Solvatochromic Behavior of a Sterically Hindered 4-aminonaphthalimide Dye

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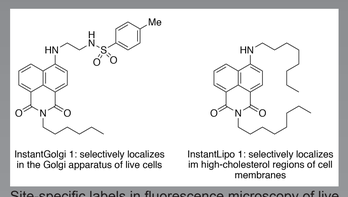
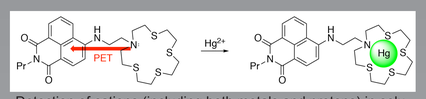
Uses of Naphthalimides

The 4-amino-1,8-naphthalimide fluorophore has been a popular scaffold for the synthesis of molecules for use as: 1) site-specific labels in fluorescence microscopy of live cells; 2) Detection of cations (including both metals and protons) in solution and in live cells; 3) sensors for cations, anions and biomolecules; and 4) logic gates. The development of sensors has focused on the formation of constructs of the general type "receptor-spacer-fluorophore" and its more complex derivatives as laid out by de Silva early this century.



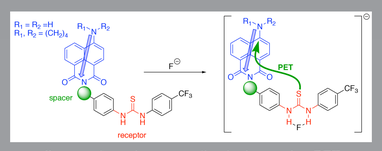
Site-specific labels in fluorescence microscopy of live cells



Detection of cations (including both metals and protons) in solution and acidic organelles (e.g., lysosomes)

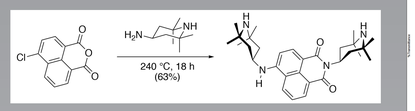
The cation sensor at left becomes luminescent in the presence of the cation or a proton due to loss of photoinduced electron transfer (PET) quenching by the lone pair on the nitrogen. Complexation of the lone pair by the metal ion or a proton removes this quenching, with the result that the fluorescence emission is enhanced. This is an example of a "turn-on" sensor.

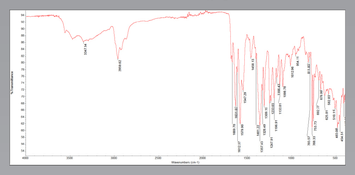
The anion sensor at right is highly luminescent in the absence of anions capable of forming hydrogen bonds to the thiourea moiety (fluortide, acetate, phosphate, etc.). Binding of the anion to the receptor group increases the available electron density on sulfur, and thus facilitates PET quenching. This is an example of a "turn-off" sensor.

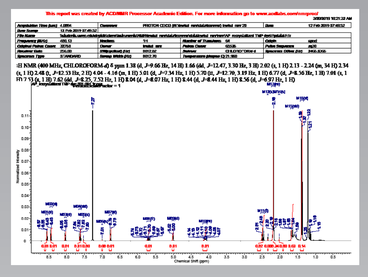


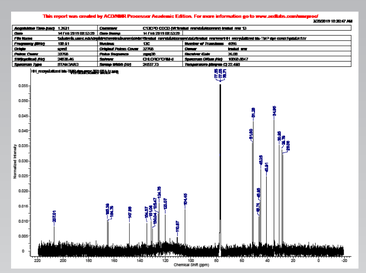
Sensors for anions operating by increasing PET on binding to the thioiurea receptor

Synthesis and Characterization of the Prototype Naphthalimide









The *bis*-TMP dye was prepared from freshly recrystallized 4-chloro-1,8-naphthalic anhydride and 4-amino-2,2,6,6-tetramethylpiperidine (TMP-NH_2) by heating a stoichiometric mixture to 240°C overnight. The product was isolated from the hydrochloride salt with sodium hydroxide in aqueous methanol. The product isolated from the resultant material was extracted with dichloromethane. The compound thus obtained was recrystallized in aqueous ethanol. The IR, ^{1}H NMR, and ^{13}C NMR spectra were in accord with the proposed structure.

IR (cm^{-1}): 3347, 2959 (NH), 1669, 1631 (C=O), 1579, 1547 (aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.38 (d, *J*=9.66 Hz, 14 H) 1.66 (dd, *J*=12.47, 3.30 Hz, 3 H) 2.02 (s, 1 H) 2.13 - 2.24 (m, 34 H) 2.34 (s, 1 H) 2.48 (t, *J*=12.53 Hz, 2 H) 4.04 - 4.16 (m, 1 H) 5.01 (d, *J*=7.34 Hz, 1 H) 5.70 (tt, *J*=12.70, 3.19 Hz, 1 H) 6.77 (d, *J*=8.56 Hz, 1 H) 7.01 (s, 1 H) 7.53 (s, 1 H) 7.62 (dd, *J*=8.25, 7.52 Hz, 1 H) 8.04 (d, *J*=8.07 Hz, 1 H) 8.44 (d, *J*=8.44 Hz, 1 H) 8.56 (d, *J*=6.97 Hz, 1 H)

^{13}C NMR ($CDCl_3$) d: 207.01, 165.38, 164.78, 147.98, 134.37, 131.08, 130.04, 125.47, 124.75, 120.07, 110.67, 104.45, 51.80, 51.26, 46.74, 45.95, 40.91, 34.95, 30.95, 28.78, 28.08 ppm

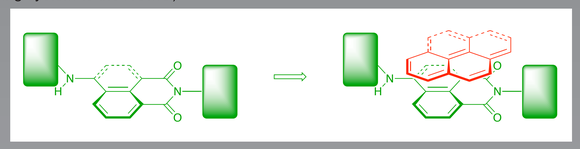
Acknowledgments

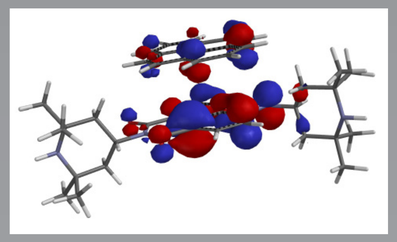
It is a pleasure to acknowledge the funding of this project by the WiSys Technology Foundation and the University of Wisconsin System through the Spark program.

The support of this project by the UW-Eau Claire Office of Research and Sponsored Programs is also gratefully acknowledged.

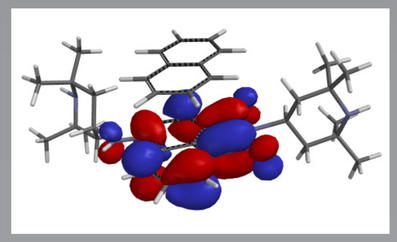
Potential New Naphthalimides to Act as Receptors

We propose that the planar naphthalimide structure provides a useful scaffold for forming new receptors for compounds capable of π-stacking. When the fluorophore contains bulky buttressing groups at each end, we expect that these groups may hinder aggregation except for compounds capable of binding into the pocket. There is evidence of strong interaction between the two molecules when perylene is the aromatic hydrocarbon (shown by the HOMO, which has lobes on both partners) but not with naphthalene (here there is no contribution from the naphthalene ring system to the HOMO).





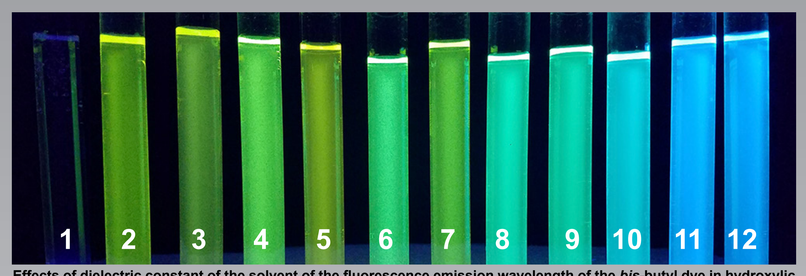
HOMO of the π-stacked complex of perylene with a hindered naphthalimide. Note the participation of both molecules in the HOMO.



HOMO of the π-stacked complex of naphthalene with a hindered naphthalimide. Note the absence of participation of naphthalene in the HOMO.

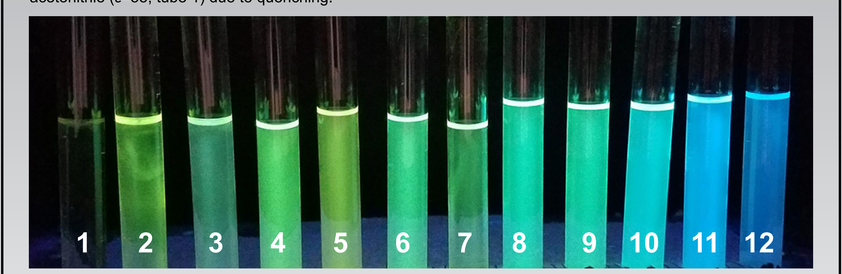
Solvatochromism of this Hindered Dye vs. a Sterically Nondemanding Dye

Solvatochromism is a property of fluorescent compounds where the fluorescence emission wavelength and intensity is dependent on the dielectric constant of the solvent. In 4-amino-1,8-naphthalimide derivatives, the solvatochromism leads to a blue shift and an increase in fluorescence intensity as the solvent dielectric constant decreases. In order to see if this was the case for the *bis*-TMP dye, we have undertaken qualitative and qunatitative studies of the effects of the solvent on its fluorescent emission in comparison to the sterically undemanding n-butyl dye.



Effects of dielectric constant of the solvent of the fluorescence emission wavelength of the *bis*-butyl dye in hydroxylic and non-hydroxylic solvents.

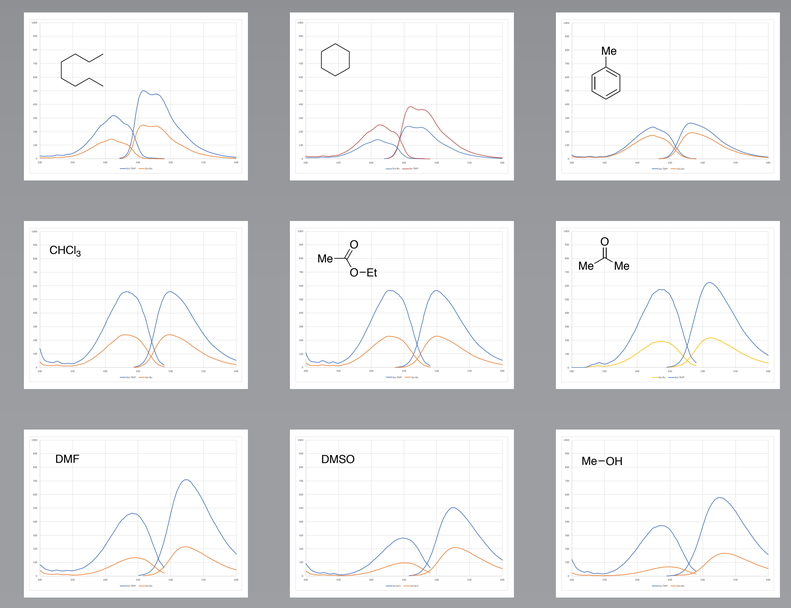
In this set of samples, the solvent varies from n-octane and cyclohexane in the two right-hand tubes to acetonitrile, DMF, DMSO, and 30% acetonitrile in the left-hand. The fluorescence of the dye in octance (ε= 2.0, tube 12) is clearly blue; in cyclohexane (ε= 2.02, tube 11) and toluene (ε=2.38, tube 10), it is remaining blue. In chloroform (ε= 4.81, tube 9), the color is starting to turn green. In ethyl acetate (ε= 6.02, tube 8), the color is basically green, and this effect is more evident in isopropyl alcohol (ε=17.9, tube 7) and acetone (ε=20.7, tube 6). In methanol (ε=32.7, tube 5), the first hint of a yellow cast begins to appear and is more evident in acetonitrile (ε=37.5, tube 4). The color remains the same in the most polar solvents, DMF (ε=36.7, tube 3) and DMSO (ε=46.7, tube 2), except for 30% acetonitrile (ε=55, tube 1) due to quenching.



Effects of dielectric constant of the solvent of the fluorescence emission wavelength of the *bis*-TMP dye in hydroxylic and non-hydroxylic solvents.

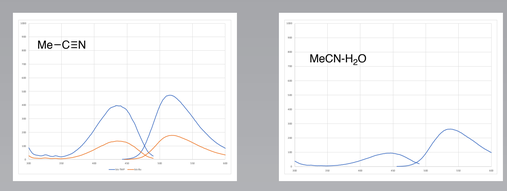
In this set of samples, the solvent varies from n-octane and cyclohexane in the two right-hand tubes to acetonitrile, DMF, DMSO, and 30% acetonitrile in the left-hand. The fluorescence of the dye in octance (ε= 2.0, tube 12) is clearly blue and is darker than that of the *bis*-butyl dye; in cyclohexane (ε= 2.02, tube 11), it is starting to get brighter and shifting to green and is more evident in toluene (ε=2.38, tube 10). In chloroform (ε= 4.81, tube 9), the color is clearly green, and this effect is more evident in ethyl acetate (ε= 6.02, tube 8) and isopropyl alcohol (ε=17.9, tube 7) and acetone (ε=20.7, tube 6). In methanol (ε=32.7, tube 5), the first hint of a yellow cast begins to appear and is shown more in acetonitrile (ε=37.5, tube 4). In the three most polar solvents, DMF (ε=36.7, tube 3), DMSO (ε=46.7, tube 2), and 30% acetonitrile (ε=55, tube 1), the emission is yellow.

Fluorescence Spectra Displaying Solvatochromic Properties of the *bis*-TMP dye vs. *bis*-butyl dye



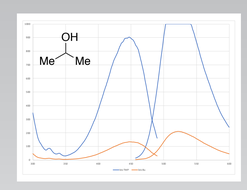
In every solvent tested, the sterically hindered *bis*-TMP dye gave a higher excitation and emission intensities than the corresponding *bis*-*n*-butyl dye. The wavelength maxima of the two dyes are the same in all solvents in this group, except for the only protic solvent, methanol. This indicates that there does not appear to be strong intramolecular transfer from the distal nitrogen to the amino nitrogen at the 4- position of the dye.

Fluorescence Quenching by Water in Acetonitrile



The addition of water to the acetonitrile solvent leads to quenching of the fluorescence, but not to a change in the excitation and emission maxima of the *bis*-TMP dye. As in other solvents, the *bis*-TMP and *bis*-*n*-butyl dyes have excitation and emission maxima at the same wavelengths.

Isopropyl Alcohol: An Unexpected Result



In all other solvents tested, the excitation and emission intensities in the *bis*-TMP dye are uniformly larger than in the corresponding *bis*-*n*-butyl dye, with a ratio between 1.3:1 and 2.5:1. In isopropyl alcohol, however, the ratio is closer to a full order of magnitude. Computations are currently being carried out, and the preliminary results suggest that isopropyl alcohol is uniquely effective at solvating the dye. As with methanol (the other protic solvent), the two dyes absorb and emit at slightly different wavelengths.

Where to Now? Future Directions

1. Continue binding studies with polycyclic aromatic hydrocarbons to determine binding affinities and photophysical effects of binding (if any).
2. Complete the synthesis of a new 1,8-naphthalimide with bulky 1-adamantanamine substituent groups at the *N*- and 4- positions.