

Solvent	Φ_a^a			τ_n/ns^b			$k_r^c/10^8 \text{ s}^{-1}$			$k_{nr}/10^8 \text{ s}^{-1}$		
	2PyIn	3PyIn	7PyIn	2PyIn	3PyIn	7PyIn	2PyIn	3PyIn	7PyIn	2PyIn	3PyIn	7PyIn
n-Hexane	0.83	0.23	0.44	1.6	0.5	1.2	5.2 (2.7)	4.6 (2.4)	3.7 (1.9)	1.1	15.4	4.7
Acetonitrile	0.88	0.75	0.55	3.0	2.2	5.4	2.9 (1.6)	3.4 (1.9)	1.0 (0.6)	0.4	1.1	0.8
1-Butanol	0.7	0.2	0.1	2.7	0.8	0.8	2.6 (1.3)	2.5 (1.2)	1.3 (0.6)	1.1	10.0	11.3
1-Propanol	0.73	0.12, 0.80 ^e	0.046	2.4	2.0 ^e	0.6	3.0 (1.6)	3.6 ^e (1.9)	0.8 (0.4)	1.1	27.1	15.9
Ethanol	0.41	0.05, 0.81 ^e	0.018	1.6	1.7 ^e	0.2	2.6 (1.3)	4.8 ^e (2.5)	0.9 (0.5)	3.7	91.2	49.1
Methanol	0.03, 0.19 ^d	0.013, 0.07 ^d	0.0034		0.2 ^d	0.03 ^f	4.4 ^d (2.3)	3.5 ^d (1.8)	1.1 (0.6)	142.3	258.1	332.2
Water	0.003	0.009	0.0027									
DMSO	0.83	0.88	0.67	3	2.3	9.6	2.8 (1.3)	3.8 (1.7)	0.7 (0.3)	0.6	0.5	0.3

^a Accuracy: $\pm 20\%$. ^b Estimated error: $\pm 0.1 \text{ ns}$. ^c The values in parentheses are corrected for the refractive index of the solvent. ^d Measured at 193 K. ^e Measured at 173 K. ^f Obtained using time-resolved picosecond single photon counting technique.⁵⁶

compounds. In aprotic solvents, both nonpolar and polar, the fluorescence quantum yields are quite high. One observes, however, a distinct decrease of the radiative rate constant in polar solvents with respect to the values obtained for nonpolar n-hexane. The effect, which is most pronounced for **7PyIn**, suggests twisting of the pyridyl unit with respect to the indole moiety in the excited state. Calculations of transition intensities as a function of α , the angle between the planes of pyridine and indole units reveal a decrease of the oscillator strength, practically to zero for $\alpha = 90^\circ$. The approximate $\cos^2 \alpha$ intensity dependence indicates that, for the same change in the twisting angle, the largest change in the radiative constant should be obtained for the chromophore for which the twisting was already substantial in S_0 . This prediction is corroborated experimentally: **7PyIn**, which reveals the largest decrease of the radiative constant upon passing from a nonpolar to a polar solvent is predicted, as discussed above, to be the most twisted of the three isomers in the ground state.

The calculations suggest that, depending on the environment, both twisting and flattening are possible in S_1 . The excited state geometry optimizations of isolated molecules indicate that pyridylindoles become more planar upon excitation; the angles of 0 (6), 0 (11), and 22 (32) degrees are obtained using CIS/6-31+G(d,p) (TD-B3LYP/TZVP) for **2PyIn**, **3PyIn**, and **7PyIn**, respectively. However, for **2PyIn** and **3PyIn**, the calculated 90° twisted structure (TD-B3LYP/TZVP) also corresponds to a minimum, with a larger dipole moment than that of the initially excited species.

Another observation, which again supports the hypothesis of twisting in the excited state, is that the values of the radiative rate constants become larger at lower temperatures in glassy solvents (cf. the data for **2PyIn** and **3PyIn** in Table 1). This can be explained by a hindrance to twisting caused by increasing solvent viscosity.

A more detailed analysis of excited state twisting and/or flattening will be discussed in a separate paper. The most relevant for this work is the effect observed for all three chromophores in protic solvents: alcohols and water. While the radiative rate constants are essentially the same as for aprotic solvents of similar polarity, an efficient nonradiative deactivation channel is switched on in hydrogen-bonding environments. Interestingly, only solvents acting as hydrogen bond donors cause this effect; no enhanced deactivation is observed for DMSO, a hydrogen bond acceptor.

The degree of fluorescence quenching by alcohols and water is different for each of the three isomers. The strongest effect is

observed for **7PyIn**, which is quenched in all alcohols and water. The weakest quenching occurs for **2PyIn**, for which the effect is observed only for methanol and water, but not for 1-butanol or 1-propanol.

In principle, the quenching abilities of the solvent can depend on several parameters, such as polarity, viscosity, acidity, and basicity. The latter does not seem to be important. This is demonstrated, on one hand, by the lack of quenching in DMSO and the observation that the more basic alcohols, 1-propanol and 1-butanol, are much weaker quenchers than less basic methanol, ethanol, and water. Also, because the polarity is similar for all the alcohols studied, this parameter is not responsible for variations in the quenching ability of different alcohols. The two crucial factors responsible for quenching are the solvent acidity and viscosity. The importance of the latter is revealed by the finding that no quenching is observed when the solvent becomes rigid, i.e. in alcohol glasses. Apparently, quenching is accompanied by large amplitude solute/solvent reorganization. On the other hand, viscosity is not the only parameter which determines the quenching rate. At room temperature, fluorescence quantum yields in water are lower than in methanol. The latter is less viscous, but also less acidic than water.

An observation relevant to the quenching mechanism was provided by experiments in which n-hexane solutions were titrated with small amounts of 1,1,1,3,3,3-hexafluoro-2-propanol, the most acidic hydrogen bonding donor of all used in this work and an extremely weak base (Fig. 6). At very small alcohol

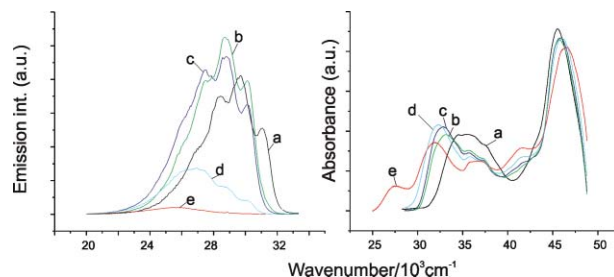


Fig. 6 Evolution of absorption and emission observed upon adding 1,1,1,3,3,3-hexafluoro-2-propanol to n-hexane solutions of **3PyIn** (a, no alcohol, b–e, alcohol concentrations: 7.9×10^{-4} , 7.1×10^{-3} , 7.9×10^{-2} , 0.31 M, respectively). The emission intensities reflect the relative fluorescence quantum yields.

concentrations, an isosbestic point and an increase in the emission intensity was observed (curves a–c). Only at higher alcohol concentrations was fluorescence quenching observed. Under these conditions, no clear isosbestic point was detected, indicating the formation of complexes of higher stoichiometry (curves d, e). Finally, absorption of the cation appeared (lowest energy band in curve e). The cation is practically nonfluorescent. Apparently, the species arising along the quenching path has to be stabilized by additional hydrogen bonding and/or a polar environment.

All the above observations suggest a model for the quenching involving the intermolecular $\text{OH}\cdots\text{N}$ hydrogen bond (Fig. 7). As a result of electron density flow onto the pyridine ring upon excitation, the hydrogen bond becomes much stronger in S_1 . The increase in basicity can enable proton transfer from water or alcohol to the pyridine nitrogen atom. Both DFT and CIS excited state geometry optimizations yield a 90° twisted geometry in S_1 for the protonated, cationic form.

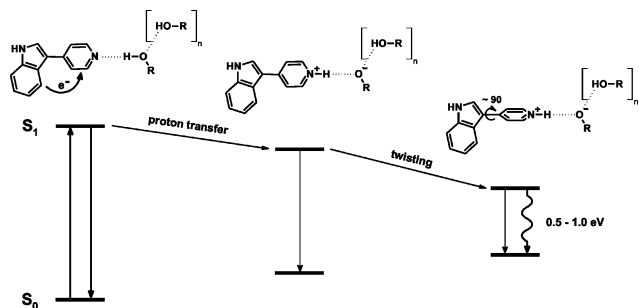


Fig. 7 Sequence of excited state processes leading to fluorescence quenching in a hydroxylic solvent.

Very low transition energies of $\text{S}_0\text{--}\text{S}_1$, of the order of 0.5–1 eV, are predicted by DFT and CIS models for the twisted cationic structures. Thus, a twisted, excited monocationic form is a good candidate for the intermediate responsible for fluorescence quenching, explaining the observed sensitivity of the process to viscosity and lack of quenching in a rigid medium.

The experiments performed in water or alcohols, acidified to ensure that only monocations were present in the ground state, revealed extremely weak fluorescence, located to the red from the emission of a neutral molecule. The spectral positions were in good agreement with the theoretical predictions of the transition energies expected for the ground state cation geometries, but not for the twisted minima in the excited state. This observation may indicate the existence of a low barrier for the excited state twisting of the cation. The barrier may also exist for the proton transfer, the initial process leading to deactivation; this would explain the dependence of quenching on the alcohol acidity.

The calculations have suggested another possible mechanism for the excited state deactivation path in complexes with water. It also includes proton/hydrogen movement, but does not require twisting of the chromophore, which makes it more general (*vide infra*) (Fig. 8). In this model, an $\text{n}\pi^*$ state is engaged, with an electron promoted from a lone-pair orbital of water to an orbital localized on the pyridine moiety. In the initially excited structure, such a state lies high in energy, about 1 eV above $\text{S}_1(\pi\pi^*)$. However, in contrast to S_0 and $\text{S}_1(\pi\pi^*)$, minimum energy path optimizations reveal a repulsive character for the potential of this state along

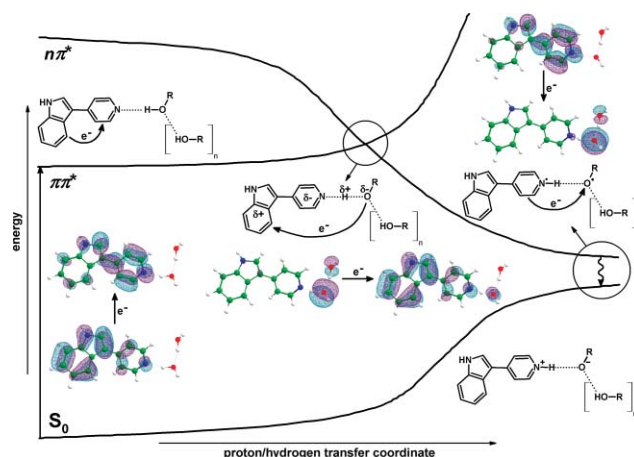


Fig. 8 Model of excited state processes for **3PyIn** leading to fluorescence quenching in a hydroxylic solvent.

the proton transfer coordinate. When the proton is about halfway between the water oxygen and the pyridine nitrogen, the energy of the $\text{n}\pi^*$ starts decreasing rapidly, which leads to a conical intersection with S_1 . Further proton movement brings the energy of the $\text{n}\pi^*$ state close to S_0 , leading to efficient internal conversion to the ground state, followed by back proton transfer.

Changing of the nature of the lowest excited singlet state at the $\pi\pi^*/\text{n}\pi^*$ conical intersection corresponds to an electron transfer from the solvent molecule, hydrogen-bonded to the pyridine nitrogen, to the excited chromophore. Formally, the overall process is equivalent to a hydrogen atom transfer. This mechanism is reminiscent of a model proposed by Sobolewski and Domcke for efficient radiationless deactivation of azaaromatic molecules based on excited state hydrogen detachment/attachment to water acting as a hydrogen bond acceptor.^{9,49} In a sense, our approach is complementary, as it deals with water acting as a hydrogen bond donor. Consequently, the present model can be called hydrogen attachment/detachment.

According to the model, the barrier for fluorescence quenching is due to the increase of the energy of the $\text{S}_1(\pi\pi^*)$ state along the proton transfer coordinate. For the 1:2 complex of **3PyIn** with water, the calculated barrier, *i.e.*, the energy required to reach the $\pi\pi^*/\text{n}\pi^*$ conical intersection, is about 5 kcal mol^{−1}. Initial computational results show that the barrier decreases with the addition of more water molecules at the pyridine site. This result is in line with the experimentally observed fluorescence changes accompanying the titration of **3PyIn** in *n*-hexane with 1,1,1,3,3,3-hexafluoro-2-propanol (Fig. 6).

Conclusions and outlook

Combined experimental and theoretical investigations of isomeric (4'-pyridyl)indoles revealed a pattern of photophysical characteristics qualitatively similar for the three derivatives. Fluorescence of all the three compounds is quenched in liquid water and alcohol solutions, but when the environment becomes rigid, the quenching is not observed. The plausible explanation of quenching involves a sequence of processes occurring in a photoexcited, intermolecularly hydrogen-bonded molecule: (i) electron density flows from the indole onto the pyridyl moiety, which leads

to a large increase of the basicity of the pyridine nitrogen atom; (ii) proton transfer along the OH...N hydrogen bond; (iii) mutual twisting of the indole and pyridyl units of the protonated form towards the perpendicular geometry; (iv) efficient S_1 – S_0 radiationless deactivation, due to a low energy of S_1 , in accordance with the energy gap law.⁵⁰

In order to gain an insight into the quenching dynamics, time-resolved experiments are planned. In view of the expected weakness of fluorescence, transient absorption techniques, involving both electronic and vibrational spectra, seem more promising.

In previous studies of fluorescence quenching in numerous bifunctional hydrogen bond donor/acceptor systems, we pointed out the role of communication between the NH donor and the pyridyl N acceptor atom *via* a triply hydrogen-bonded bridge in chromophore–alcohol or water complexes of 1:2 stoichiometry.^{3,26,38} The present results indicate that quenching can occur even without forming such bridges, although the process is then less efficient. Actually, **7PyIn** was more effectively quenched than the other two isomers. Optimizations of 1:2 water complexes, starting with the geometry involving two independent linear hydrogen bonds, always converged to a cyclic structure, even though the arrangement of the OH group of water with respect to the pyridine ring was not favorable for a strong hydrogen bonding (the OH bond nearly perpendicular to the pyridyl plane). One cannot exclude that, even for **2PyIn** and **3PyIn**, longer alcohol or water bridges are important for the quenching. In any case, a simultaneous presence of both a proton acceptor and proton donor groups is necessary for the quenching to occur: 2-phenylindole, which lacks the pyridyl nitrogen, is strongly fluorescent in methanol, ethanol, 1-propanol and 1-butanol. The role of the NH group “devoid” of communication *via* intermolecular hydrogen bonds with the pyridine nitrogen may then be to release electronic charge upon excitation, which then flows towards the pyridyl ring. This behavior is demonstrated by a huge, negative pK_a change in S_1 .

The mechanism of quenching which involves the twisting of the pyridyl *vs.* the indole unit seems very probable for the presently studied pyridylindoles. On the other hand, it cannot explain the quenching observed for planar bifunctional systems such as 1-H-pyrrolo[3,2-*h*]quinoline, 7,8,9,10-pyrido[2,3-*a*]carbazole, pyrido[2,3-*a*]carbazole,^{25,26} and dipyrro[2,3-*a*:3',2'-*i*]carbazole.^{36,37} The alternative model proposed in this work, based on coupled proton/electron transfer, does not require twisting and, therefore, seems more general. The observed viscosity dependence in this case could be explained by the solvent rearrangement necessary to accommodate changes in charge distribution occurring at different stages of deactivation at different locations in the complex.

It should be stressed that the spectral and photophysical characteristics of pyridylindoles are very different from those of the parent indole chromophore.⁵¹ In the latter, the two lowest excited singlet states, L_b and L_a , differ in polarity. This may lead to the inversion of state ordering in polar *vs.* nonpolar solvents, where the more polar L_a state, being stabilized to a much larger degree than L_b , becomes the lowest singlet excited state. On the contrary, the calculations for pyridylindoles reveal similar dipole moments for S_1 and S_2 and thus their ordering should not change in solvents of different polarity. However, changes in solvent polarity may lead to different behavior of an excited

pyridylindole. In nonpolar solvents, flattening is a preferred path, as in 2-phenylindole, which is known from supersonic jet studies to become planar in S_1 , whereas in the ground state, the angle between the indole and phenyl moieties is 27°. ⁵² In polar solvents, however, the twisting may become preferable in S_1 , which leads to significantly longer fluorescence decay. These unique properties of pyridylindoles may be used for practical purposes. Derivatives of phenylindole, such as 4',6-diamidino-2-phenylindole (DAPI) can bind to DNA;⁵³ the fluorescence of DAPI bound to the minor groove of double stranded DNA is about twenty-fold greater than in the unbound state. DAPI is therefore used as a fluorescence probe in DNA imaging, drug testing and various assays.^{54,55} The detailed understanding of the photophysics and structure of pyridylindoles and their hydrogen-bonded complexes may open up possibilities for their use as fluorescent probes for biological materials. Such studies are now underway in our laboratory using supersonic jet techniques.

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