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The Polarity Sensitivity Factor (PSF) of some Fluorescent Probe Molecules Used for Studying Supramolecular Systems and other Heterogeneous Environments

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Abstract

Fluorescence spectroscopy provides an excellent technique for investigating heterogeneous systems, due to its high sensitivity and the large effect of the local environment on molecular emission. In addition, the use of polarity-sensitive fluorescent probes as guests in supramolecular host-guest inclusion complexes can be exploited in fluorescent sensors. This paper identifies, tabulates, and quantifies a series of useful polarity-sensitive fluorescent probes, with a wide range of polarity-dependent fluorescence responses. The degree of polarity sensitivity is quantified using the Polarity Sensitivity Factor (PSF), developed in our laboratory. In most cases, such polarity-sensitive probes show increased emission as the local polarity is decreased (PSF>1); ten such probes are described. However, less commonly, "reverse polarity dependence" can occur in which probe emission decreases with decreasing polarity (PSF<1); four such probes are described. The mechanism for the observed polarity-induced fluorescence changes will also be discussed in selected representative cases. The purpose of this paper is to present details on a broad arsenal of polarity-sensitive fluorescence probes with varying properties, with potentially useful applications in the study of heterogeneous systems including inclusion phenomena, and in practical applications as fluorescent sensors, which will be useful to researchers studying supramolecular and other heterogeneous systems using fluorescence spectroscopy.

Keywords: fluorescence; fluorescent probes; polarity sensitivity; solvent effects; sensors

Introduction

Fluorescence spectroscopy[1] has been extensively and effectively applied to the study of heterogeneous environments,[2,3] such as proteins,[4] membranes[5] and other biological structures,[6] supramolecular inclusion systems,[7,8] and polymers.[9] In particular, polarity-sensitive fluorescent dyes such as anilinonaphthalene sulfonates and coumarins have been extensively used to probe the local environment in such heterogeneous systems, for example probing the local polarity, viscosity, or water accessibility.[10] In order to use fluorescence spectroscopy for these purposes, it is essential that an appropriate polarity-sensitive fluorescent probe be chosen.

In this article, a series of polarity-sensitive fluorescent probes will be evaluated for their spectroscopic properties, in particular their UV-visible absorption and emission properties, and the effect of solvent polarity[11] on these properties, including wavelength maxima (red-shift or blue-shift) and emission intensities (brightness). One of the most important features of polarity-sensitive fluorescent probes is the degree to which their emission intensity is affected by changes in polarity. Different fluorescent probes show very different sensitivity to polarity in terms of their emission intensity. In order to express this polarity sensitivity of fluorescence probes, we developed the concept of the "Polarity Sensitivity Factor", or PSF, as a quantitative expression of the degree to which the fluorescence emission of a given probe changes in a nonpolar relative to a polar environment.[8,12,13] The PSF is given by:

$$PSF = (F_{EtOH}/F_{H2O}) \times (A_{H2O}/A_{EtOH}) \quad (1)$$

where F_{EtOH} and F_{H2O} are the integrated fluorescence emission spectra of the fluorescent probe of interest in ethanol and water solution, respectively, and A is the absorbance of each

solution at the (identical) excitation wavelength. Water and ethanol were chosen as the polar and less polar solvents respectively because these types of polarity-sensitive probes were mainly used in our laboratory to study supramolecular host–guest inclusion complexes in aqueous solution, and the polarity of the internal cavity of the most common supramolecular hosts, cyclodextrins, is similar to that of ethanol solution.[14] The PSF is basically the fluorescence enhancement (or suppression) of the probe molecule in ethanol relative to water, and is directly related to the relative quantum yield in ethanol vs. water, differing only in the constant factor of the square of the relative refractive index of ethanol and water. For fluorescent probes which show increased fluorescence with decreased solvent polarity, the PSF will be greater than 1, but for those which show decreased fluorescence with decreased solvent polarity, the PSF will be less than 1. The relative polarity sensitivity of a given fluorescent probe is therefore indicated by the magnitude of the PSF. Knowledge of the PSF of a given probe, as well as its absorption and emission wavelengths, is essential for choosing that probe for a particular application.

There have been previous reviews on the utility of fluorescent probes for studying heterogeneous systems,[15-19] including specific families of fluorescent probes, such as anilinonaphthalene sulfonates[15] and coumarins.[19] There have also been other useful previous reviews of fluorescent probes, including an IUPAC report on fluorescent probes to be used as standards for fluorescence quantum yield measurements[20] and a review of fluorescent probes that are useful for the detection of reactive oxygen species.[21] This paper reports and tabulates for the first time the PSF values of a wide range of polarity-sensitive fluorescence probes, and serves as a useful resource for identifying appropriate probes for future applications in the fields of supramolecular and other heterogeneous chemistry.

Results and Discussion

A total of fourteen polarity-sensitive fluorescent probes were studied and quantified for this work. Of these fourteen, ten exhibited increased emission with decreasing solvent polarity (i.e. were more highly fluorescent in ethanol as compared to water solution at equal absorbance), while four exhibited decreased emission with decreasing solvent polarity (i.e. were more highly fluorescent in water as compared to ethanol solution at equal absorbance).

Fluorescent Probes which Exhibit Increased Emission with Decreasing Solvent Polarity

The majority of fluorescent probes studied fall into this category. Probes exhibiting this polarity dependence can be utilized in "switch-on" fluorescence sensors upon inclusion into an appropriate host cavity (or "switch-off" upon host exclusion by a competing guest of interest). The ten probes of this type studied and quantified for this work are shown in Figure 1 on the next page. The measured values of the absorption maximum in water, the emission maximum in water and in ethanol, and the PSF for these ten probes are listed in Table 1.

As can be seen from this table, the absorption maxima of these probes span the UV/visible spectrum, from 308 to 631 nm, allowing for a probe with PSF>1 to be chosen for any excitation range required. Furthermore, the emission spans a broad range from 375 to 680 nm, yielding a range of colours for sensor applications. There is also a wide range of PSF values; in most applications a PSF value as large as possible would be optimal. For the majority of these ten cases (**1**-**3**, **5**-**7**), the increase in fluorescence intensity upon decrease of solvent polarity is accompanied by a blue shift in the absorption spectrum. For example, in the case of 1,8-ANS **2**, the fluorescence maximum shifted from 560 nm in water to 473 nm in ethanol. In such cases the increase in emission can be attributed, at least in part, to the increased S_1-S_0 energy gap in ethanol as compared to water; by the energy gap law,[22] this

results in a significantly lower rate constant for internal conversion as a nonradiative decay path, and therefore a correspondingly larger quantum yield for fluorescence.

The largest PSF values (by far) are found for the well-known and highly-utilized anilinonaphthalene sulfonate family of fluorescent probes **1** to **3**. The incredibly high fluorescence polarity sensitivity of ANS probes has been well studied in the literature,[23-28] and has been explained as a combination of the effect of polarity on the S_1-S_0 energy gap and specific solvent-solute interactions in protic solvents.[25] In the specific case of 1,8-ANS 2, Ebbesen and Ghiron[25] concluded that in mixed water-ethanol solutions, the fluorescence wavelength is dependent on the macroscopic solvent polarity, while the quantum yield is dependent on the specific interactions of the fluorophore with water molecules in the mixed solvent. Thus, the large PSF values observed here can be attributed to the very low fluorescence quantum yield of the ANS probes in water, due to direct water-probe interactions which depopulate the S_1 excited state and hence reduce the fluorescence emission.

Our research group has done extensive studies of 1,8-ANS **2** and 2,6-ANS **3** as probes of the supramolecular host-guest inclusion complexes of both cyclodextrins[12,29] and cucurbit[n]urils.[30-31] Figure 2 shows a photograph of 1,8-ANS **2** in the absence and presence of hydroxypropyl-β-cyclodextrin, illustrating the large effect of supramolecular host inclusion on the emission intensity of this probe in water, and its potential for application in switch-on or switch-off sensor technology. The effect of inclusion on emission wavelength (spectral shift) is also seen in this figure, with the colour changing from a pale green in water to a bright blue when complexed with the cyclodextrin. The largest PSF measured was that for the ANS dimer probe bis-ANS **1,**[32] which had a PSF of 216 ± 9. This means that bis-ANS is 216 times more fluorescent in ethanol than it is in water, which gives an incredibly large and easily measured signal change. As can be seen from Figure 1, bis-ANS itself is a

relatively large probe molecule, which would be most suitable for investigating large host cavities, or protein structures. 1,8-ANS and 2,6-ANS on the other hand are smaller, and are the appropriate size for investigation of the most common families of supramolecular hosts, including cyclodextrins and cucurbit[n]urils. Moreover, as we reported previously[12] 1,8-ANS and 2,6-ANS also have significant PSF values of 197 and 120, respectively, and have different shapes, with 2,6-ANS being more streamlined, and suited for narrow host cavities or material channels. Thus, the ANS family of fluorescent probes is particularly well suited for applications in fluorescent sensor design, as well as for the study of heterogeneous systems, including supramolecular host-guest complexation.

Two eosin dyes[33-36] were studied, eosin B **4** and eosin Y **9**. As can be seen in Figure 1, these dye molecules differ only in the replacement of two bromo substituents in eosin Y by nitro substituents in eosin B. However, as can be seen in Table 1, they have very different fluorescence properties, and most dramatically, highly different polarity sensitivities, arising from the presence or absence of the nitro groups. Eosin B is highly sensitive to polarity, with a PSF of 48 ± 14, whereas eosin Y shows a much lower sensitivity to polarity, with a PSF of 2.7 ± 0.2. Eosin B fluoresces more to the red than does eosin Y, but the two have similar absorption properties. In general, eosin B is the preferred choice for studying heterogeneous systems or for sensor applications, as it shows a much greater change in emission intensity with change in polarity.

Curcumin **5** is an extremely interesting molecule.[37-39] It is the main component of the spice turmeric, and a major component of curry powders. It has extensive bioactive properties, including antibiotic and anticancer properties. It is highly fluorescent, and hence turmeric itself exhibits strong, visible green fluorescence under UV irradiation, especially when dissolved in ethanol, as shown in Figure 3. As seen in Figure 1, curcumin has a symmetrical, elongated structure which makes it an interesting guest for host complexation: it

tends to form 2:1 host:guest complexes in which a host encapsulates each end of the molecule; this 2:1 complexation was observed in the case of both cyclodextrin[40] and cucurbit[n]uril[13] hosts. It also shows a high polarity sensitivity, with a PSF value of 39 ± 2, as reported by our group previously.[13] Its high degree of polarity sensitivity, long symmetrical shape and tendency to form 2:1 host:guest inclusion complexes make curcumin a distinctive fluorescent probe, with potential specific utility in unique types of sensor applications.

The other polarity sensitive probes which show increased fluorescence in decreased polarity all have PSF values of 10 or less. Thus, while potentially useful as probes of heterogeneous environments, these probes would be less appealing in sensor technologies, as the signal changes obtained upon host inclusion or exclusion would not be as large as those obtained using any of the probes described above. However, each has a unique shape and fluorescence properties, which may potentially make them more useful in specific applications. Nile Blue A **6**[41-43] is an oxazine dye, closely related to the more commonly used Nile Red. However, whereas Nile Red is insoluble in water (thus not allowing for a determination of its PSF), Nile Blue A is water soluble. In general, oxazine dyes have been used as laser dyes and in other optical applications, and show significant solvent-dependent fluorescence.[41] Nile Blue A shows a moderate polarity sensitivity, with a measured PSF of 9.8 ± 1.7. It also shows the longest-wavelength absorption and emission of all of the ten probes studied in this section, which is potentially useful for the development of sensors in the red region of the visible spectrum.

Dansyl lysine **7** is a fluorescent probe used for studying membrane structures, and has been proposed as a highly selective probe for cholesterol-free domains within membranes.[44] It shows a moderately strong PSF of 10 ± 2. As an illustration of this type of probe which shows increased fluorescence in decreased polarity, the fluorescence spectrum of dansyl lysine in water and in ethanol is shown in Figure 4; this figure clearly shows the increased

emission intensity in ethanol as compared to water, giving a PSF of an order of magnitude in this case. The blue shifting of the emission spectrum in ethanol relative to water is also clearly illustrated in this figure, in this case from 575 nm in water to 536 nm in ethanol.

The coumarins are a well-studied family of fluorescent probes,[19,45] with widely varying fluorescence properties and solvatochromism. In fact, coumarins can exhibit either increased or decreased fluorescence emission upon decreased polarity. The example studied in this section, which shows an increased fluorescence upon decreasing solvent polarity, is the parent coumarin itself, **8**.[46] This is a relatively small fluorescent probe, suitable for small host cavities or heterogeneous pockets, and exhibits a PSF of 3.5 ± 0.5. Note that a coumarin with the opposite polarity sensitivity is described in the next section.

The final probe studied which showed increased fluorescence in ethanol as compared to water is the interesting aromatic hydrocarbon azulene **10**.[47-49] This molecule shows a beautiful blue fluorescence, and is interesting as the best-known example of an exception to Kasha's Rule: its fluorescence originates from the S_2 higher excited singlet state, whereas most molecules exhibit measurable fluorescence only from their first excited state, S_1 (Kasha's Rule).[1,49] Thus, azulene is an ideal probe for investigating the effects of supramolecular host inclusion or other heterogeneous systems on higher excited states. In addition, azulene is extremely small and compact as compared to the other probes investigated, other than coumarin, and thus is ideally suited for investigating small host cavities of material pores, and in compact molecular sensors based on small molecular hosts. A very small PSF of 1.5 ± 0.2 was measured for azulene; although this is relatively small, this still represents a 50% increase in the S_2 fluorescence in ethanol as compared to water, making azulene a potentially useful upper excited state polarity-sensitive fluorescent probe.

Fluorescent Probes which Exhibit Decreased Emission with Decreasing Solvent Polarity

Only four of the fluorescent probes studied (**11**-**14**) exhibited this "reverse" polarity dependence of decreased intensity with decreased polarity; their structures are shown in Figure 5. Probes exhibiting this polarity dependence can be utilized as fluorescent "switch-off" inclusion sensors, or more importantly as "switch-on" fluorescent sensors upon exclusion from an appropriate host cavity. Thus, in many ways, this rarer type of polarity-sensitive fluorescent probe is of greater potential for supramolecular fluorescence sensor applications, as the sensor can be designed to switch its fluorescence on upon inclusion of a target competing species, which can itself be non-fluorescent. It is better to have such a switch-on sensor which is dark in the absence of the target molecule, but switches on in its presence, as this is much easier to measure, and the baseline (null response) is extremely small.

7-Alkoxycoumarins such as 7-methoxycoumarin (7MC, **11**) are probably the best-known examples of fluorescent guests which show the reverse polarity sensitivity of most probes,[50-51] namely showing decreased emission in decreased polarity. We have previously reported extensive studies of the host-guest inclusion of 7MC into various CDs.[52] 7-MC showed the smallest value for a PSF less than 1, namely 0.09 ± 0.02; thus the emission of 7MC in water is approximately ten times larger than it is in ethanol. Furthermore, the absolute value of the fluorescence quantum yield ϕ_F of 7-MC in water is relatively high, with a value of 0.21 ± 0.02 as determined in this work. This makes it a highly useful probe for potential sensor applications, particularly as a switch on exclusion probe, as both the change in intensity, and the intensity itself in the "on" configuration (exclusion into water), will be large and easily measurable. We have previously studied the application of 7MC in a prototype drug delivery fluorescent sensor system, based on polymer stars with cyclodextrin

cores.[53] However, the use of 7MC in biomedical sensor applications is severely limited, as it is known to be toxic to humans.[54]

The mechanism for the increased fluorescence of 7MC in water as compared to ethanol is (not unexpectedly) quite different for that of the probes which show decreased fluorescence in water as compared to ethanol, the latter of which involved mainly the effect of the decreased S_1-S_0 energy gap on the rate of internal conversion back to the ground state. In the case of 7MC, the proposed mechanism involves the existence of a $\pi\pi^*$ triplet state T_2 in close proximity in terms of energy to the $\pi\pi^*$ S_1 state in less polar environments such as ethanol. This results in a highly efficient ($^1\pi\pi^* \rightarrow {}^3\pi\pi^*$) intersystem crossing (ISC) process from the excited singlet to the nearby triplet state, resulting in a significant competing process with fluorescence for depopulation of the excited state, and hence a low fluorescence quantum yield.[50] However, in a polar medium such as water, the energy of the S_1 state is lowered significantly below that of the T_2 state, and thus ISC can occur only to the $n\pi^*$ T_1 state, which is a much less efficient ISC process ($^1\pi\pi^* \rightarrow {}^3n\pi^*$). Thus, the fluorescence quantum yield is higher in water than in ethanol ("reverse" polarity dependence), and a PSF < 1 is observed.

7-Azaindole (7AI, **12**)[55-60] is another example of a fluorescent probe which shows decreased emission with decreased polarity. It is highly important in biochemical sciences, as it is the fluorophore of 7-azatryptophan, a derivative of the essential amino acid tryptophan which exhibits more favourable spectroscopic properties than does tryptophan itself, including longer wavelength absorption and longer fluorescence lifetime.[57] The emission spectrum of 7AI in water and in ethanol is shown in Figure 6; the enhanced fluorescence of 7AI in water as compared to ethanol is clearly seen in this Figure. The PSF of 7AI was determined to be 0.34 ± 0.04. Thus, 7AI is a potentially useful fluorescent probe for a switch on sensor based on host exclusion in aqueous solution, however its PSF is significantly closer

to 1 than is the case for 7MC, i.e. it has a lower polarity sensitivity. Its fluorescence intensity is 3 times larger in water than in ethanol, compared to 7MC for which the fluorescence intensity is 10 times larger in water than in ethanol. Furthermore, the fluorescence quantum yield ϕ_F of 7AI in water was determined to be 0.031 ± 0.004, significantly lower than that of 7MC. However, as is clear from its MSDS sheet, 7AI is nontoxic, and so can be used in medicinal sensor applications.

The potential usefulness of 7-azaindole as a nontoxic polarity-sensitive probe with higher fluorescence in higher polarity media led us to explore the fluorescence properties of N-methyl-7-azaindole (NM7AI, **13**). It has been shown in the literature that a significant excited state decay pathway of 7AI in water is the result of the interaction of the NH group with solvent water molecules, and that this deactivation pathway can be removed by alkyl substitution at the amine nitrogen to yield N-methyl-7-azaindole.[55] The fluorescence quantum yield ϕ_F of NM7AI in water was determined to be 0.58 ± 0.05, significantly higher than that of 7AI, and in fact twice as large as that of 7MC. Thus, sensors designed using NM7AI would potentially have a very bright "on" configuration. Unfortunately, however, the PSF of NM7AI was even closer to 1 than in the case of 7AI, with a value of 0.56 ± 0.01. Thus, the fluorescence of NM7AI in water is slightly less than a factor of 2 more intense than that in ethanol.

As a final example of a probe in this category of reverse polarity sensitivity, anthracene-9-carboxylic acid (A9C) **14** was studied.[61-65] This is a highly complex molecule in aqueous solution, as it not only establishes an acid-base equilibrium, but it has also been shown in the literature that significant dimerization occurs, with the dimer having significantly different fluorescence properties than the monomer.[61-63] Photodimerization has also been shown to occur.[64] Our measurements revealed a moderate decrease in the total fluorescence intensity in ethanol as compared to water, with a PSF = 0.58 ± 0.04; thus the

increase in integrated fluorescence intensity of A9C in water relative to ethanol is less than a factor of 2. Due to this relatively small change in emission, and the extremely complicated chemical equilibria, photophysics and photochemistry involved, this probe was not studied further, and is not recommended for applications in the study of heterogeneous media or fluorescence sensor design.

Experimental

(4,4'-dianilino)-1,1'binaphthyl-5,5'disulfonic acid, dipotassium salt (bis-ANS, 1) and N-e-(5-dimethylaminonaphthalene-1-sulfonyl)-L-lysine (dansyl lysine, 7) were received from Molecular Probes, Inc. and used as received. Eosin B (4), Nile Blue A (6), coumarin (8), eosin Y (9), azulene (10), 7-methoxycoumarin (11), 7-azaindole (12), and anthracene-9-carboxylic acid (14) were received from Sigma-Aldrich, Ltd. and used as received. N-methyl-7-azaindole (13) was prepared according to the literature method.[55,66] Analysis of 13: 1H NMR (300 MHz, $CDCl_3$) δ = 8.25 (dd, J = 4.5, 1.3 Hz, 1H), 7.82 (dd, J = 7.8, 1.5 Hz, 1H), 7.11 (d, J = 3.6 Hz, 1H), 6.97 (dd, J = 7.8, 4.5 Hz, 1H), 6.36 (d, J = 3.6 Hz, 1H), 3.81 (s, 3H).

All absorption measurements were performed using a CARY Bio UV-Vis spectrophotometer manufactured by Varian. The wavelength of maximum absorption for the first (S_0 to S_1) absorption band was recorded as $\lambda_{A,max}$. Solutions for emission measurements were prepared to give an absorbance at the excitation wavelength (determined based on the absorption spectra) near 0.30 a value high enough for sufficient excitation of the samples but low enough to prevent self-absorption and other non-linear effects. Emission scans were performed on a Perkin Elmer (PE) LS 55 Fluorescence Spectrometer or a Photon Technologies International (PTI) RF-M2004 Fluorescence Spectrometer. All fluorescence emission spectra were corrected for the instrument detector response. The area underneath the

resulting fluorescence curve was recorded as the total fluorescence (F) and the wavelength at which maximum fluorescence occurred was recorded as $\lambda_{F,max}$. Blank scans were also performed on nanopure water and ethanol. The PSF was then calculated using Equation 1 (which takes into account difference in absorbance between the water and ethanol solutions in each case). Fluorescence quantum yields were determined using the relative method, using 9,10-diphenylanthrace in cyclohexane as the fluorescent standard ($\phi_F = 0.90$).[20]

Conclusions

A total of 14 polarity-sensitive fluorescent probes were studied and quantified in this work, with a broad range of polarity responses, and absorption and emission wavelength ranges. All of these probes have potential applications in the study of heterogeneous systems and the design of supramolecular fluorescent sensors. PSF was shown to be a useful measure of the degree of polarity sensitivity of fluorescent probes, and allowed for the quantification of the relative polarity sensitivity of each probe.

Ten of the probes studied showed the more common fluorescence polarity sensitivity of exhibiting increased intensity upon decrease in polarity (PSF > 1). Of these, the anilinonaphthalene sulfonates showed by far the greatest polarity sensitivity, with PSF values ranging from 120 in the case of 2,6-ANS to 216 in the case of bis-ANS. These ANS probes would be most useful in supramolecular fluorescent sensor design in which a “switch-off” response is desired upon exclusion from the host cavity. However, the seven other probes studied with this type of polarity sensitivity are also potentially useful, with PSF values ranging from 1.5 to 45. All of these probes have unique shapes, sizes, and spectral properties which might be useful for specific applications; for example the size and shape can be chosen to give an optimal fit with a specific material or host cavity size and shape to be investigated or utilized.

A number of polarity–sensitive probes showing higher emission in water than in ethanol ("reverse polarity sensitivity") were also studied. The quantum yield and PSF varied greatly, with the probe showing the highest quantum yield (NM7AI) showing the least significant PSF. The most useful probe of this type would be 7MC, which showed the highest degree of polarity sensitivity (PSF = 0.09) and a significant quantum yield. 7MC would be most useful in supramolecular fluorescent sensor design in which a "switch-on" response is desired upon exclusion from the host cavity. However, it is known to be toxic, so in the case of biomedical sensor application (for example involving drug delivery), 7AI or NM7AI are recommended.

Overall, these 14 polarity sensitive fluorescent probes and their details presented herein present a useful arsenal of probes for researchers studying a wide range of fluorescence applications.

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References

(1) Lakowicz, J.R. Principles of Fluorescence Spectroscopy, 3rd Ed.: Springer, Berlin, **2006**.

(2) Applied Fluorescence in Chemistry, Biology and Medicine, Eds.: W. Rettig, B. Strehmel, S. Schrader, H. Seifert: Springer, Berlin, **1999**.

(3) B. Valeur in Molecular Luminescence Spectroscopy, Part 3, Ed.: S. G. Schulman; Wiley- Interscience: **1993.**

(4) Hawe, A.; Sutter, M.; Jiskoot, W. *Pharmaceut. Res.* **2007**, *25*, 1487.

(5) Epand, R.M.; Kraayenhof, R. *Chem. Phys. Lipids* **1999**, *101*, 57.

(6) Terai, T.; Nagano, T. *Curr. Opin. Chem. Biol.* **2008**, *12*, 515.

(7) Wagner, B.D. Chapter 1 in Handbook of Photochemistry and Photobiology, Volume 3: Supramolecular Photochemistry, H.S. Nalwa, Ed.; American Scientific Publishers: Stevenson Ranch, CA **2003**, pp. 1–57.

(8) Wagner, B.D. Chapter 2 in Cyclodextrins Materials Photochemistry, A. Douhal, Ed.; Elsevier B.V.: Netherlands, **2006**, pp. 27–59.

(9) Bosch, P.; Catalina, F.; Corrales, T.; Peinado, C. *Chem. Eur. J.* **2005**, *11*, 4314-.

(10) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319.

(11) Katritzky, A.R; Fara, D.C.; Yang, H.; Tämm, K. *Chem. Rev.* **2004**, *104*, 175.

(12) Wagner, B.D.; Fitzpatrick, S.J. *J. Inclus. Phenom. Macro. Chem.* **2000**, *38*, 467.

(13) Rankin, M.A.; Wagner, B.D. *Supramol. Chem.* **2004**, 16, 513.

(14) Cox, G.S.; Turro, N.J.; Yang, N.C.; Chen, M.-J. *J. Am. Chem. Soc.* **1984**, *106*, 422.

(15) Slavík, J. *Biochim. Biopys. Acta* **1982**, *694*, 1.

(16) Johnston, L; Wagner, B. Chapter 13 in Physical Methods in Supramolecular Chemistry, J. Eric D. Davies and J. Ripmeester, Eds.; Pergamon Press: Oxford, UK, **1996** (Volume 8 of Comprehensive Supramolecular Chemistry).

(17) Vaz, W.L.C.; Melo, E. *J. Fluoresc.* **2001**, *11*, 255.

(18) Kapanidis, A.N.; Weiss, S. *J. Chem. Phys.* **2002**, *117*, 10953.

(19) Wagner, B.D. *Molecules* **2009**, *14*, 210.

(20) Eaton, D.F. *Pure & Appl. Chem.* **1988**, *60*, 1107.

(21) Gomes, A.; Fernandes, E.; Lima, J.L.F.C. *J. Biochem. Biophys. Methods* **2005**, *65*, 45.

(22) Englman; R.; Jortner, *Molec. Phys.* **1970**, *18*, 145.

(23) Kosower, E.M.; Dodiuk, H; Kanety, H. *J. Am. Chem. Soc.* **1978**, *100*, 4179.

(24) Robinson, G.W.; Robbins, R.J.; Fleming, G.R.; Morris, J.M.; Knight, A.E.W.; Morrison, R.J.S. *J. Am. Chem. Soc.* **1978**, *100*, 7145.

(25) Ebbesen, T.W.; Ghiron, C.A. *J. Phys. Chem.* **1989**, *93*, 7139.

(26) Mendz, G.L.; Vandenberg, R.J.; Easterbrook-Smith, S.B. *Magnet. Res. Chem.* **1990**, *28*, 104.

(27) Jordts, M.; Kerth, A.; Drescher, S.; Ott, M.; Blume, A. *J. Colloid Interface Sci.* **2017**, *501*, 294.

(28) Städe, L.W.; Nielsen, T.T.; Duroux, L.; Wimmer, R.; Shimizu, K.; Larsen, K.L. *Beilstein J. Org. Chem.* **2015**, *11*, 514.

(29) Wagner, B.D.; MacDonald, P.J. *J. Photochem. Photobiol. A:Chem.* **1998**, *114*, 151.

(30) Wagner, B.D.; Fitzpatrick, S.J.; Gill, M.A.; MacRae, A.I.; Stojanovic, N. *Can. J. Chem.* **2001**, *79*, 1101.

(31) Wagner, B.D.; Stojanovic, N.; Day, A.I.; Blanch, R.J. *J. Phys. Chem.* **2003**, *107*, 10741.

(32) Musci, G.; Metz, G.D.; Tsunematsu, H.; Berliner, L. *J. Biochem.* **1985**, *24*, 2034.

(33) Robertson, T.A.; Bunel, F.; Roberts, M.S. *Cells* **2013**, *2*, 591.

(34) Acharya, S.; Rebery, B. *Arab. J. Chem.* **2009**, i, 7.

(35) Yan, X.; Chen, Z.; Cui, Y.; Hu, Y.; Yu, R. *Anal. Chim. Acta* **2016**, *921*, 38.

(36) Shi, H,; Cui, Y.; Gong, Y.; Feng, S. *Spectrochim. Acta, Part A* **2016**, *161*,150.

(37) Khopde, S.M.; Priyadarsini, K.I.; Palit, D.K.; Mukherjee, T. *Photochem. Photobiol.* **2000**, *72*, 625.

(38) Salem, M.; Rohani, S.; Gillies, E.R. *RSC Adv.* **2013**, *4*, 10815.

(39) Paul, S.; Panda, A.K. *RSC Adv.* **2014**, *4*, 32383.

(40) Baglole, K.N.; Boland, P.G.; Wagner, B.D. *J. Photochem. Photobiol. A:Chem.* **2005**, *173*, 230.

(41) Tajalli, H.; Ghanadzadeh Gilani, A.; Zakerhamidi, M.S.; Tajalli, P. *Dyes and Pigments* **2008**, *78*, 15.

(42) Wang, X.; Sun, R.; Zhu, W.; Sha, X.; Ge, J. *J Fluoresc.* **2017**, *27*, 819.

(43) Mironov, L.Y. *Opt. Spectrosc.* **2016**, *121*, 867.

(44) Humphries, G.M.K.; Lovejoy, J.P. *Biophys. J.* **1983**, *42*, 307.

(45) Mattoo, B.N. *Trans. Faraday Soc*. **1956**, *52*, 1184.

(46) Song, P.-S.; Gordon, W.H. III. *J. Phys. Chem.* **1970**, *74*, 4234.

(47) Binsch, G.; Heilbronner, E.; Jankow, R.; Schmidt, D. *Chem. Phys. Lett.* **1967**, *1*, 135.

(48) Wagner, B.D.; Tittelbach-Helmrich, D.; Steer, R.P. *J. Phys. Chem.* **1992**, *96*, 7904.

(49) Itoh, T. *Chem. Rev.* **2012**, *112*, 4541.

(50) Muthuramu, K.; Ramamurthy, V. 7-alkoxy *J. Photochem.* **1984**, *26*, 57.

(51) Żamojć, K.; Wiczk, W.; Zaborowski, B.; Jacewicz, D.; Chmurzyński, L. *J Fluoresc.* **2014**, *24*, 713.

(52) Wagner, B.D.; Fitzpatrick, S.J.; McManus, G.J. *J. Inclus. Phenom. Macro. Chem*. **2003**, *47*, 187.

(53) Tennekone, G.K.; Wagner, B.D.; Shaver, M.R. *Green Materials* **2014**, *2*, 31.

(54) Vassallo, J.D.; Hicks, S.M.; Daston, G.P.; Lehman-McKeeman, L.D. *Toxicolog. Sci.* **2004**, *80*, 249.

(55) Avouris, P.; Yang, L. L.; El-Bayoumi, M. A. *Photochem. Photobiol.* **1976**, *24*, 211.

(56) Chen, Y.; Rich, R.L. Gai, F. Petrich, J.W. *J. Phys. Chem.* **1993**, *97*, 1770.

(57) Smirnov, A.V.; English, D.S.; Rich, R.L.; Lane, J.; Teyton, L.; Schwabacher, A.W.; Luo, S.; Thornburg, R.W.; Petrich, J.W. *J. Phys. Chem. B* **1997**, *101*, 2758.

(58) Zhao, S.-B.; Wang, S. *Chem. Soc. Rev.* **2010**, *39*, 3142.

(59) Mehata, M.S.; Singh, A.K.; Sinha, R.K. *J. Mol. Liq.* **2017**, *231*, 39.

(60) Carmona, C.; García-Fernández, E.; Hidalgo, J.; Sánchez-Coronilla, A.; Balón, M. *J Fluoresc.* **2014**, *24*, 45.

(61) Ito, Y.; Fujita, H. *J. Org. Chem.* **1996**, *61*, 5677.

(62) Momiji, I.; Yoza, C.; Matsui, K. *J. Phys. Chem. B* **2000**, *104*, 1552.

(63) Abdel-Mottaleb, M.S.A.; Galai, H.R.; Dessouky, A.F.M.; El-Naggar, M.; Mekkawi, D.; Ali, S.S.; Attya, G.M. *Int. J. Photoenergy* **2000**, *2*, 47.

(64) Zhu, L.; Al-Kaysi, R.O.; Dillon, R.J.; Tham, F.S.; Bardeen, C.J. *Cryst. Growth Des*. **2011**, *11*, 4975.

(65) Rodríguez-Córdoba, W.; Noria-Moreno, R.; Navarro, P.; Peon, J. *J. Lumin.* **2014**, *145*, 697.

(66) Robison, M.M.; Robison, B.L. *J. Am. Chem. Soc.* **1955**, *77*, 6554.

Figure Captions

Fig. 1. The structures of the ten fluorescent probes studied which show increased fluorescence in ethanol as compared to water (PSF > 1).

Fig. 2. The fluorescence of 1,8-ANS in water under 350 nm UV illumination in the absence (left) and presence (right) of 10 mM hydroxypropyl-β-cyclodextrin.

Fig. 3. The fluorescence of turmeric powder dropped into ethanol under 350 nm UV illumination.

Fig. 4. The fluorescence spectrum of dansyl lysine in water (H_2O, red) and in ethanol (EtOH, blue).

Fig. 5. The structures of the four fluorescent probes studied which show decreased fluorescence in ethanol as compared to water (PSF < 1).

Fig. 6. The fluorescence spectrum of 7-azaindole in water (H_2O, red) and in ethanol (EtOH, blue).

Table 1. The spectroscopic parameters and PSF of fluorescent probes which exhibit increased emission in ethanol as compared to water (PSF > 1). PSF error limits represent one standard deviation of at least 3 replicate trials cases.

#	Name	$\lambda_{A,max}$/nm	$\lambda_{F,max,H2O}$/nm	$\lambda_{F,max,EtOH}$/nm	PSF
1	bis-ANS	386	561	500	216 ± 9
2	1,8-ANS[a]	352	560	473	197
3	2,6-ANS[a]	319	470	414	120
4	eosin B	520	548	585	48 ± 14
5	curcumin[b]	425	568	554	39 ± 2
6	Nile Blue A	631	680	663	9.8 ± 0.7
7	dansyl lysine	327	575	536	10 ± 2
8	coumarin	308	390	486	3.5 ± 0.5
9	eosin Y	518	542	550	2.7 ± 0.2
10	azulene	338	375	376	1.5 ± 0.2

[a]From reference 12.

[b]From reference 13.

Table 2. The spectroscopic parameters and PSF of fluorescent probes which exhibit decreased emission in ethanol as compared to water (PSF < 1). PSF error limits represent one standard deviation of at least 3 replicate trials in all cases.

#	Name	$\lambda_{A,max}$/nm	$\lambda_{F,max,H2O}$/nm	$\lambda_{F,max,EtOH}$/nm	PSF
11	7-methoxycoumarin	322	391	388	0.09 ± 0.02
12	7-azaindole	288	382	355	0.34 ± 0.04
13	N-methyl-7-azaindole	288	395	372	0.56 ± 0.01
14	Anthracene-9-carboxylic acid	365	410	411	0.58 ± 0.04

Figure 1

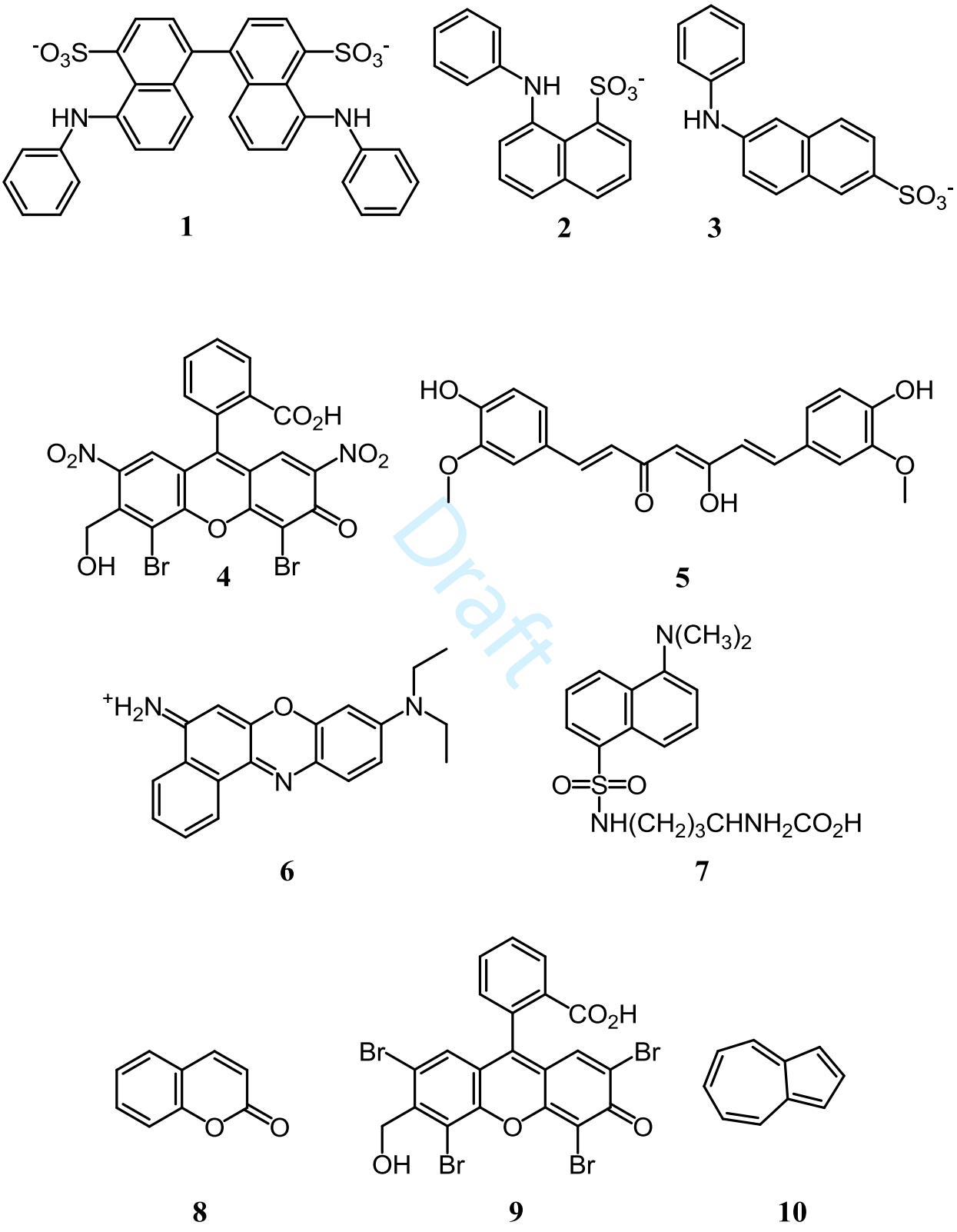


Figure 2

Figure 3

Figure 4

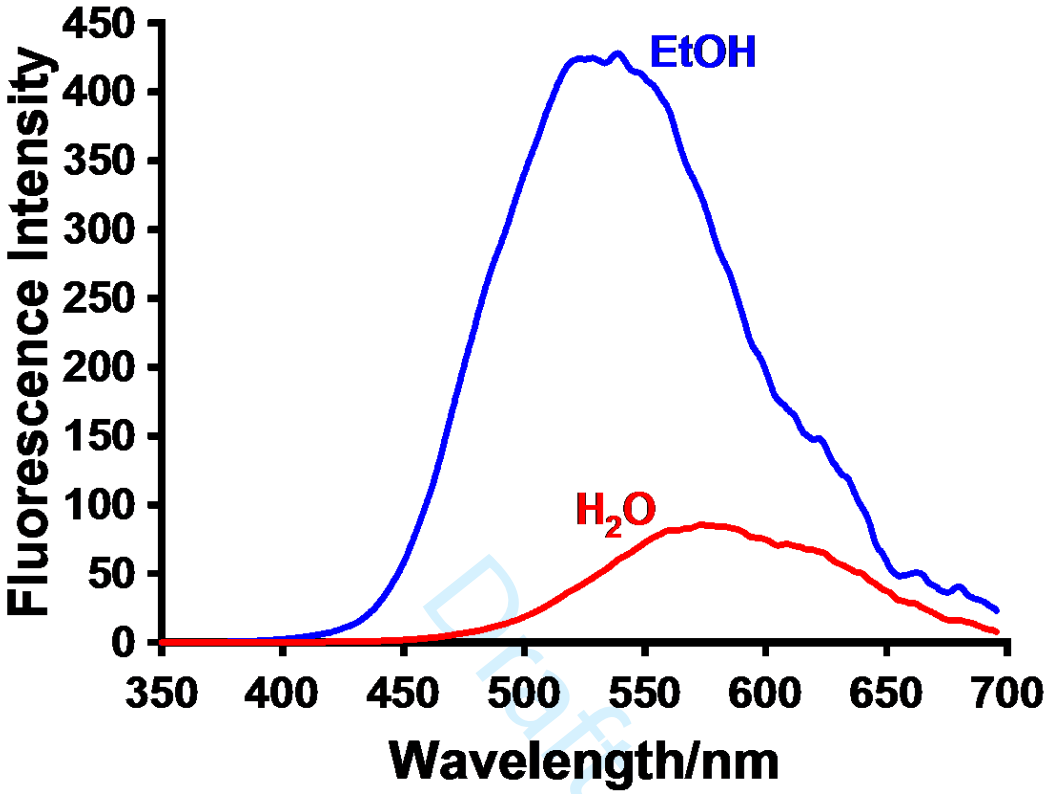
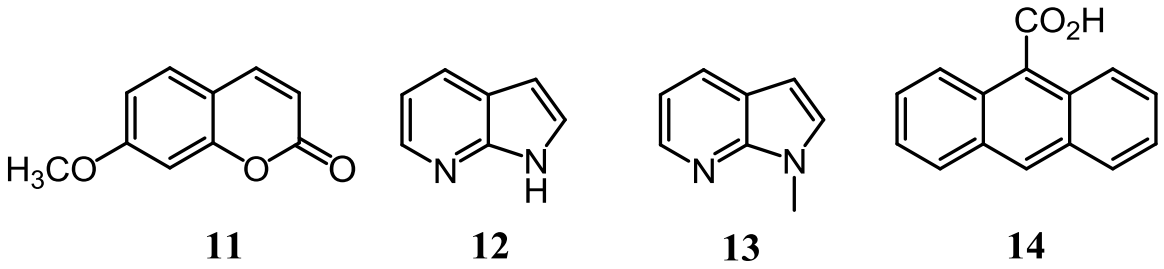


Figure 5



11 **12** **13** **14**

Figure 6