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A Dual Channel Fluorescence Chemosensor for Anions Involving Intermolecular Excited State Proton Transfer**

Kihang Choi and Andrew D. Hamilton*

Effective fluorescence chemosensors must convert molecular recognition into changes in fluorescence that are both highly sensitive and easy to detect. A key issue in sensor design is the connection of substrate binding in a recognition domain to photophysical changes in a fluorophore with optimal sensitivity.[1] In recent years there has been great interest in anion recognition and sensing, because of their importance in biological and environmental settings.[2] Many fluorescence anion sensors utilizing competitive binding,[3] photo-induced electron transfer,[4] metal-to-ligand charge transfer,[5] and excimer/exiplex formation[6] mechanisms have been developed. Surprisingly, the strategy of linking a fluorophore with emission from an internal charge transfer (CT) excited state to an ion-binding domain, while widely used for cation sensing, has been rarely exploited for anion sensing.[1a, 7] Anion binding close to the fluorophore could lead to the stabilization of positive charge developed in the fluorophore excited state and to the opening of another fluorescence emission channel through intermolecular excited state proton transfer (ESPT)[8] (Figure 1). Herein we report the preparation of anion sensors using this strategy and show that one of them can function as a dual-channel sensor system.

As the anion-binding domain we used macrocycle **1** which can bind certain anions strongly and selectively through hydrogen bonding to the three amide NH groups.[9] In a first design, we attached covalently a fluorophore onto the periphery of **1**. 4-Trifluoromethyl-7-aminocoumarin, which possesses an excited state where negative charge is transferred from the nitrogen atom to the coumarin ring,[10] was connected to the monocarboxy derivative of **1** to give **2** (Boc = *tert*-butoxycarbonyl).[11, 12] This fluorophore-appended macrocycle showed modest changes in the intensity of its fluorescence emission upon addition of different anions. The binding constants were determined by fluorescence titration and showed high selectivity for tetrahedral anions such as $H_2PO_4^-$ (Table 1), which mirrors the properties of **1**. Although this high selectivity (especially for phosphate over chloride) is desirable for sensing applications,[2a,e] the changes in emission intensity and wavelength (+2 nm for $H_2PO_4^-$) induced by anion binding were small. It appears that the negative charge on the bound anion is not effectively positioned to interact with the increasing positive charge on the nitrogen atom of the fluorophore, and has little effect on the excited-state energy.[13]

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Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.

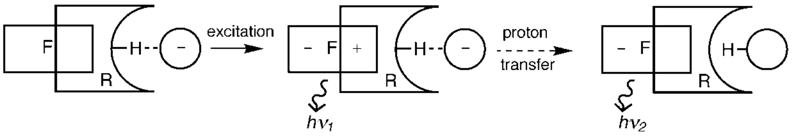
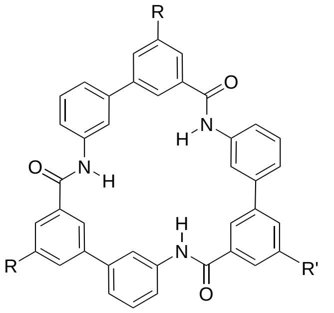
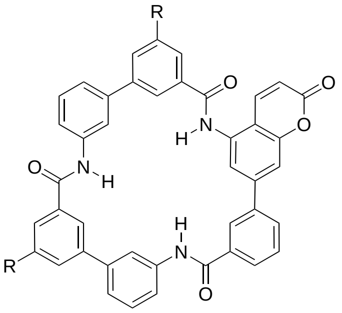


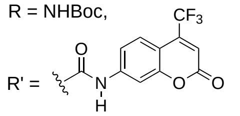
Figure 1. Two possible fluorescence emission channels from a CT excited state. An anion receptor (R) is connected to the electron-donating terminal of a fluorophore (F). Anion binding stabilizes the CT excited state by electrostatic interaction. The proton that becomes acidic by the localized positive charge on F is transferred to the anion and the second emission channel is opened.



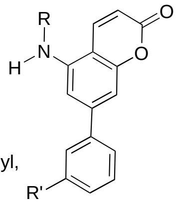


1 R = R' = NHBoc

2 R = NHBoc, R' =



3 R = NHBoc



4 R = Teoc, R' = CO_2H

5 R = 3,5-dimethylbenzoyl, R' = H

The sensitivity problem was addressed by integrating the fluorophore into the macrocyclic ring in order to involve the coumarin NH group directly in hydrogen bonding to the anion. Preparation of this second-generation sensor centered on the synthesis of a fluorescent biphenyl monomer that could be incorporated into the modular macrocycle synthesis. Compound **4** (Teoc = 2-trimethylsilylethoxycarbonyl) was selected as the fluorescent monomer and synthesized in five steps from methyl 3,5-dihydroxy-2-formylbenzoate.[14] This monomer was successfully incorporated into macrocycle **3** with a final cyclization yield of 30%.[11, 15]

The anion-binding properties of **3** were similar to those of **2** (Table 1), with no significant difference in the association constants or the high selectivity for $H_2PO_4^-$ ions. However, the changes in the intensity of the fluorescence emission and wavelength were much stronger and anion dependent (Figure 2). The binding of all tetrahedral anions studied caused an increase in the fluorescence intensity and a red-shift of the peak maximum (10–20 nm). This red-shift is presumably the result of stabilization of the fluorophore excited state relative to the ground state on anion binding. There was no change in the UV absorption spectrum of **3** on anion binding, which suggests that the intensity enhancement may be a consequence of a restriction in the conformational flexibility that would otherwise lead to nonradiative decay through rotatory motion of the amide group.[10] The most remarkable change was the appearance of a second emission band at longer λ_{em} values. Titration of **3** with $H_2PO_4^-$ ions gave a simultaneous increase in the intensity of two bands, which, excitation spectra showed, came from the same ground state (Figure 3a).[16] The maximum intensity change of this second band was largest for the most basic $H_2PO_4^-$ ion, modest for $PhPO_3H^-$ ions, and absent with the least basic tetrahedral anion studied, pTsO$^-$.

This correlation of the second emission band with bound anion basicity suggested that proton transfer from the fluorophore excited state was occurring. Although detailed photophysical studies are required to fully understand this phenomenon, the fluorescence properties of control molecule

Table 1. Association constants K_a (M^{-1}) and maximum intensity changes F/F_O of macrocycle **2** and **3** with tetrabutylammonium salts.[a]

Anion	**2** K_a	(F/F_O)	**3** K_a	(F/F_O)
Cl^-	< 1000		< 1000	
pTsO$^-$	N/A[b]	(0.98)	3.8×10^3	(2.55)
$PhPO_3H^-$	5.9×10^5	(0.63)	6.3×10^5	(5.61)[c]
$H_2PO_4^-$	2.7×10^6	(0.56)	2.0×10^6	(13.7)[c]

[a] [**2**] = 2 μM, [**3**] = 3 μM in 1/1 DMSO/1,4-dioxane. The emission change with the biggest $F - F_O$ value was curve-fitted to a 1/1 binding mode, and the F/F_O value at this wavelength is included in parenthesis. The 1/1 binding stoichiometry was determined by the linearity of Benesi–Hildebrand plots.[17] Estimated errors of association constants are within ±15%. [b] Not available because of the small intensity change. [c] Calculated from ESPT emission bands.

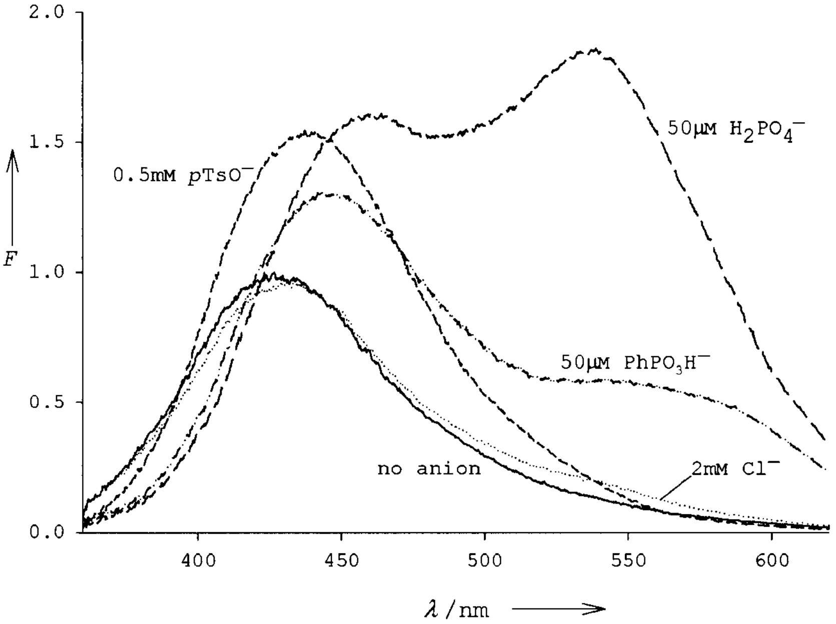


Figure 2. Emission spectra (λ_{ex} = 320 nm) of **3** (3 μM in 1/1 DMSO/1,4-dioxane) with different anions.

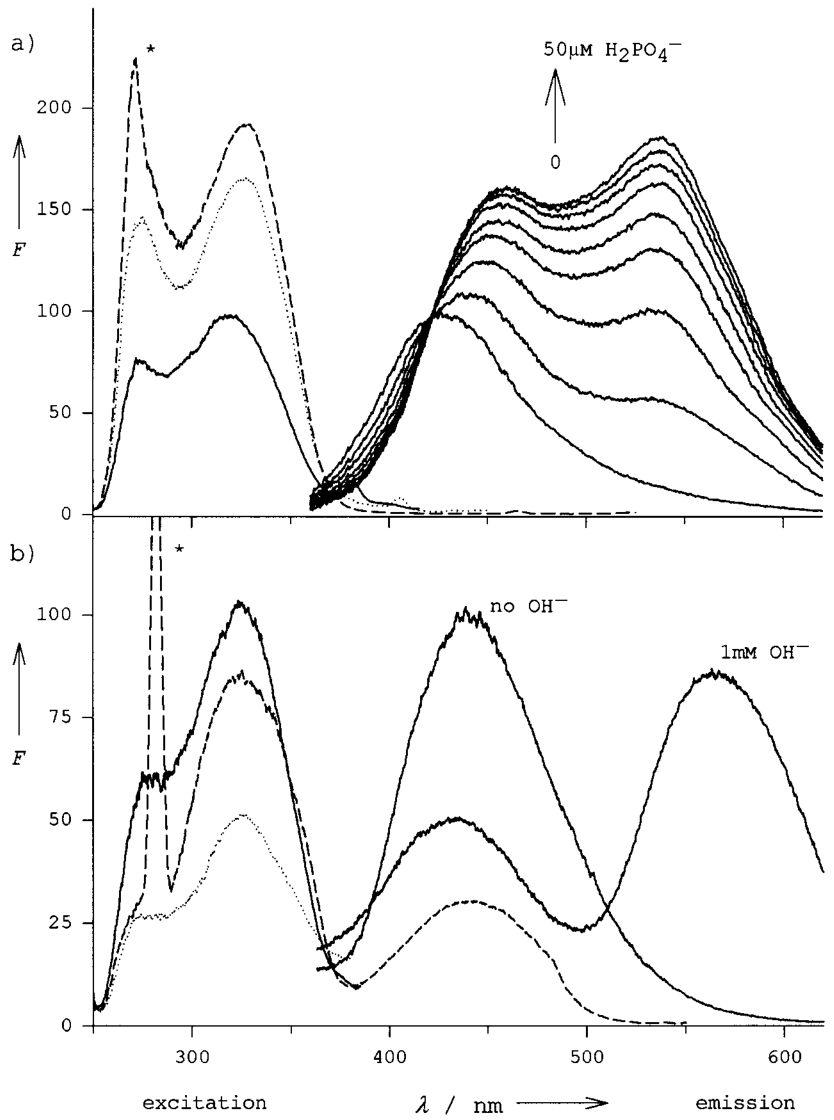


Figure 3. Excitation and emission spectra (λ_{ex} = 320 nm) of **3** and **5**. a) Excitation spectrum of **3** (3 μM) without (——, λ_{em} = 429 nm) and with 50 μM $H_2PO_4^-$ (••••, λ_{em} = 459 nm; ----, λ_{em} = 537 nm). b) Excitation spectrum of **5** (1 μM) without (——, λ_{em} = 440 nm) and with 1 mM OH^- (••••, λ_{em} = 434 nm; – – –, λ_{em} = 565 nm). (*: artifact caused by the second-order transmission.)

5 containing only one labile proton and no anion binding domain are informative. The addition of 1 mM tetrabutylammonium hydroxide increased the intensity of the UV absorption of **5** overall and a new lowest absorption band appeared around 440 nm. These changes were reversible and indicated partial deprotonation of the amide proton in the ground state. Compound **5** showed similar λ_{ex} and λ_{em} values as **3** and the second emission band was also observed under basic conditions (Figure 3b). The excitation spectrum (λ_{em} = 565 nm) clearly shows that two pathways for this second band exist: direct excitation from the deprotonated ground state (λ_{ex} = 443 nm) and excitation from the nondeprotonated ground state (λ_{ex} = 320 nm) followed by deprotonation (proton transfer) from the excited state. The difference in the pK_a values between the ground and excited states was calculated as 14 by application of the Förster equation.[8a, 12b] Addition of $H_2PO_4^-$ ions (up to 1 mM) to the solution induced little change in the UV and fluorescence spectra of **5**, which indicates that both the decrease in the pK_a value of the excited state and the increased local concentration of bound anion are necessary for proton transfer to weakly basic anions to occur at low anion concentration. An excitation spectrum of **3** complexed with $H_2PO_4^-$ ions shows (Figure 3a) that proton transfer occurs only from the excited state.

In conclusion, we have presented a new mechanism for anion sensing by fluorescence spectroscopy that is based on proton transfer from a fluorophore excited state. The increased acidity of the fluorophore in the CT excited state was combined with selective recognition of the anion to open an ESPT channel in addition to a CT channel. Emissions from the two channels showed different anion dependencies and, as a result, more selective and sensitive sensing was achieved.

Experimental Section

Fluorescence spectra were obtained on a Hitachi F-4500 spectrophotometer with 5 nm excitation and emission slit widths. All stock solutions of anions and compounds were prepared in DMSO and the concentration was adjusted by adding DMSO and 1,4-dioxane (anhydrous grades from Aldrich) before recording the spectra. Association constants were determined by titrating a sensor solution with a tetrabutylammonium salt solution containing the sensor at its initial concentration. The intensity changes were analyzed by nonlinear regression methods using SigmaPlot (Version 5.0, SPSS Inc.). See the Supporting Information for the synthesis and characterization of compounds **2**–**5**.

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