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A new solvatochromic fluorophore for exploring nonpolar environments created by biopolymers†

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The fluorescence of a new aminocyanonaphthalene exhibits exquisite sensitivity to its environment and responds to a solvent change from water to hexane with greater than a 100-fold increase in intensity and 100 nm shift in $\lambda_{max.em}$. These properties should support many applications including the detection of abasic sites within duplex DNA as illustrated below.

Solvatochromic probes have emerged as powerful tools for identifying and characterizing microenvironments created by macromolecules and molecular assemblies.[1,2] Their use has been particularly significant when characterizing interfaces between regions of dramatically different polarity such as those between bulk solution and surfaces of proteins and nucleic acids. Investigators in this field have successfully adapted classical fluorophores for convenient incorporation into biopolymers of interest. For example, 4-*N*,*N*-dimethylamino-1,8-naphthalimide[3] and 6-propionyl-2-(*N*,*N*-dimethyl)-aminonaphthalene (prodan)[4] have been elaborated into amino acids for direct coupling within peptides and proteins to monitor ligand binding. Similarly, the dansyl group[5] and 6-(dimethylamino)-2-acylnaphthalene (dan)[6] have been attached to nucleobases and incorporated into duplex DNA to examine the polarity of the major and minor grooves. Progress has additionally been reported on an alternative approach involving nucleotide analogues that remain nearly isosteric yet are emissive and sensitive to their local environment.[2] A furan-conjugated pyrimidine was generated for this purpose and responded with a 50 nm shift in $\lambda_{max.em}$ and greater than a 3-fold gain in emission as the surrounding microscopic polarity increased from dioxane to water.[7]

In a complementary approach, low molecular weight ligands can be prepared with solvatochromic properties to interrogate macromolecular surfaces, to monitor association with macromolecular receptors and even simply to detect the presence of a specific receptor of interest. A bisbenzimide derivative (Hoechst 33258) was one of the first fluorescent probes to characterize the polarity of the minor groove through its solvent dependent 100-fold gain in emission intensity and 13% change in Stokes shift.[8] Likewise, certain aminonaphthyridine derivatives selectively coordinate to unpaired nucleobases within a duplex[9] and support a sensitive fluorescence-based method for detecting abasic sites in DNA.[10] More recently, a pyrazinecarboxamide has been reported for detecting abasic sites containing an unpaired thymine as indicated by the more than 2-fold decrease of its fluorescence.[11]

An abasic site was integral to our laboratory's model for studying excess electron transport in duplex DNA and was based in part on the expectation that this site would serve to bind and position diaminonaphthyl donors.[12–14] Unfortunately, the emissive properties of these initial compounds were not sufficiently sensitive to their environment to report on their interaction with DNA.[15] A new solvatochromic derivative based on naphthalene has now been synthesized and used to examine its DNA association. This derivative exhibits ideal characteristics for detecting abasic sites in duplex DNA and should also find utility in studying nonpolar surfaces of other macromolecules quite generally since its fluorescence intensity increases by 140-fold when transferred from an aqueous solution to a microenvironment with a polarity equivalent to hexane. Its Stokes shift also increases by more than 40% under equivalent conditions and provides another complementary measure of binding and polarity change with a very high dynamic range.

Typically, fluorophores with strong dipoles have the greatest potential to exhibit significant dependence on solvent,[1,2] and consequently, 1,5-diaminonaphthalene was converted to a strong dipolar derivative by enhancing the electron donating ability of one amine and replacing the other amine with an electron withdrawing group. Enhanced donor strength was gained by monomethylation of the amine. Dimethylation would ordinarily be expected to offer even greater electron donation, but its resonance contribution is diminished in this system by an out-of-plane twist required to accommodate the hydrogen at the *peri*-position of the fused ring.[16] Introduction of an electron withdrawing group was first achieved by replacing the second amino with a nitro group. However, preliminary studies on 1-methylamino-5-nitronaphthalene indicated that this fluorophore expressed very weak emission and little dependence on solvent.[17] This may perhaps be due to a non-radiative relaxation pathway available to the nitro group.[18] Alternative use of a cyano group proved more successful and was inspired

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† Electronic supplementary information (ESI) available: Synthesis of **2**, absorption spectra of **2** in each solvent, fluorescence quantum yield measurement, fluorescence emission stability, titration of **2** with all dsDNAs, and K_d analysis. See DOI: 10.1039/c0cc04917d

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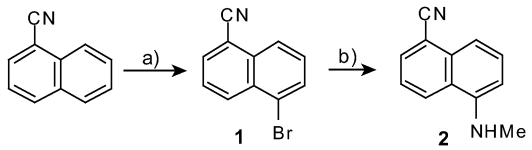

Scheme 1 Synthesis of **2**. *Reagents and conditions*: (a) $FeBr_3$, Br_2, 90 °C, 3 h, 60%; (b) ±-BINAP, $Pd(OAc)_2$, Cs_2CO_3, CH_3NH_2, toluene, 80 °C, 24 h, 69%.

Table 1 Photophysical parameters of **2** in the indicated solvents[a]

Solvent	$\lambda_{max.abs}$ (ε)[b]	$\lambda_{max.em}$[c]	$\Delta\bar{\nu}$	Φ_f[d]
H_2O	346 (3.62), 362 (3.76)	575	43.7	0.006
MeOH	346 (3.24), 389 (4.26)	534	53.2	0.15
EtOH	346 (3.00), 392 (4.00)	527	55.2	0.23
n-BuOH	346 (3.20), 394 (4.28)	523	56.5	0.28
MeCN	346 (3.10), 386 (4.24)	527	55.2	0.25
EtOAc	346 (3.20), 385 (4.34)	499	65.3	0.53
DMSO	346 (3.22), 404 (4.48)	533	53.5	0.46
DMF	346 (3.10), 397 (4.36)	524	56.2	0.47
$CHCl_3$	346 (2.58), 383 (3.50)	500	64.9	0.70
CH_2Cl_2	346 (2.94), 383 (4.02)	502	64.1	0.73
Toluene	346 (3.44), 383 (4.54)	485	71.9	0.72
Hexane	346 (3.40), 378 (4.34)	476	76.9	0.69

[a] λ, ε, $\bar{\nu}$, and $E_T(30)$ are given in nm, $mM^{-1}cm^{-1}$, $\times 10^3$ cm^{-1}, and kcal mol^{-1}, respectively. [b] Extinction coefficients were obtained independently by measuring absorbance of a series of solutions with known concentrations at $\lambda_{max.abs}$. [c] λ_{ex} = 346 nm. [d] Φ_f was obtained independently in each solvent using optically matched solutions of anthracene in ethanol as a reference (Φ_f = 0.27).[25] Errors are less than 10% for each individual value.

by the substantial effect of solvent on the fluorescent properties of monocyanoanilines.[19,20] 1-Cyano-5-methylaminonaphthalene (**2**) was synthesized in two steps by a Friedel–Crafts bromination of commercially available 1-cyanonaphthalene followed by a palladium-catalyzed cross coupling between the resulting 1-bromo-5-cyanonaphthalene (**1**) and methylamine (Scheme 1).[21]

The photophysical properties of **2** were examined in a range of solvents from water to hexane. Solvent polarity affects its light absorption by a relatively modest 50% (Fig. S1).[21] The long wavelength λ_{max} varies between 360 and 400 nm, and the short wavelength λ_{max} at 346 nm remains nearly constant (Table 1). However, its absorptivity varies by as much as 50%. Most importantly, the emission spectra reveal a very substantial influence of solvent (Fig. 1). An aqueous solution of **2** emits only weakly with a maximum at 575 nm whereas a hexane solution of **2** emits over 100-fold more intensely at its maximum of 476 nm (Table 1). Solvents of intermediate polarity display intermediate effects, and both the quantum yield for emission and the Stokes shift respond with near linearity to $E_T(30)$, a measure of microscopic solvent polarity (Fig. 1).[22,23] Proton transfer does not appear to control the excited state properties of **2** since there is no discontinuity in either fluorescence emission or Stokes shift as a function of protic *versus* aprotic solvents (Fig. 1).

The large dipole created by the electron donating and withdrawing substituents of **2** resulted in a large shift of $\lambda_{max.em}$ ranging over 99 nm (from water to hexane). This magnitude is nearly equivalent to the change in $\lambda_{max.em}$ of 130 nm for the widely applied prodan (from water to cyclohexane)[23,24] and greater than the change in $\lambda_{max.em}$ of 86 nm for dansyl amide (from water to toluene) when comparing an analogous change of 90 nm for **2** (from water to toluene).[23] Furthermore, **2** is sufficiently stable photochemically to be used routinely. Continuous irradiation of **2** in sodium phosphate (10 mM) pH 7 and 100 mM NaCl at 346 nm for 1 h resulted in less than a 10% change in its fluorescence emission (Fig. S3).[21]

The striking solvatochromic properties of **2** provide a sensitive indicator for testing the potential for the naphthyl-based electron donors to bind within an abasic site. Accordingly, the fluorescence of **2** under aqueous conditions was monitored during titration with a series of oligonucleotide duplexes containing abasic sites equivalent to those used earlier in studies on electron transport in DNA in which the unpaired base varied between C, G, A and T (Scheme 2).[14] Since the environment within helical DNA is less polar than its surrounding solvent, association between **2** and these duplexes was predicted to increase fluorescence emission and induce a hypsochromic shift of the $\lambda_{max.em}$. Both results were clearly evident by the 13- to 18-fold increase in emission and 42 nm hypsochromic shift in $\lambda_{max.em}$ after addition of any of the duplexes containing an abasic site (Fig. 2 and S4–S7).[21]

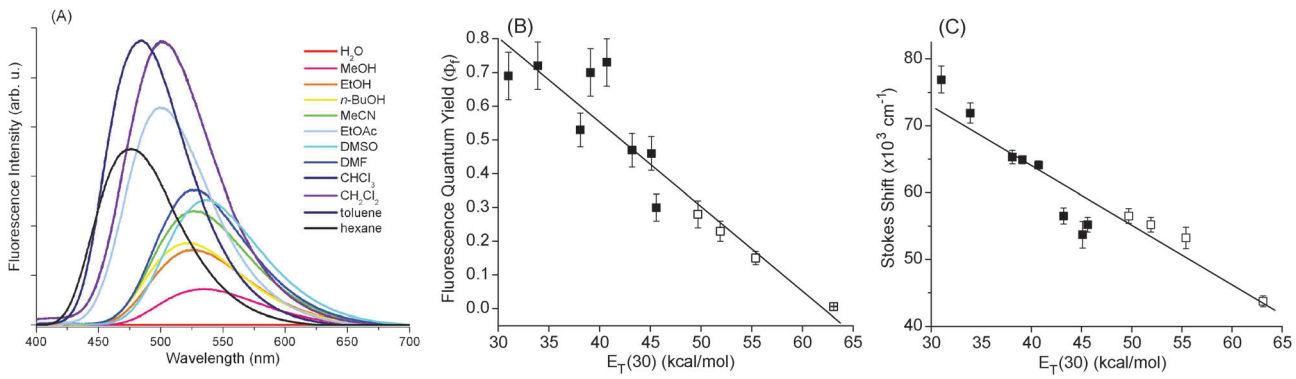

Fig. 1 Solvent dependent properties of **2** in aprotic (■) and protic (□) solvents. (A) Fluorescence emission of **2** (50 μM, λ_{ex} of 346 nm) was recorded under ambient conditions. (B) The quantum yield for emission and (C) the Stokes shift were compared to published values of $E_T(30)$, a measure of microscopic solvent polarity.[22] The quantum yields were calculated as illustrated in the Supplementary Information from multiple independent determinations, and the Stokes shifts are averages of 3 independent measurements. Linear fits in (B) and (C) were generated by Origin 6.0.

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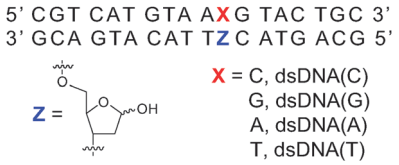

Scheme 2 Nucleotide sequences of duplex DNA with abasic sites.

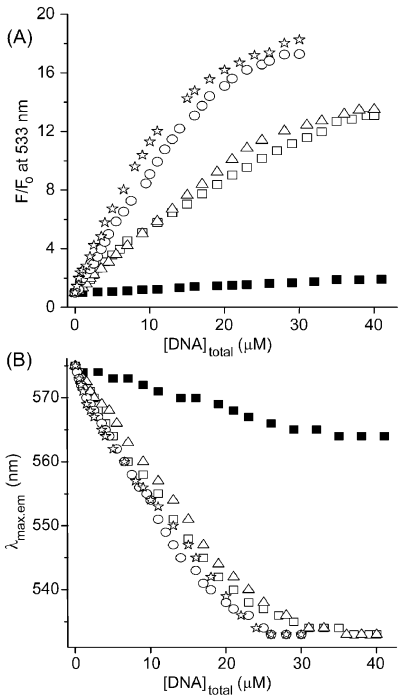

Fig. 2 The fluorescence of **2** responds selectively to the presence of abasic sites in duplex DNA. Solutions of **2** (10 μM) in 10 mM sodium phosphate pH 7 and 100 mM NaCl (measured $E_T(30)$ = 64.5 kcal mol^{-1}) were titrated with an oligonucleotide duplex (■, X = C, Z = G, Scheme 2) or its derivatives containing an abasic alternatively opposite a G (dsDNA(G), ☆), A (dsDNA(A), ○), C (dsDNA(C), △), and T (dsDNA(T), □). (A) The relative fluorescence emission (F/F_o) and (B) $\lambda_{max.em}$ of **2** (λ_{ex} 346 nm) were monitored as a function of total DNA concentration.

Additionally, the response of **2** to each of these duplexes was saturable and followed an expected 1 : 1 binding isotherm.[21] Fluorescence emission correlated well to this process and yielded K_d values of 7–8 μM for X = pyrimidine and 5 μM for X = purine. Interestingly, the $\lambda_{max.em}$ data did not clearly distinguish between X = pyrimidine or purine but still suggested K_d values between 3–10 μM. These affinities are also similar to that measured for a neutral pyrazinecarboxamide derivative (3.5 μM) used previously as a control when developing reagents to identify specific abasic sites through suppression of fluorescence rather than enhancement of fluorescence as exhibited by **2**.[11]

Selectivity of **2** for an abasic site over the ubiquitous alternatives of standard intercalation and groove binding was demonstrated by further titration with the parent oligonucleotide duplex lacking the abasic site (dsDNA) in which X = G and Z = C (Scheme 2). Only a slight increase in fluorescence emission of less than 2-fold was observed in the presence of this canonical DNA duplex at its maximum concentration of 40 μM (Fig. 2). A corresponding hypsochromic shift in $\lambda_{max.em}$ of less than 10 nm was more pronounced but still much smaller than that induced by the presence of an abasic site.

In summary, we have successfully created a novel solvatochromic fluorophore that should find application in many studies examining the surface and structure of biopolymers. The fluorescence is highly sensitive to solvent polarity and provides a dynamic range of 100-fold for emission and 100 nm for $\lambda_{max.em}$. This probe was particularly useful to validate a previously untested assumption that the naphthalene-based electron donors are bound at an abasic site within duplex DNA. Derivatives of **2** should also be easily prepared in the future for conjugation to a broad array of low molecular ligands and large macromolecules.

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