CHEMISTRY
A EUROPEAN JOURNAL

# Supporting Information

# Photophysics of a Series of Efficient Fluorescent pH Probes for Dual Emission Wavelength Measurements in Aqueous Solutions

Sandrine Charier,[a] Odile Ruel,[a] Jean-Bernard Baudin,[a] Damien Alcor[a]
Jean-François Allemand,[a,b] Adrien Meglio,[a] Ludovic Jullien,[a],* Bernard Valeur[c]

[a] *S. Charier, O. Ruel, J.-B. Baudin, D. Alcor, A. Meglio, L. Jullien,*
*Département de Chimie (C.N.R.S. U.M.R. 8640), École Normale Supérieure,*
*24, rue Lhomond, 75231 Paris Cedex 05, France,*
*Fax: (+33) 1 44 32 33 25, E-mail: Ludovic.Jullien@ens.fr*

[b] *J.-F. Allemand,*
*Département de Physique (C.N.R.S. U.M.R. 8550), École Normale Supérieure,*
*24, rue Lhomond, 75231 Paris Cedex 05, France*

[c] *B. Valeur,*
*C.N.R.S U.M.R 8531, Laboratoire de Chimie Générale, Conservatoire National des Arts et Métiers,*
*292, rue Saint-Martin, 75141 Paris cedex 03, France*
*and Laboratoire PPSM, École Normale Supérieure de Cachan,*
*61, avenue du Président Wilson, 94235 Cachan cedex, France*

August 22, 2005

## Supporting Information

- Appendix
  - Interpretation of the solvatochromism in absorption and in fluorescence emission
  - Application to the evaluation of the protonation constant of the excited state of the present oxazoles
- Supplementary Materials
  - Absorption and emission spectra of **4-PYPO** (Figure 1S), **2-PYMPO** (Figure 2S), **2-QUIMPO** (Figure 3S), **4-PYMPO-NHNH$_2$** (Figure 4S), **4-PYMPO-NHNHAc** (Figure 5S), **4-PYHPO** (Figure 6S), **2-PYMPO-CO$_2$H** (Figure 7S), **2-PYMPO-CO$_2$Me** (Figure 8S), **2-PYMPO-CH$_2$OH** (Figure 9S), **4-BOMPO** (Figure 10S), **4-PYMPOMe$^+$,TsO$^-$** (Figure 11S), **4-PYMPOM** (Figure 12S)
  - Stern-Volmer analysis of the lifetimes extracted from the measurements performed on **4-PYMPO** solutions below pH=3 (Figure 13S)

– Analysis of the solvatochromism in the present oxazole series in relation with acid base reactions (Figures 14S and 15S).

# Appendix

## Interpretation of the solvatochromism in absorption and in fluorescence emission

We interpret the solvatochromism observed in absorption and in emission with the theoretical model that was recently introduced by Hynes et al [1] to account for photoinduced charge transfer for neutral and charged push-pull conjugated molecules induced by optical excitation. In this model, the role of solvent polarity is twofold. A more polar solvent first affects the energy gap between the ground and excited states with relaxed solvation shells ($V_{eq}^{adia}$ in Figure 14Sa). This term tends to shift both the absorption and fluorescence spectra in the same direction, while keeping the Stokes shift $\mathcal{S}$ equal. Second, a more polar solvent increases the solvent reorganization energy $\Lambda_s$, both in the ground ($\Lambda_s^g$) and in the excited states ($\Lambda_s^{ex}$), and this tends to increase the Stokes shift $\mathcal{S} = \Lambda_s^{ex} + \Lambda_s^g$ (Figure 14Sa).

In neutral basic states of the present series, the solvatochromic behavior is compatible with an increase of the dipole moment in its excited state as it is observed in related neutral push-pull molecules [2]. The equilibrated excited state is more stabilized by an increase of solvent polarity than the equilibrated ground state: $V_{eq}^{adia}$ decreases when the solvent polarity is increased. At the same time, the solvent reorganization energies $\Lambda_s^{ex}$ and $\Lambda_s^g$ are increased in a more polar solvent. Consequently, solvatochromism is more pronounced in emission than in absorption. Indeed, an increase of polarity almost does not affect the wavelength of absorption whereas it promotes a red shift in emission. Using the two valence bond states description of the model [1], the ground and excited electronic basic states are mixtures of the two valence bond states: **N** (neutral form) and **Z** (zwitterionic form bearing a negative and a positive charge on the nitrogen and oxygen atoms respectively) (Figure 14Sb). Then it is possible to use Eq.(1) to extract from the solvatochromism study an order of magnitude of the change upon light absorption $\Delta\delta = \delta^{ex} - \delta^g$ in the negative and positive partial charge borne by the nitrogen and oxygen terminal atoms.

$$\mathcal{S} = \Lambda_s^{ex} + \Lambda_s^g \approx 2\Delta\delta^2 \left(\frac{1}{\epsilon_\infty} - \frac{1}{\epsilon_r}\right)\left(\frac{1}{2r_D} + \frac{1}{2r_A} - \frac{1}{R_{DA}}\right) \tag{1}$$

In the preceding equation, $\epsilon_\infty$ and $\epsilon_r$ respectively designate the high frequency optical and zero frequency static dielectric constants of the solvent ($\epsilon_\infty = n^2$ where $n$ is the refractive index of the solvent), $r_D$ and $r_A$ are the radii of the electron donating and accepting groups, and $R_{DA}$ is the donor–acceptor distance. Figure 15S displays the dependence of the Stokes shift $\mathcal{S}$ on the solvent parameter ($\frac{1}{\epsilon_\infty} - \frac{1}{\epsilon_r}$). The dependence is reasonably linear and it is possible to extract $\Delta\delta$ from the slope for **4-PYMPO**, **2-PYMPO**, and **4-PYMPO-NH$_2$** by using reasonable estimates for $r_D$ and $r_A$ ($r_D = r_A = 4$Å; [1]), and by measuring $R_{DA}$ on a molecular model ($R_{DA} = 11$Å for **4-PYMPO** and **4-PYMPO-NH$_2$**, and $R_{DA} = 9$Å for **2-PYMPO**). We found: $\Delta\delta = 0.4 z_e$ ($z_e = 1.6\ 10^{-19} C$) for the three investigated compounds.

In **4-PYMPOMe$^+$,TsO$^-$** considered as representative of the acidic state of the present oxazole, the observations are in line with the redistribution of the positive charge of the pyridinium on the molecular backbone upon light absorption. The ground and the excited states would be rather similarly affected by a change of solvent polarity: in contrast to the situation evoked in the preceding paragraph, $V_{eq}^{adia}$ would be essentially independent on the solvent. At the same time, $\Lambda_s^{ex}$ and $\Lambda_s^g$ would increase in a more polar solvent. In the stilbazolium series analyzed in reference [1], a blue (respectively red) shift was observed in absorption (respectively fluorescence emission) upon increasing solvent polarity. In the present series, one only observes a small blue shift in absorption and almost no change in fluorescence emission by increasing solvent polarity. Such a difference of behavior may originate from an increase of the delocalisation of the positive charge over the oxazole molecular backbone upon light absorption ($V_{eq}^{adia}$ slightly increasing with polarity). In a two valence bond state description that now involves cationic forms bearing a positive charge either on the nitrogen (**R**) or on the oxygen atoms (**L**) (Figure 14Sb), it is again possible to rely on solvatochromism to derive from Eq.(1) an order of magnitude of the change in the positive partial charges borne by the nitrogen ($\Delta\delta = (1-\delta^{ex}) - (1-\delta^g)$) and the oxygen ($-\Delta\delta = \delta^{ex} - \delta^g$) terminal atoms upon light absorption. With the parameter set used above for the 4-pyridyl series, we obtained: $\Delta\delta = 0.5 z_e$.

## Application to the evaluation of the protonation constant of the excited state

To address quantitatively the corresponding issue, we turn again to the theoretical model that was used above to analyze solvatochromism [1]. In relation to Figure 14Sa, one can write Eq.(2):

$$2.3 k_B T[pK_a(\mathcal{BH}^{+*}/\mathcal{B}^*) - pK_a(\mathcal{BH}^+/\mathcal{B})] = hc\left(\frac{1}{\lambda_{abs}(\mathcal{B})} - \frac{1}{\lambda_{abs}(\mathcal{BH}^+)}\right) + (\Lambda_s^{ex}(\mathcal{BH}^+) - \Lambda_s^{ex}(\mathcal{B})) \quad (2)$$

where $k_B$ is the Boltzmann constant, $T$ the absolute temperature, $h$ is the Planck constant, and $c$ is the light velocity. Eq.(2) is closely related to the corresponding equation arising from the Förster cycle [3], except for taking explicitly into account the reorganization energies of the solvent. In principle, $\Lambda_s^{ex}(\mathcal{BH}^+)$ and $\Lambda_s^{ex}(\mathcal{B})$ could be obtained from time-resolved experiments by probing the wavelength of fluorescence emission as a function of time shortly after excitation [4]. To derive Eq.(3) in the Text, we assumed that the curvatures of the free energy surfaces of the electronic ground and excited states displayed in Figure 14Sa were identical. Then $\Lambda_s^{ex}(\mathcal{BH}^+) = \Lambda_s^g(\mathcal{BH}^+) = \frac{1}{2}\mathcal{S}(\mathcal{BH}^+)$ and $\Lambda_s^{ex}(\mathcal{B}) = \Lambda_s^g(\mathcal{B}) = \frac{1}{2}\mathcal{S}(\mathcal{B})$ where $\mathcal{S}(\mathcal{BH}^+)$ and $\mathcal{S}(\mathcal{B})$ designate the experimentally determined Stokes shifts for $\mathcal{BH}^+$ and $\mathcal{B}$ .

Application of Eq. (2) on experimental data suggests in particular that $pK_a(\mathcal{BH}^{+*}/\mathcal{B}^*) - pK_a(\mathcal{BH}^+/\mathcal{B})$ is larger for **4-PYMPO** than for **4-PYMPO-NH$_2$** (see Table 3). This result was anticipated from the analysis of the solvatochromic study. Indeed the stabilization of the

positive charge borne by the nitrogen atom of the pyridinium ring by the conjugated ortho amino group should be more pronounced in the ground state ($\delta'^{g} \approx 0$ in the reaction of proton exchange displayed in Figure 14Sb) [1] than in the excited state ($\delta'^{ex} \approx 0.5$ in Figure 14Sb).

# Supplementary Materials

## Equations associated to the dynamics of a closed system submitted to the processes displayed in Figure 6

In a closed system submitted to the processes displayed in Figure 6, the concentrations obey the system of Eqs.(3–6) where no bracket as well as no charge were introduced in the concentration notations in a purpose of simplification.

$$\frac{dBH}{dt} = -(\kappa_{BH\to B} + \kappa_{BH\to BH^*})BH + \kappa_{B\to BH}B + \kappa_{BH^*\to BH}BH^* \quad (3)$$

$$\frac{dB}{dt} = \kappa_{BH\to B}BH - (\kappa_{B\to BH} + \kappa_{B\to B^*})B + \kappa_{B^*\to B}B^* \quad (4)$$

$$\frac{dBH^*}{dt} = \kappa_{BH\to BH^*}BH - (\kappa_{BH^*\to BH} + \kappa_{BH^*\to B^*})BH^* + \kappa_{B^*\to BH^*}B^* \quad (5)$$

$$\frac{dB^*}{dt} = \kappa_{B\to B^*}B + \kappa_{BH^*\to B^*}BH^* - (\kappa_{B^*\to B} + \kappa_{B^*\to BH^*})B^* \quad (6)$$

with $\kappa_{BH\to B} = k_{13}+k_{23}OH+k_{3'3}Y$; $\kappa_{B\to BH} = k_{31}H+k_{32}+k_{33'}YH$; $\kappa_{BH\to BH^*} = \frac{2.3\varepsilon_{BH}(\lambda_{exc})lI_0(\lambda_{exc}))}{V}$ with one-photon excitation, whereas $\kappa_{BH\to BH^*} = \frac{\delta_{BH}(\lambda_{exc})I_0^2(\lambda_{exc}))}{2S^2}$ with two-photon excitation; $\kappa_{BH^*\to BH} = \frac{1}{\tau_{BH^*}}$; $\kappa_{BH^*\to B^*} = k^*_{13} + k^*_{23}OH + k^*_{3'3}Y$; $\kappa_{B^*\to BH^*} = k^*_{31}H + k^*_{32} + k^*_{33'}YH$; $\kappa_{B\to B^*} = \frac{2.3\varepsilon_B(\lambda_{exc})lI_0(\lambda_{exc}))}{V}$ with one-photon excitation, whereas $\kappa_{B\to B^*} = \frac{\delta_B(\lambda_{exc})I_0^2(\lambda_{exc}))}{2S^2}$ with two-photon excitation; $\kappa_{B^*\to B} = \frac{1}{\tau_{B^*}}$ where the $k_{ij}$, $\varepsilon(\lambda_{exc})$, $\delta(\lambda_{exc})$, $l$, $S$, $V$, $I_0$, and $\tau$ respectively designate the rate constants associated to the reaction $i \to j$, the molar absorption coefficient and the cross-section for two-photon absorption of the considered species at the excitation wavelength, the length of the light pathway in the sample, the surface of the light beam, the volume of irradiated sample, the intensity of the incident light at the excitation wavelength, and the lifetime of the considered excited state.

## The steady-state

### Expressions of the concentrations in $BH$, $B$, $BH^*$ and $B^*$

The concentrations at steady-state upon illumination are obtained by solving Eq.(7):

$$\frac{dBH}{dt} = \frac{dB}{dt} = \frac{dBH^*}{dt} = \frac{dB^*}{dt} = 0 \quad (7)$$

Under the present conditions of weak illumination ($I_0(\lambda_{exc}) \approx 10^{-9}$ einstein/s), the processes involving the excited states only slightly perturbate the processes involving the ground states and simplifying approximations can be made. In particular, the rates associated to the reactions $BH \rightleftharpoons BH^*$ and $B \rightleftharpoons B^*$ can be neglected in front of the other terms in Eqs.(3,4). Then, one has in the steady state:

$$BH_\infty \approx \frac{C_B}{1 + \frac{K_a}{H}} \quad (8)$$

$$B_\infty \approx \frac{K_a}{H}BH_\infty \quad (9)$$

where $C_B$ and $K_a$ respectively designate the total concentration in species derived from $\mathcal{B}$, and the proton dissociation constant of $\mathcal{BH}^+$ in the ground state. $BH_\infty$ and $B_\infty$ are essentially equal to the equilibrium values in the absence of light.

After reporting the expressions of $BH_\infty$ and $B_\infty$ in Eqs.(5,6), one finally obtains:

$$BH^*_\infty \approx \frac{\kappa_{BH\to BH^*}(\kappa_{B^*\to BH^*}+\kappa_{B^*\to B})+\kappa_{B\to B^*}\kappa_{B^*\to BH^*}\frac{K_a}{H}}{\kappa_{BH^*\to BH}(\kappa_{B^*\to BH^*}+\kappa_{B^*\to B})+\kappa_{B^*\to B}\kappa_{BH^*\to B^*}}\frac{C_B}{1+\frac{K_a}{H}} \tag{10}$$

$$B^*_\infty \approx \frac{\kappa_{BH\to BH^*}\kappa_{BH^*\to B^*}+\kappa_{B\to B^*}(\kappa_{BH^*\to B^*}+\kappa_{BH^*\to BH})\frac{K_a}{H}}{\kappa_{BH^*\to BH}(\kappa_{B^*\to BH^*}+\kappa_{B^*\to B})+\kappa_{B^*\to B}\kappa_{BH^*\to B^*}}\frac{C_B}{1+\frac{K_a}{H}} \tag{11}$$

Using typical orders of magnitude for the rate constants ($k_{23}\approx k^*_{23}\approx k_{31}\approx k^*_{31}\approx 10^{10}$ M$^{-1}s^{-1}$, $\kappa_{BH^*\to BH}\approx\kappa_{B^*\to B}\approx 10^9\ s^{-1}$), the latter expressions can be simplified in the three following $pH$ regimes:

$pH < pK_a(\mathcal{BH}^+/\mathcal{B}) < pK_a(\mathcal{BH}^{+*}/\mathcal{B}^*)$

$$BH_\infty \approx C_B \tag{12}$$

$$B_\infty \approx \frac{K_a}{H}C_B \tag{13}$$

$$BH^*_\infty \approx \frac{\kappa_{BH\to BH^*}}{\kappa_{BH^*\to BH}}C_B \tag{14}$$

$$B^*_\infty \approx \frac{1}{1+\frac{\kappa_{B^*\to BH^*}}{\kappa_{B^*\to B}}}\frac{K_a}{H}\frac{\kappa_{B\to B^*}}{\kappa_{B^*\to B}}C_B \tag{15}$$

$pK_a(\mathcal{BH}^+/\mathcal{B}) < pH < pK_a(\mathcal{BH}^{+*}/\mathcal{B}^*)$

$$BH_\infty \approx \frac{H}{K_a}C_B \tag{16}$$

$$B_\infty \approx C_B \tag{17}$$

$$BH^*_\infty \approx \frac{\kappa_{B\to B^*}}{\kappa_{BH\to BH^*}}\frac{\kappa_{B^*\to BH^*}}{\kappa_{B^*\to BH^*}+\kappa_{B^*\to B}}\frac{\kappa_{BH\to BH^*}}{\kappa_{BH^*\to BH}}C_B \tag{18}$$

$$B^*_\infty \approx \frac{\kappa_{B^*\to B}}{\kappa_{B^*\to BH^*}+\kappa_{B^*\to B}}\frac{\kappa_{B\to B^*}}{\kappa_{B^*\to B}}C_B \tag{19}$$

$pK_a(\mathcal{BH}^+/\mathcal{B}) < pK_a(\mathcal{BH}^{+*}/\mathcal{B}^*) < pH$

$$BH_\infty \approx \frac{H}{K_a}C_B \tag{20}$$

$$B_\infty \approx C_B \tag{21}$$

$$BH^*_\infty \approx \frac{\kappa_{B^*\to BH^*}}{\kappa_{BH^*\to B^*}}B^*_\infty \tag{22}$$

$$B^*_\infty \approx \frac{\kappa_{B\to B^*}}{\kappa_{B^*\to B}}C_B \tag{23}$$

**Theoretical expressions of the ratios of the absorbances and of the fluorescence emissions at two wavelengths with pH**

Upon assumption of weak illumination, the ratio $\rho^{a_i}_{\lambda_1,\lambda_2}$ of the absorbances of $\{\mathcal{BH}^+,\mathcal{B}\}$ solutions at two different wavelengths $\lambda_1$ and $\lambda_2$ after one- ($i=1$, $\alpha^i=\varepsilon$) or two-($i=2$, $\alpha^i=\delta$) photon

excitation can be calculated from Eqs.(8,9):

$$\rho^{a_i}_{\lambda_1,\lambda_2} = \frac{\alpha^i_{BH}(\lambda_1)BH_\infty + \alpha^i_B(\lambda_1)B_\infty}{\alpha^i_{BH}(\lambda_2)BH_\infty + \alpha^i_B(\lambda_2)B_\infty} = \frac{\alpha^i_{BH}(\lambda_1)10^{-pH} + \alpha^i_B(\lambda_1)10^{-pK_a(\mathcal{BH}^+/\mathcal{B})}}{\alpha^i_{BH}(\lambda_2)10^{-pH} + \alpha^i_B(\lambda_2)10^{-pK_a(\mathcal{BH}^+/\mathcal{B})}} \tag{24}$$

At a given excitation wavelength $\lambda_{exc}$, the ratio $\rho^{e_i}_{\lambda_1,\lambda_2}$ of the fluorescence emissions of $\{\mathcal{BH}^+, \mathcal{B}\}$ solutions at two different wavelengths $\lambda_1$ and $\lambda_2$ can be generally written:

$$\rho^{e_i}_{\lambda_1,\lambda_2} = \frac{\kappa_{BH^*\to BH}(\lambda_1)BH^*_\infty + \kappa_{B^*\to B}(\lambda_1)B^*_\infty}{\kappa_{BH^*\to BH}(\lambda_2)BH^*_\infty + \kappa_{B^*\to B}(\lambda_2)B^*_\infty} \tag{25}$$

When the rate for protonation or deprotonation of the excited states is slower than the rate for desexcitation, one has: $\kappa_{BH\to BH^*}BH \approx \kappa_{BH^*\to BH}BH^*$ and $\kappa_{BH\to BH^*}BH \approx \kappa_{BH^*\to BH}BH^*$. Then Eq.(25) transforms into Eq.(26):

$$\rho^{e_i}_{\lambda_1,\lambda_2} = \frac{\mathcal{I}_{BH^*}(\lambda_1)\phi_{BH^*}\alpha^i_{BH}(\lambda_{exc})10^{-pH} + \mathcal{I}_{B^*}(\lambda_1)\phi_{B^*}\alpha^i_B(\lambda_{exc})10^{-pK_a(\mathcal{BH}^+/\mathcal{B})}}{\mathcal{I}_{BH^*}(\lambda_2)\phi_{BH^*}\alpha^i_{BH}(\lambda_{exc})10^{-pH} + \mathcal{I}_{B^*}(\lambda_2)\phi_{B^*}\alpha^i_B(\lambda_{exc})10^{-pK_a(\mathcal{BH}^+/\mathcal{B})}} \tag{26}$$

where $\phi$ and $\mathcal{I}(\lambda)$ respectively designate the quantum yield of fluorescence and the normalized emission spectrum of the considered species ($\int_0^\infty \mathcal{I}(\lambda)d\lambda = 1$).

Under the conditions of applications of the Eqs.(24,26), the pH can be extracted from Eq.(27)

$$pH = pK_a(\mathcal{BH}^+/\mathcal{B}) + log_{10}\frac{\rho^{(e,a)_i}_{\lambda_1,\lambda_2} - \rho^{(e,a)_i}_{\lambda_1,\lambda_2}(\mathcal{BH}^+)}{\rho^{(e,a)_i}_{\lambda_1,\lambda_2}(\mathcal{B}) - \rho^{(e,a)_i}_{\lambda_1,\lambda_2}} + log_{10}\frac{S^{(e,a)_i}_{\lambda_2}(\mathcal{BH}^+)}{S^{(e,a)_i}_{\lambda_2}(\mathcal{B})} \tag{27}$$

where $S^{(e,a)_i}_{\lambda_2}(\mathcal{BH}^+)$ (respectively $S^{(e,a)_i}_{\lambda_2}(\mathcal{B})$) designates the value of the signals in absorption or in emission when $\mathcal{BH}^+$ (respectively $\mathcal{B}$) is present only.

## The transient state

### Time-dependent fluorescence intensity after a pulse excitation of the basic state

After excitation by a pulse of light, the system relaxes according to the Eqs.(28,29):

$$\frac{dBH^*}{dt} = -(\kappa_{BH^*\to BH} + \kappa_{BH^*\to B^*})BH^* + \kappa_{B^*\to BH^*}B^* \tag{28}$$

$$\frac{dB^*}{dt} = \kappa_{BH^*\to B^*}BH^* - (\kappa_{B^*\to B} + \kappa_{B^*\to BH^*})B^* \tag{29}$$

Upon selective excitation of the basic state $A$ (initial concentration in $B^*$: $B^*_0$), Eqs.(28,29) provide Eqs.(30,31):

$$B^* = \frac{B^*_0}{\beta_1 - \beta_2}\{[(\kappa_{B^*\to B} + \kappa_{B^*\to BH^*}) - \beta_2]e^{-\beta_1 t} + [\beta_1 - (\kappa_{B^*\to B} + \kappa_{B^*\to BH^*})]e^{-\beta_2 t}\} \tag{30}$$

$$BH^* = \frac{\kappa_{B^*\to BH^*}B^*_0}{\beta_1 - \beta_2}[e^{-\beta_2 t} - e^{-\beta_1 t}] \tag{31}$$

with

$$2\beta_1 = \frac{2}{\tau_{1,pH}} = \kappa_{B^*\to B} + \kappa_{B^*\to BH^*} + \kappa_{BH^*\to BH} + \kappa_{BH^*\to B^*}$$

$$+\left[\left(\kappa_{BH^*\to BH}+\kappa_{BH^*\to B^*}-\kappa_{B^*\to B}-\kappa_{B^*\to BH^*}\right)^2+4\kappa_{B^*\to BH^*}\kappa_{BH^*\to B^*}\right]^{\frac{1}{2}} \quad (32)$$

$$2\beta_2 = \frac{2}{\tau_{2,pH}} = \kappa_{B^*\to B}+\kappa_{B^*\to BH^*}+\kappa_{BH^*\to BH}+\kappa_{BH^*\to B^*}$$

$$-\left[\left(\kappa_{BH^*\to BH}+\kappa_{BH^*\to B^*}-\kappa_{B^*\to B}-\kappa_{B^*\to BH^*}\right)^2+4\kappa_{B^*\to BH^*}\kappa_{BH^*\to B^*}\right]^{\frac{1}{2}} \quad (33)$$

If the lifetimes of the excited states $B^*$ and $BH^*$, $\tau_{B^*}$ and $\tau_{BH^*}$, are known (or similarly $\kappa_{B^*\to B}=\frac{1}{\tau_{B^*}}$ and $\kappa_{BH^*\to BH}=\frac{1}{\tau_{BH^*}}$) , both $\kappa_{B^*\to BH^*}$ and $\kappa_{BH^*\to B^*}$ can be extracted from the values of the rate constants $\beta_1$ and $\beta_2$ that are determined from the decay of fluorescence emission of the solution:

$$\kappa_{B^*\to BH^*} = \frac{\tau_{BH^*}}{\tau_{B^*}}\frac{1}{\tau_{B^*}-\tau_{BH^*}}(\beta_1\tau_{B^*}-1)(\beta_2\tau_{B^*}-1) \quad (34)$$

$$\kappa_{BH^*\to B^*} = \frac{\tau_{B^*}}{\tau_{BH^*}}\frac{1}{\tau_{BH^*}-\tau_{B^*}}(\beta_1\tau_{BH^*}-1)(\beta_2\tau_{BH^*}-1) \quad (35)$$

## References


[1] D. Laage, W. H. Thompson, M. Blanchard-Desce, J. T. Hynes, *J. Phys. Chem. A*, **2003**, *107*, 6032–6046.

[2] Z. Diwu, Y. Lu, C. Zhang, D. H. Klaubert, R. P. Haugland, *Photochem. Photobiol.*, **1997**, *66*, 424–431.

[3] B. Valeur, *Molecular Fluorescence Principles and Applications*, Wiley VCH, Weinheim, **2002**.

[4] G. R. Fleming, M. H. Cho, *Annual Review of Physical Chemistry*, **1996**, *47*, 109–134.

[5] C. Frugoni, *Gazz. Chim. Ital.* **1957**, *87*, 403–407.

## Figures

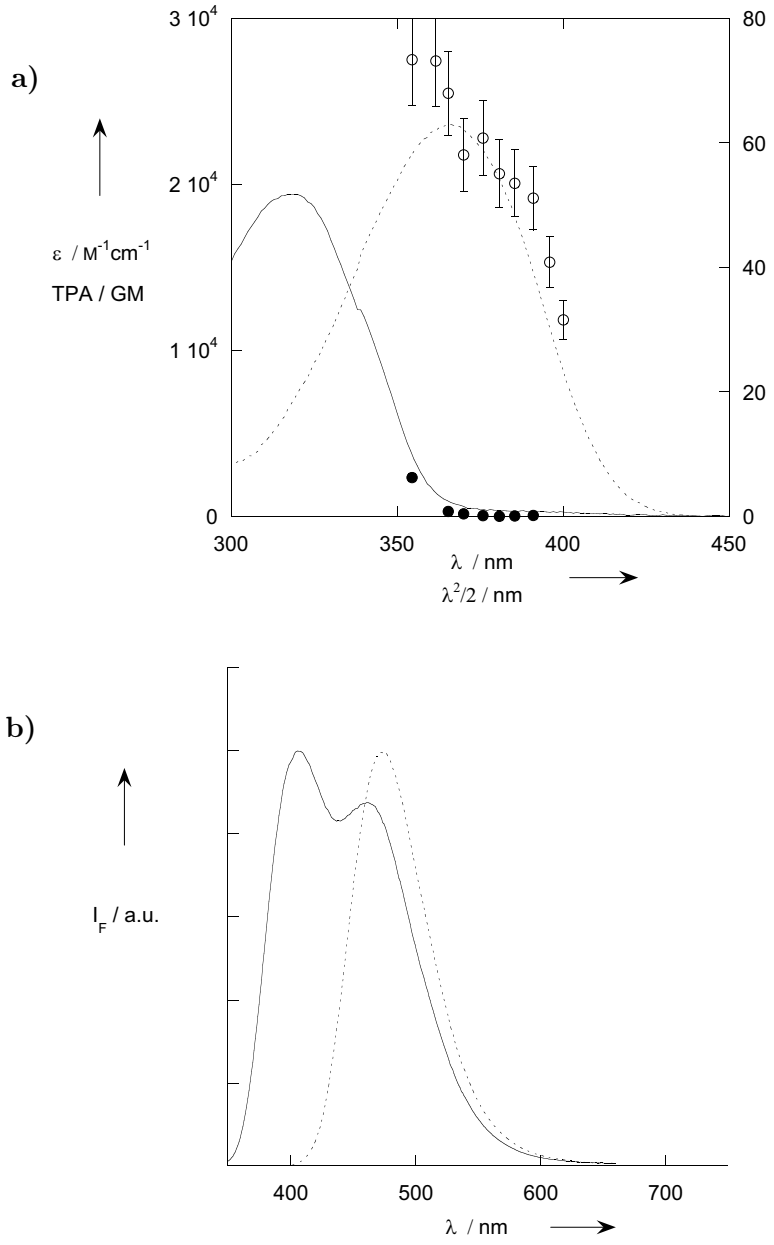


**Figure 1S**. Photophysical properties of **4-PYPO** at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines) and two-photon excitation (markers) spectra; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=338 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=2: dotted line and empty markers; pH=12: solid line and filled markers.

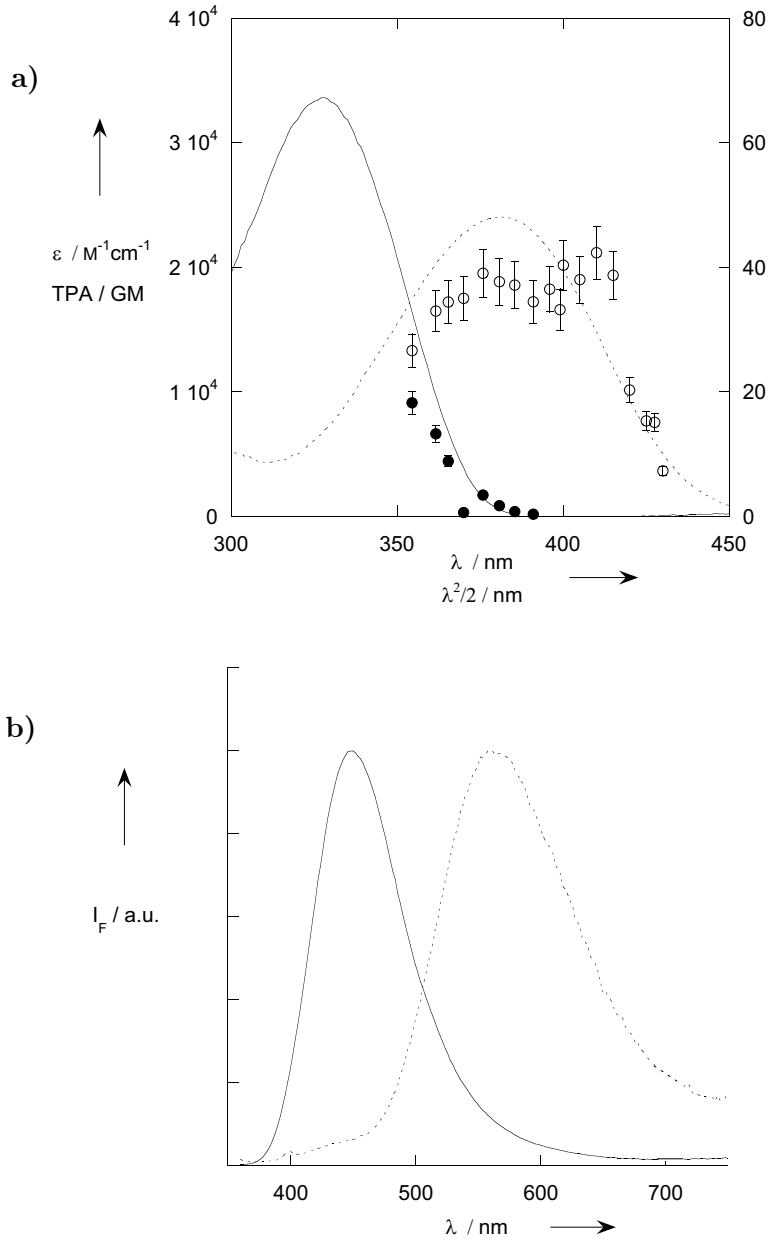


**Figure 2S**. Photophysical properties of **2-PYMPO** at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines) and two-photon excitation (markers) spectra; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=350 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=1: dotted line and empty markers; pH=10: solid line and filled markers.

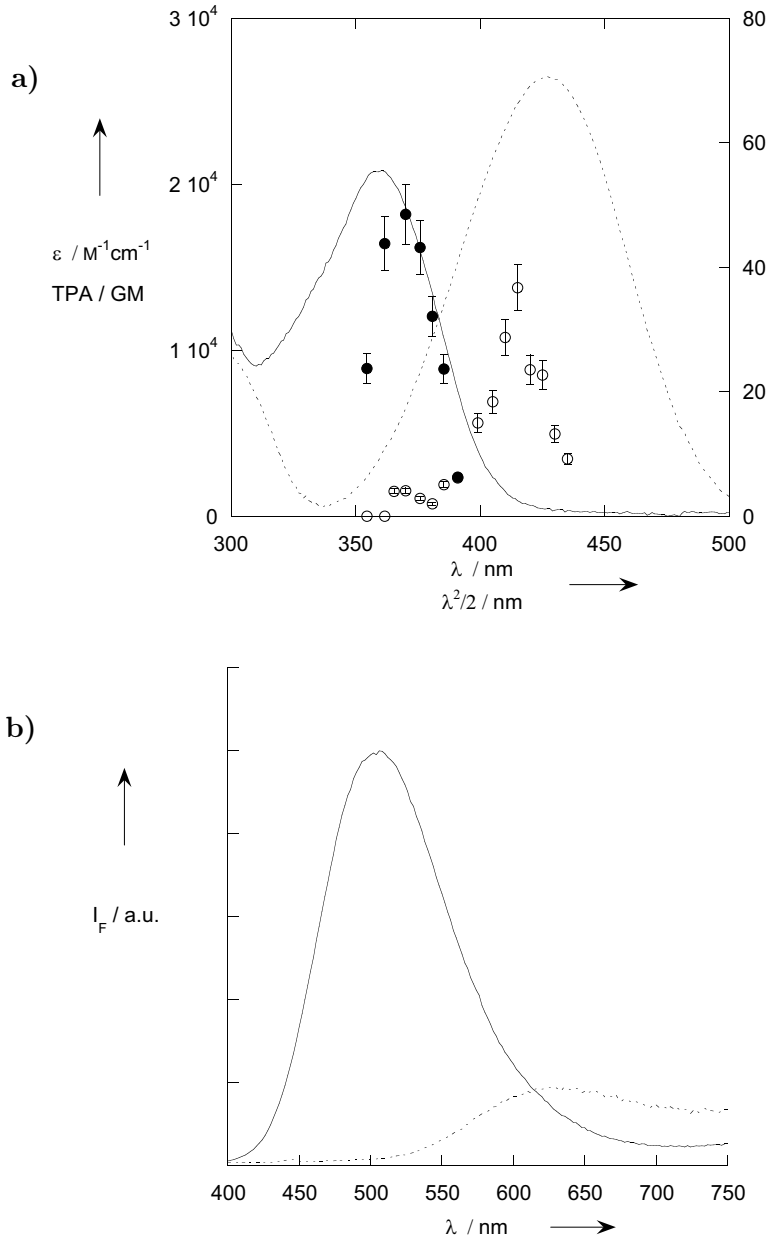


**Figure 3S**. Photophysical properties of **2-QUIMPO** at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines) and two-photon excitation (markers) spectra; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=385 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=1: dotted line and empty markers; pH=10: solid line and filled markers.

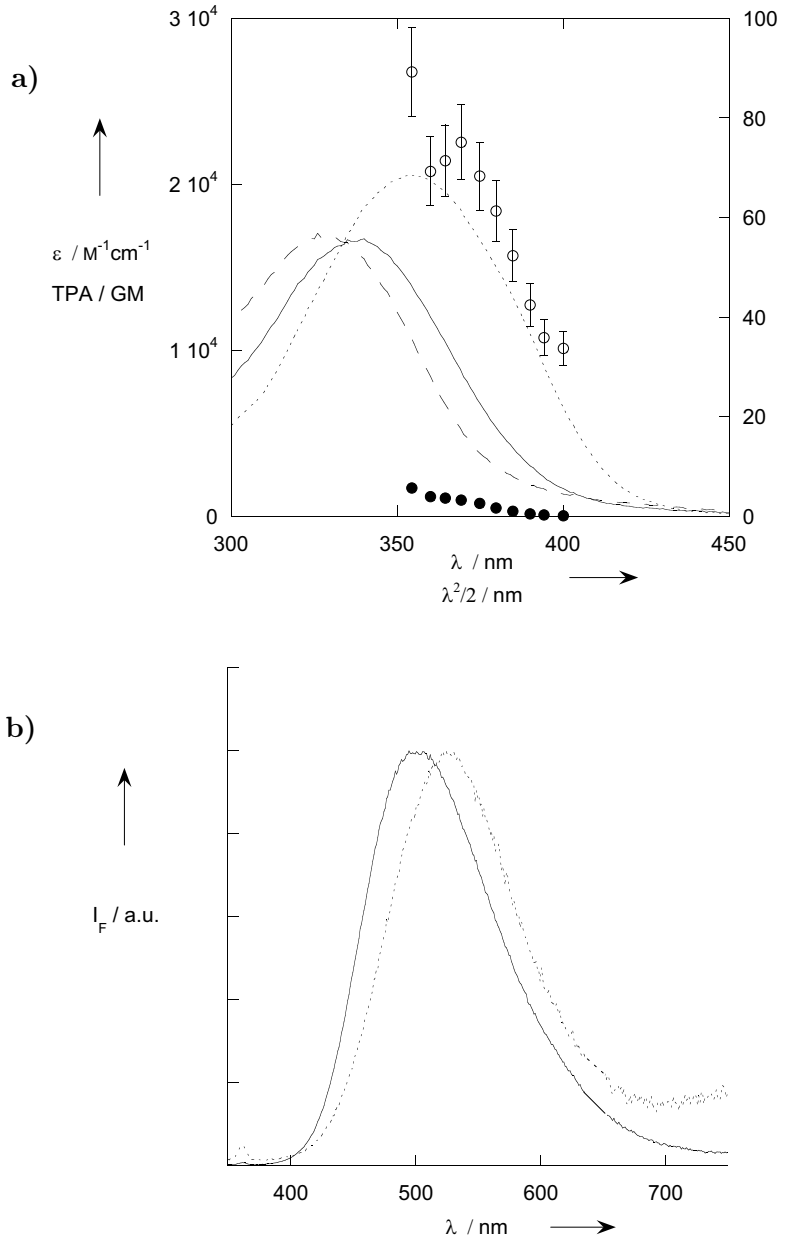


**Figure 4S**. Photophysical properties of **4-PYMPO-NHNH$_2$** at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines) and two-photon excitation (markers) spectra; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=320 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=2: dotted line and empty markers; pH=9: solid line and filled markers; pH=12: dashed line.

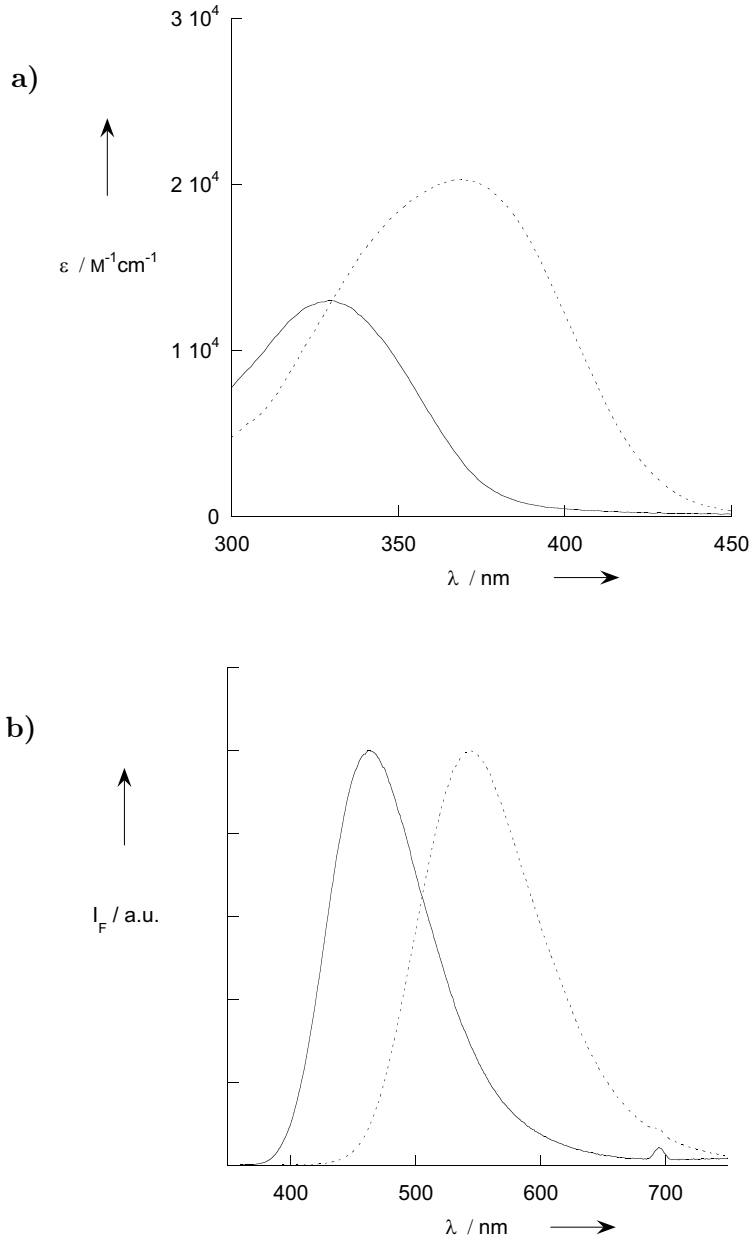


**Figure 5S**. Photophysical properties of **4-PYMPO-NHNHAc** at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines) spectrum; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=346 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=2: dotted line; pH=7: solid line.

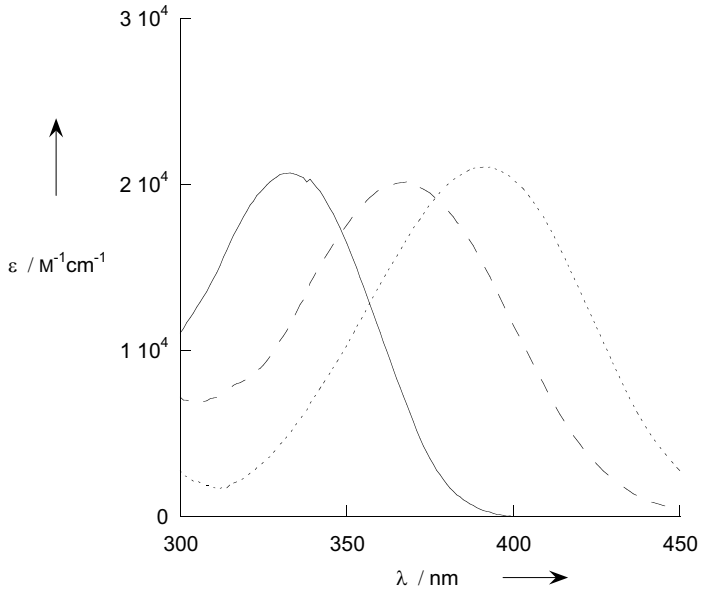


**Figure 6S**. Photophysical properties of **4-PYHPO** at 293 K. Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines). Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=2: dotted line; pH=6: solid line; pH=12: dashed line.

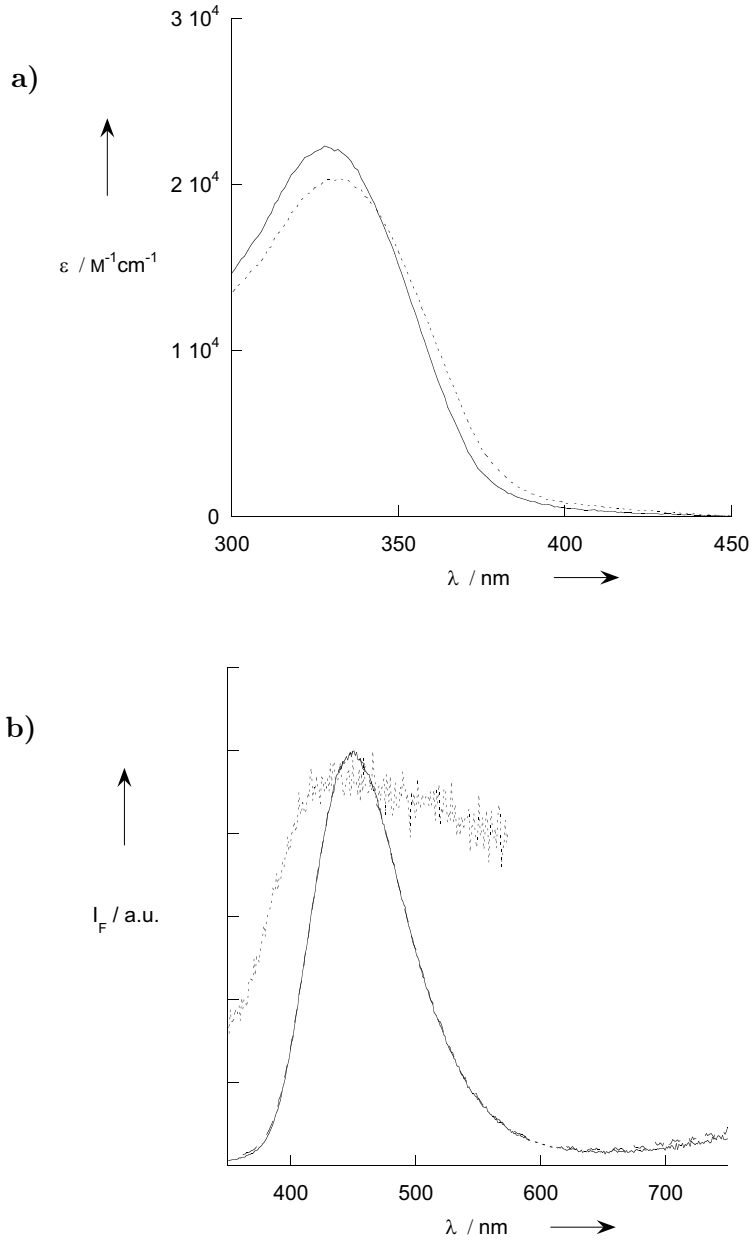


**Figure 7S**. Photophysical properties of **2-PYMPOCO$_2$H** at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines); **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=300 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=1: dotted line; pH=5: dashed line; pH=10: solid line.

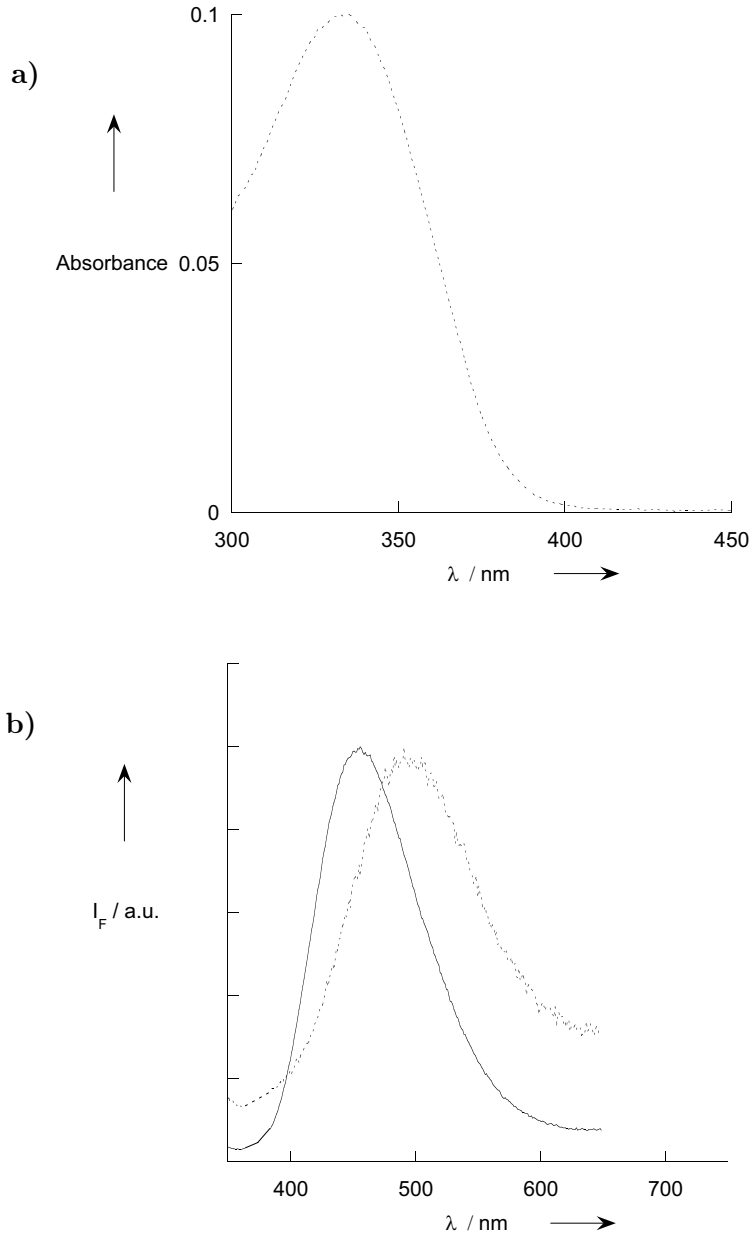


**Figure 8S**. Photophysical properties of **2-PYMPOCO$_2$Me** at 293 K. **a**: Single-photon absorption at saturation; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=330 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=1: dotted line; pH=8: solid line.

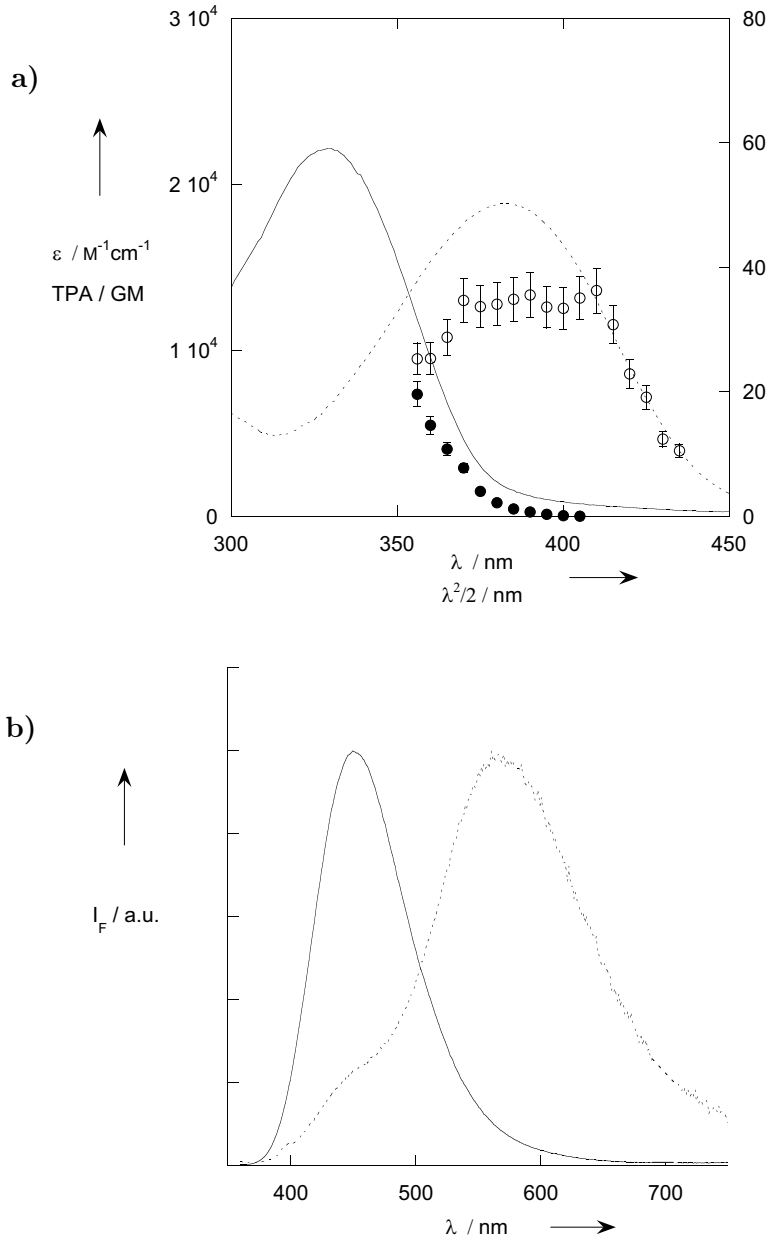


**Figure 9S**. Photophysical properties of **2-PYMPOCH$_2$OH** at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines) and two-photon excitation (markers) spectra; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=348 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=1: dotted line and empty markers; pH=9: solid line and filled markers.

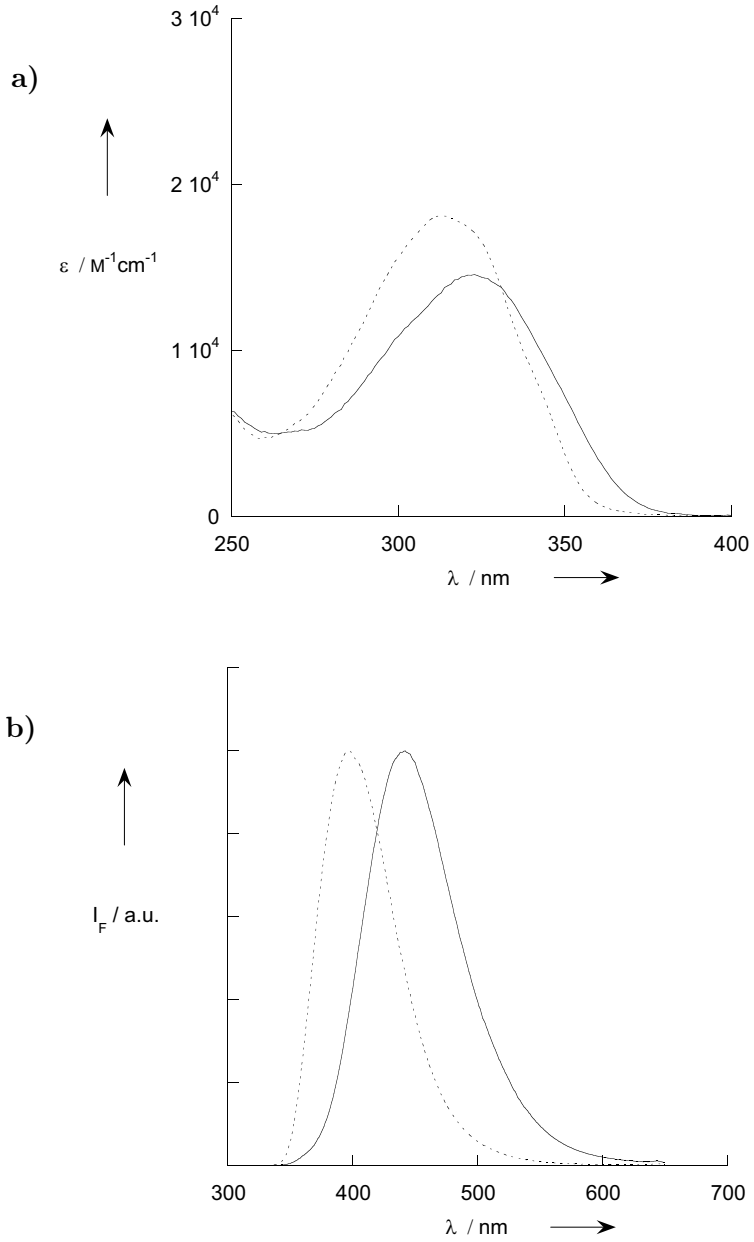


**Figure 10S**. Photophysical properties of **4-BOMPO** at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines) spectrum; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=320 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=5: dotted line; pH=10: solid line.

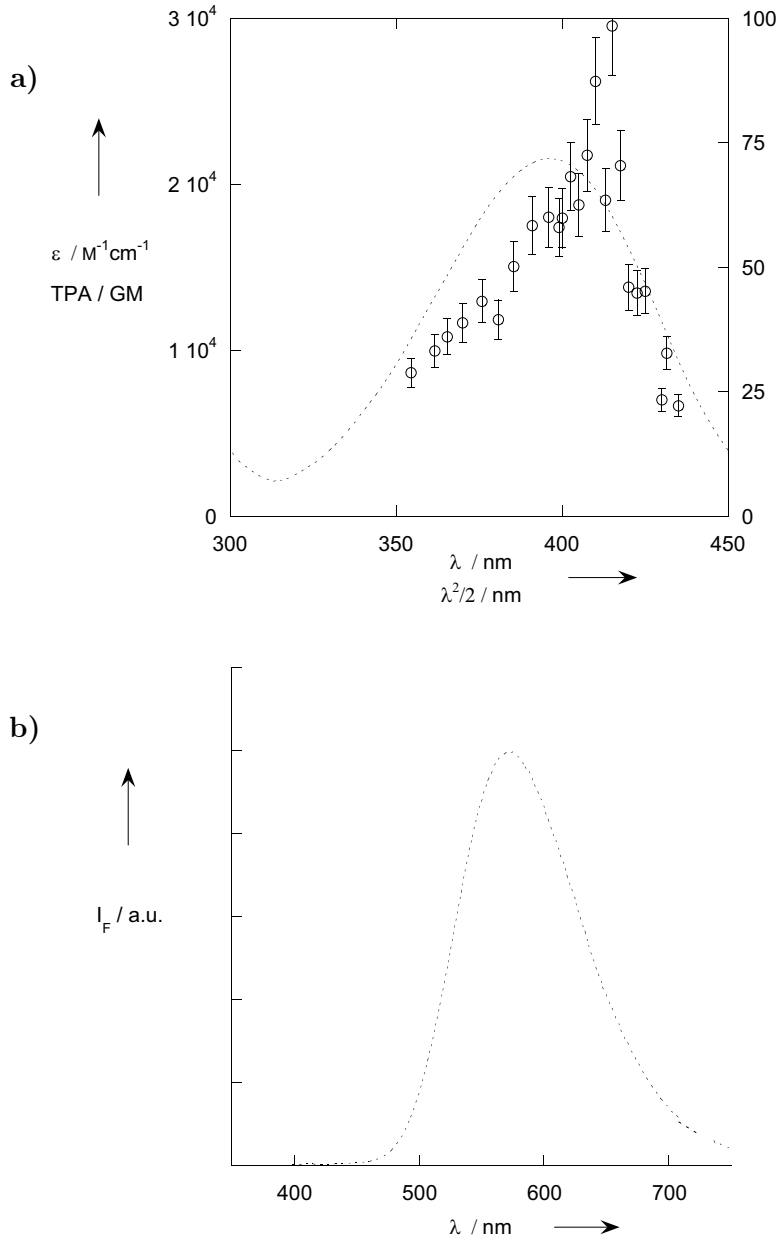


**Figure 11S**. Photophysical properties of **4-PYMPOMe**$^+$ at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; line) and two-photon excitation (markers) spectra; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=360 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M pH=7.

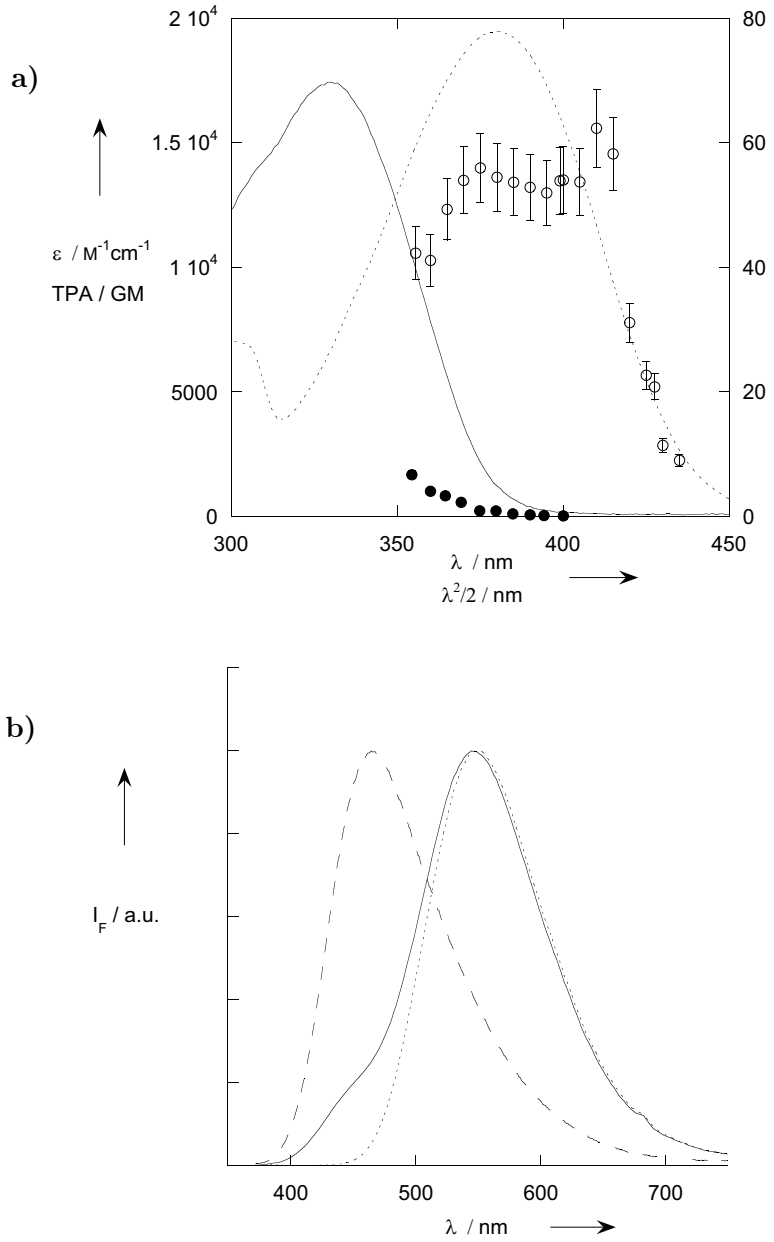


**Figure 12S**.Photophysical properties of **4-PYMPOM** at 293 K. **a**: Single-photon absorption (molar absorption coefficient $\varepsilon$ in $M^{-1}cm^{-1}$; lines) and two-photon excitation (markers) spectra; **b**: Normalized steady-state fluorescence emission $I_F$ after one-photon excitation at $\lambda_{exc}$=340 nm. Solvent: Britton-Robinson buffer [5] (acetic acid, boric acid, phosphoric acid) at 0.1 M. pH=2.5: dotted line and empty markers; pH=9: solid line and filled markers; after addition of concentrated sodium hydroxide pH>14: dashed line.

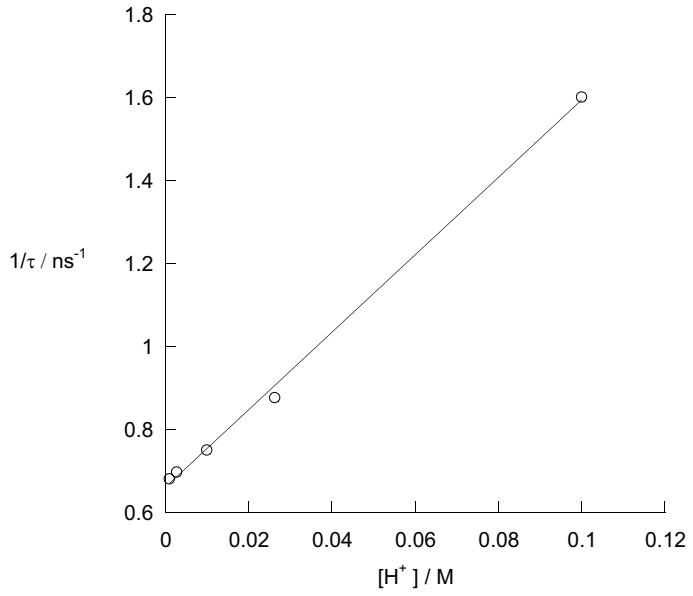


**Figure 13S**. Stern-Volmer analysis of the lifetime extracted from the measurements performed on **4-PYMPO** solutions below pH=3. Markers: experimental data; solid line: fit according to the law: $\beta_{1,pH} = \frac{1}{\tau_{4-PYMPOH^{+*}}} + k_Q[Q]$. The proton plays here the role of the quencher Q.

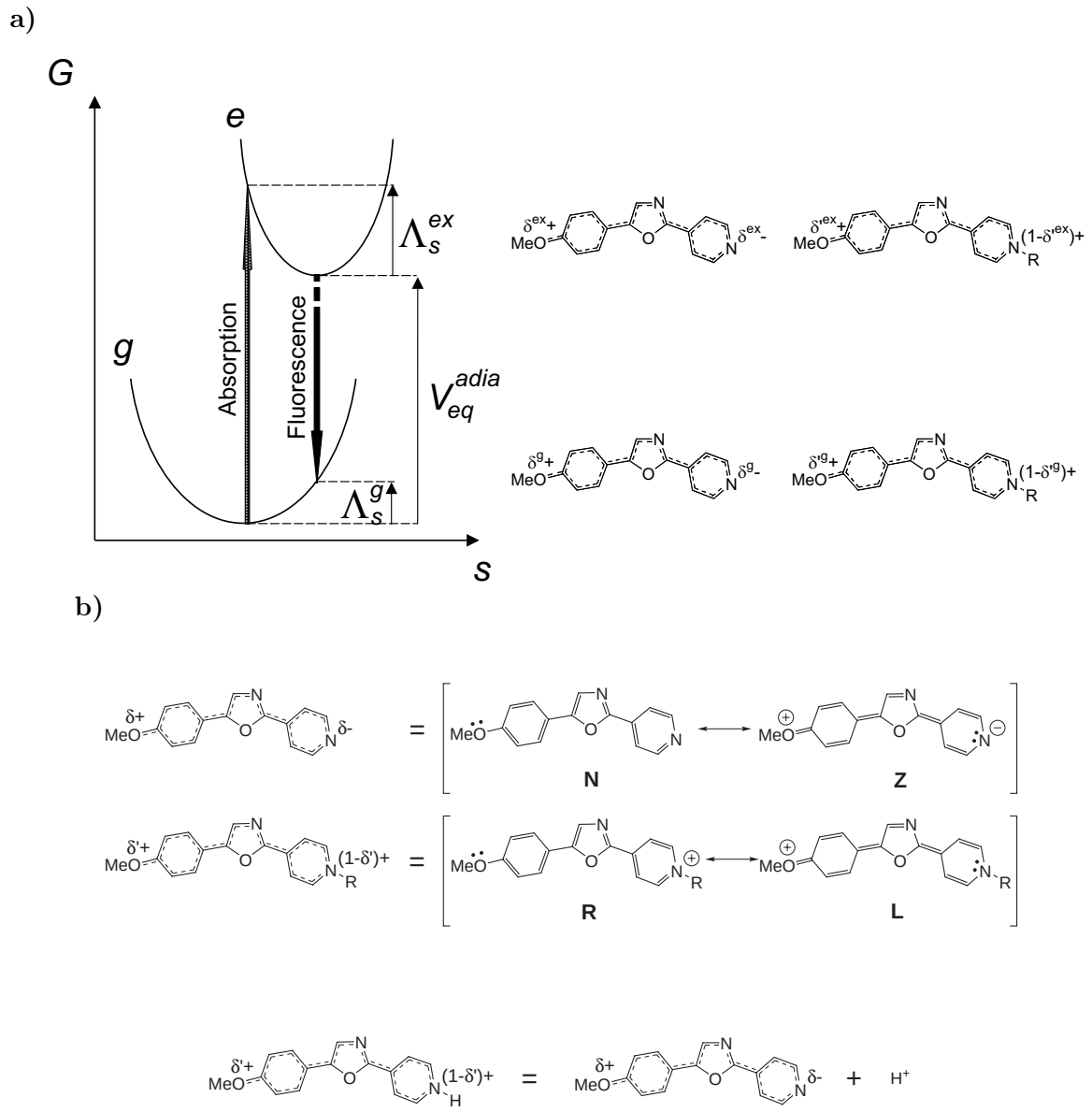


**Figure 14S**. **a**: Schematic picture of the electronic ground- and excited state free energy surfaces in the solvent coordinate that measures the slow nuclear polarization of the solvent [1] and ground and electronic states of representative basic and acidic states of the investigated oxazoles in the two valence bond description reported in **b**; **b**: Description of the ground and electronic states of representative basic and acidic states of the investigated oxazoles as a mixture of two states: **N** and **Z** for the neutral basic form, **R** and **L** for the positive acidic form.

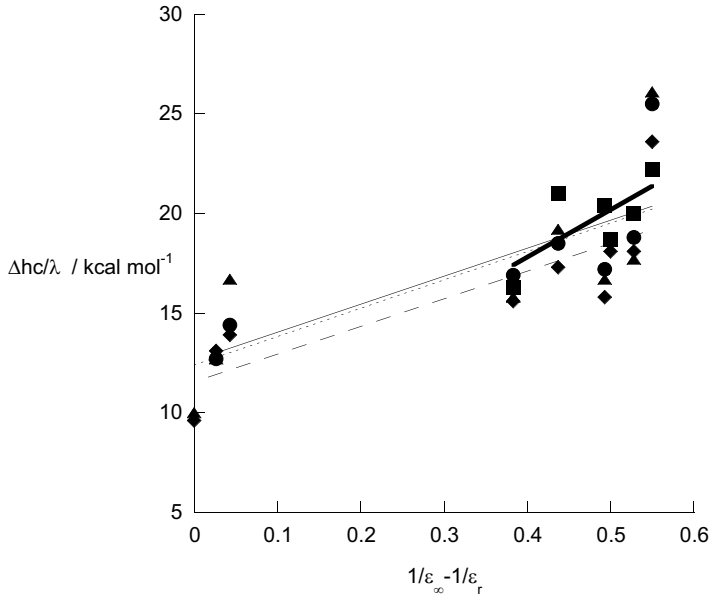


**Figure 15S**. Dependence of the Stokes Shift $\Delta E_{abs} - \Delta E_{em} = \Delta hc/\lambda$ on $\frac{1}{\varepsilon_\infty} - \frac{1}{\varepsilon_r}$ and data fit according to Eq.(1). **4-PYMPO** (circles; thin solid line), **4-PYMPOMe$^+$,TsO$^-$** (squares; thick solid line), **2-PYMPO** (diamonds; thin dashed line),**4-PYMPOH$^+$-NH$_2$** (triangles; thin dotted line), **4-PYMPOH$^+$** (large squares), **2-PYMPOH$^+$** (large diamonds), **4-PYMPOH$^+$-NH$_2$** (large triangles). T=298 K. See Table 1S.