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A Multifunctional Tripodal Fluorescent Probe: "Off−On" Detection of Sodium as well as Two-Input AND Molecular Logic Behavior

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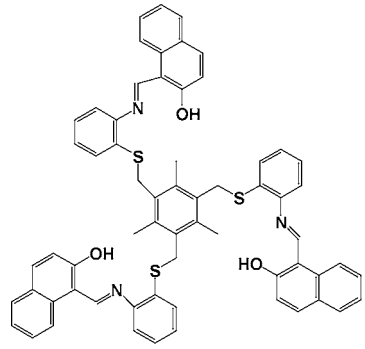
ABSTRACT



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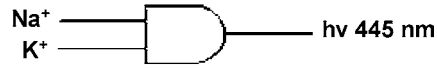

A simple tripodal sensor (3) with multifunctional capability has been synthesized in three steps. The sensor, a naphthalene-functionalized Schiff base, displays selectivity for sodium over other important physiological and environmentally important cations through changes in the emission spectra at λ_{max} 355 nm when excited at 270 nm. Interestingly, the combined addition of both sodium and potassium produced a new band at λ_{max} 445 nm, while the addition of sodium or potassium alone produced negligible changes at this wavelength. Therefore, the conditions of a two-input AND logic operator were also satisfied.

Fluorescence spectroscopy continues to play an important role in molecular sensing due to its high sensitivity, rapid response rate, and relative inexpense. Commercially available fluorescent probes are routinely used to measure the concentrations of physiologically relevant analytes.[1] However, the majority of such probes have been restricted to measuring the concentration of one specific target. Quite often though, abnormal cellular events result from the synergistic interaction between two or more analytes.[2] Thus, sensors with multifunctional capability which can identify situations where two (or more) analytes are present at abnormal levels have enormous benefits.

The concept of molecular logic, pioneered by de Silva and co-workers in the early 1990s, has enabled the design of

(1) See http://probes.invitrogen.com/handbook/.

(2) (a) Balaji, R. V.; Colvin, R. A. *Neurochem. Res.* **2005**, *30*, 171–176. (b) Galla, J. H. *J. Am. Soc. Nephrol.* **2000**, *11*, 369–375. (c) King, G. L.; Brownlee, M. *Endocrin. Metabol. Clin. North Am.* **1996**, *25*, 255–270.

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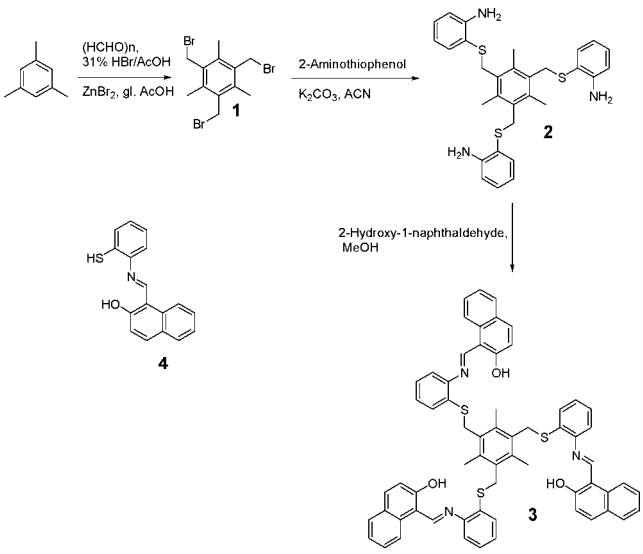
luminescent molecules that only respond to defined levels of multiple inputs.[3] These elementary logic operations, central to the functioning of electronic circuitry, can be performed by cleverly designed organic molecules, and can access areas inaccessible by even the smallest electronics. Instead of using voltage inputs and outputs, molecular logic is based on chemical inputs and a fluorescence output. A range of fluorescent molecular logic gates (i.e., AND, INHIBIT etc) have now been prepared with a variety of different input types.[4] However, to the best of our knowledge, there have been no reports of fluorescent AND logic gates with Na^+ and K^+ inputs that can also function as a single ion Na^+ sensor. Both Na^+ and K^+ are important blood electrolytes, and their plasma concentration must be maintained at defined levels for normal physiological functioning.[5] Fluctuations in plasma levels of Na^+ and K^+ (hypo-, hypernatremia and hypo-, hyperkalemia) can be potentially fatal if left untreated.[6] Although there are many single-ion sensors available for both Na^+ and K^+,[7] probes that can monitor both of these ions simultaneously are rare. Here, we design a multifunctional tripodal Schiff base fluorescent sensor that not only is capable of monitoring Na^+ levels (at $\lambda_{max} = 355$ nm) but also functions as a two-input AND logic gate using inputs of Na^+ and K^+ and an emission output at $\lambda_{max} = 445$ nm.

The sensor **3**, a tripodal Schiff base, was formed in three steps as shown in Scheme 1. Compound **1** was obtained from

Scheme 1. Synthesis of **3**



mesitylene after reaction with formaldehyde and a HBr/AcOH mixture in the presence of $ZnBr_2$. Compound **1** was then reacted with 2-mercaptoaniline under basic conditions to produce the amine **2**, which, after a condensation reaction with 2-hydroxynapthaldehyde gave the target compound **3** in 38.2% yield (see the Supporting Information). The synthesis, characterization, and photophysical properties of control compound **4** have been detailed by us in a previous communication.[8]

The fluorescence spectrum of **3** was recorded in THF/H_2O (9:1) HEPES (pH 7.0) buffered solution and displayed two main peaks with $\lambda_{max} = 310$ and 355 nm when excited at 270 nm (Figure 1b). The effects of cations on the fluorescence properties of **3**were investigated by the addition of a variety of physiological and environmentally relevant ions as their chloride salts. A substantial fluorescence enhancement was observed for sodium (Figure 1) with only minor changes observed for the other ions. Figure 1b shows the

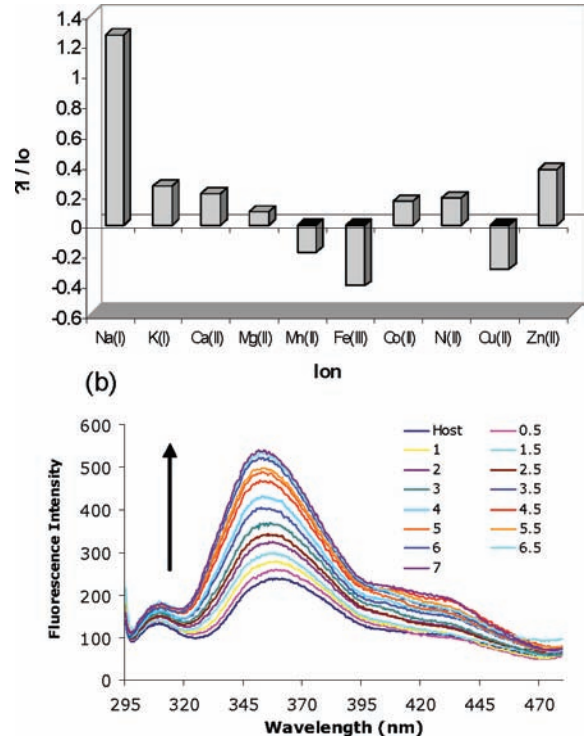


Figure 1. (a) Bar chart reflecting changes in the relative intensity ($\Delta I/I_o$) of **3** (20 μM) at 355 nm upon the addition of metal ions (100 μM). (b) Emission spectra of **3** (20 μM) upon addition of increasing amounts of Na^+. Solvent = THF/H_2O (9:1,v/v) HEPES buffer solution (pH 7.0 $\pm$ 0.1). Concentrations (μM) of Na^+ are shown in the inset.

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fluorescence spectra of **3** recorded at different concentrations of sodium (0−140 μM) and shows an enhancement of the 355 nm band accompanied by a slight blue shift (~5 nm). This enhancement of fluorescence is most likely due to the prevention of rapid isomerization of the C=N bond of the receptor upon Na^+ binding, which otherwise leads to nonradiative decay.[9] The selectivity of this type of Schiff base receptor for the hard Na^+ ion was not totally unexpected as we have previously shown that monopod **4** selectively binds Mg^{2+} ions through an excited-state intramolecular proton transfer (ESIPT) process.[8] Job plot analysis[10] (see the Supporting Information, Figure S6) revealed the binding stoichiometry between **3** and Na^+ to be 1:1 (host/guest) with an association constant $K_a = (2.1 \pm 0.7) \times 10^3\ M^{-1}$ as determined from the Benesi−Hildebrand plot[11] (see the Supporting Information, Figure S7).

To determine if **3** could sense Na^+ in competitive environments, we carried out a series of titrations in which 1 equiv of either K^+, Ca^{2+}, and Mg^{2+} was added to a solution of **3** (20 μM) in the presence of 1 equiv of Na^+. In each case, only minor effects were observed at 355 nm while a substantial enhancement was observed at 445 nm for the solution containing both Na^+ and K^+ (Figure 2a). However, addition of up to 12 equiv of K^+ alone to a solution of **3** produced only minor changes at 445 nm (Figure 2b). These results suggest that Na^+ must first bind to **3** before K^+ is permitted to bind. It also suggests that two independent binding states of **3** are formed, one responsible for Na^+-only induced changes at 355 nm with the other state accounting for the joint Na^+/K^+ changes at 445 nm. We know that single pod **4** undergoes ESIPT to generate *keto* and *enol* tautomers enabling ratiometric fluorescence determination of Mg^{2+}.[8] The emission wavelengths observed for these two states were 355 nm (*enol*) and 445 nm (*keto*), similar to those for the Na^+-only and joint Na^+/K^+ effects observed for **3** above.

The similarity in the emission profiles of **3** (toward Na^+ and K^+) and **4** (toward Mg^{2+}) suggests a similar phenomenon is occurring for **3** with the *enol* form of **3** being responsible for the Na^+ binding and the *keto* form enabled K^+ binding. The binding stiochiometry between the **3**/Na^+ complex and K^+ was also determined as 1:1 host/guest using Job's plot (see the Supporting Information, Figure S8). To investigate this, further molecular modeling studies were undertaken.[12] The energy-minimized structure of **3** is presented in Figure 3a and shows two receptor pods located perpendicular to the mesitylene platform with the third pod in the same plane. ^1H NMR spectroscopy of **3** also suggested there was an unsymmetrical arrangement of the receptor pods with more than one signal being observed for both the methyl and methylene protons of mesitylene (see the Supporting Information, Figure S4). However, the ^1H NMR spectrum of **2**, the amine precursor of **3**, showed only one signal for the methyl and methylene protons (see the Supporting Information, Figure S2). These results compare favorably with

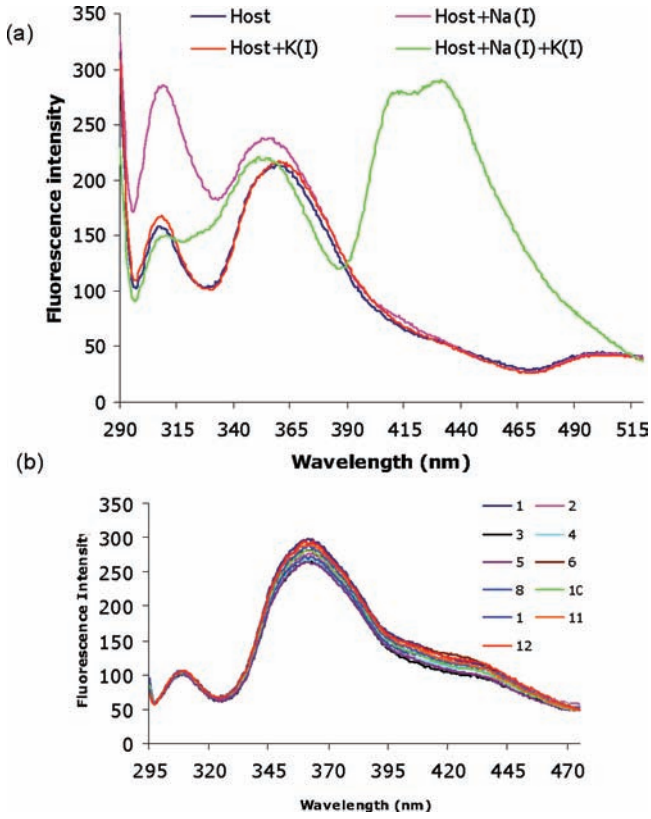


Figure 2. (a) Emission spectra for **3** (20 μM) in the absence of ions (blue line), in the presence 20 μM Na^+ (pink line), in the presence of 20 μM K^+ (red line), and in the presence of both 20 μM Na^+ and 20 μM K^+ (green line). (b) Plot of emission intensity of **3** (20 μM) in the presence of up to 12 equiv of K^+. Solvent = THF/H_2O (9:1,v/v) HEPES buffer solution (pH 7.0 ± 0.1).

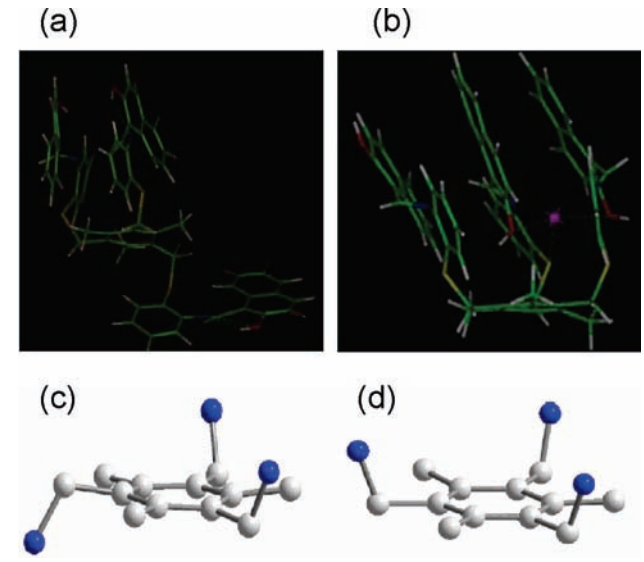
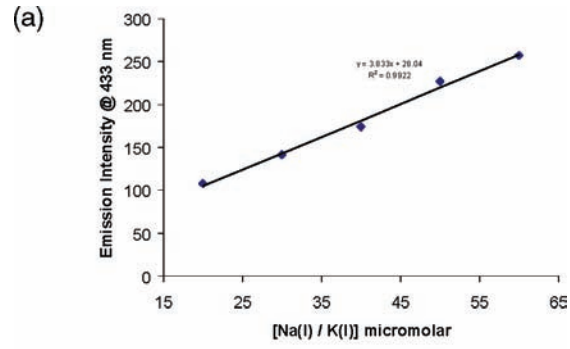


Figure 3. Energy-minimized molecular models of (a) free **3** and (b) **3** in the presence of Na^+. (c, d) Possible arrangements of the receptors (in blue) about the mesitylene platform with (c) illustrating a *cis,trans,trans* arrangement and (d) a *cis,cis,cis* arrangement.

reported crystal structures of similar compounds.[13,14] The molecular model of **3** also revealed hydrogen bonding between the napthalene hydroxyl and the imine nitrogen within each pod confirming the likelihood of ESIPT between the two units.

In the presence of Na^+, however, the energy-minimized structure changed with the three receptor pods now perpendicular to the plane of the mesitylene platform (Figure 3b). The model also predicts only two pods are used to bind Na^+ leaving the third pod free. On the basis of these results, we propose that Na^+ binds to **3** through two receptor pods in the *enol* form inducing a change in conformation. This creates a second binding site for K^+ using the free receptor pod. Given that the emission λ_{max} of the **3**/Na^+/K^+ complex was 445 nm suggests the third pod is in the *keto* form when binding K^+. Therefore, we present **3** as multifunctional probe that can monitor changes in Na^+ concentration at 355 nm yet also function as a two-input AND logic gate by signaling the simultaneous presence of Na^+ and K^+ (at 445 nm). To determine the response of **3** to the combined addition of both Na^+ and K^+ a titration was performed in which equal concentrations of both ions were added to a solution of **3** (20 μM). The results from this titration are shown in Figure S9 (Supporting Information) and reveal a gradual enhancement of both the 355 and 445 nm bands upon increasing Na^+/K^+ addition. When the intensity at 433 nm was plotted against combined Na^+/K^+ concentration (Figure 4a) linearity was observed in the 10−60 μM range.

In summary, we demonstrate the first reported example of a single molecule capable of functioning as a sensor for Na^+ and also as an AND logic gate for Na^+ and K^+ using spectrally distinct output wavelengths. Molecular modeling and experimental evidence suggest that the Na^+ binding results from two of the three available Schiff base pods chelating the Na^+ ion in the *enol* form. The remaining pod binds K^+ in the *keto* form, only when Na^+ is already bound to demonstrate AND logic behavior. Thus, this sensor demonstrates a versatility not commonly observed among molecular logic gates.



(b)

Input 1 (Na+)	Input 2 (K+)	Output Flu(445 nm)
0	0	0
1	0	0
0	1	0
1	1	1

Figure 4. (a) Plot of fluorescence intensity at 433 nm for **3** against concentration for mixed Na^+ and K^+ addition in THF/H_2O (9:1,v/v) HEPES buffer solution (pH 7.0 ± 0.1). Each concentration shown has an equal amount of Na^+ and K^+ present; i.e., 20 μM contains 10 μM Na^+ and 10 μM K^+ (excitation at λ_{max} = 275 nm). (b) Truth table for AND molecular logic displayed by **3** for Na^+ and K^+ at 445 nm.

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Supporting Information Available: Details of the synthesis and other spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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