A Simple and General Strategy for the Design of Fluorescent Cation Sensor Beads[¶]
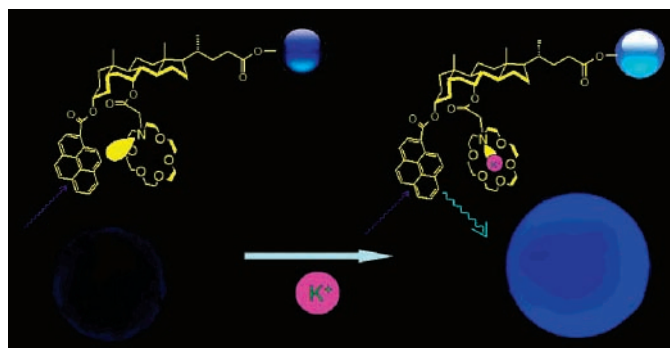
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Received May 3, 2006

ABSTRACT

Chenodeoxycholic acid based PET sensors for alkali metal ions have been immobilized on Merrifield resin and on Tentagel. The fluorescence of the sensor beads is enhanced upon binding the cations. The modular nature of the sensor allows designing different sensors based on this concept.

Photoinduced electron transfer (PET) based cation sensors *in solutions* have been explored extensively in the recent past.[1,2] Such a molecular device attached to a polymer bead and capable of sensing cations can have practical application for qualitative detection and quantitative determination of metal ions. Not surprisingly, the development of polymer-bead-attached sensors as reusable cation sensors is of contemporary interest.[3]

We recently reported a bile acid-derived sensor (**1**) in which *through space* photoinduced electron transfer (PET)

[¶] Dedicated to Prof. Ronald Breslow on the occasion of his 75th birthday.

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occurred from the N-atom of the cation receptor (1-aza-18-crown-6) to the fluorophore unit (pyrene), resulting in the quenching of the fluorescence of the pyrene unit.[4] Fluorescence titration with different salts in a methanolic solution of **1** showed an increase in the fluorescence intensity upon the addition of K^+.[4]

In the present work, we demonstrate a simple, general, and effective strategy to immobilize and utilize such sensors on polymer beads in aqueous environments. Compound **1** has an unused side chain carboxylic acid ideally suited for immobilization. Consequently, sensor **2** was synthesized with a benzyl ester on the side chain, which was hydrogenolyzed (Pd−C/EtOAc) to obtain compound **3** (Scheme 1).

Scheme 1. Synthesis of the Sensor Beads
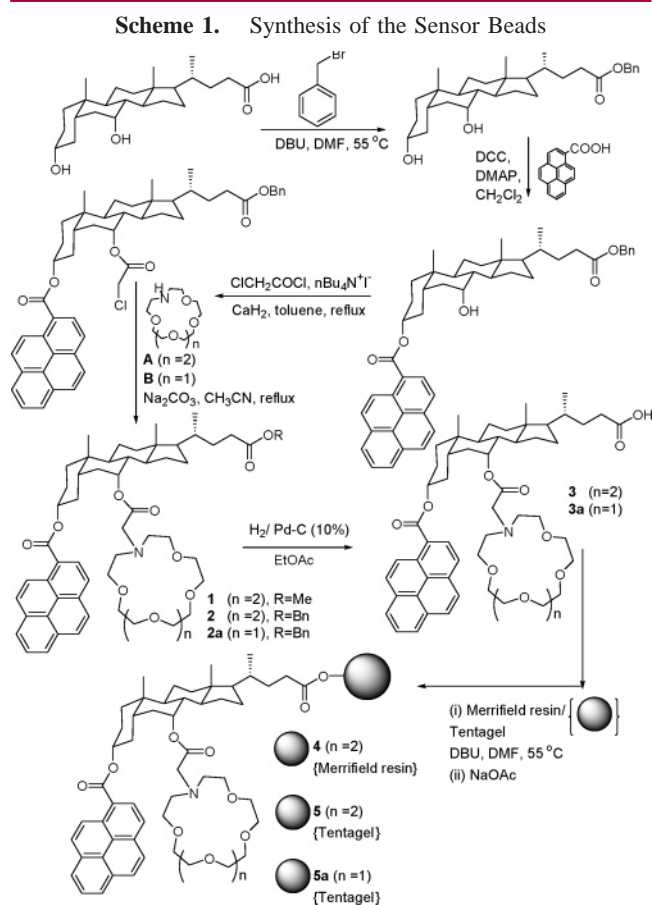
As a proof-of-concept, compound **3** was immobilized on Merrifield resin by stirring a DMF solution of **3** with the

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resin (1.0−1.5 mmol Cl/g) in the presence of DBU (17% loading). The excess chloromethyl groups of the Merrifield resin were capped by acetylation (DBU, Na-acetate) to generate sensor bead **4** (Scheme 1).

Fluorescence microscopy was selected as the method of choice for the binding measurements. Bead **4** (5 mg) was stirred with $KClO_4$ (500 μM) in acetonitrile/toluene (1:4) for 6 h, and the fluorescent image of the beads was taken in a fluorescence microscope while irradiating the beads at 365 nm. The images clearly showed that after the treatment with $KClO_4$ the beads swelled and the fluorescence intensity of **4** increased due to the *inhibition* of PET after K^+ binding (Figure 1a,b). Reversibility of the fluorescence enhancement
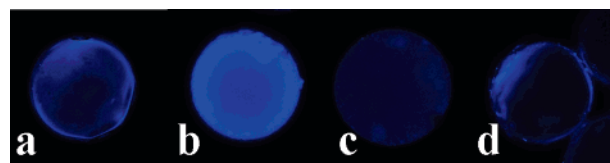
Figure 1. Fluorescence microscope image of (a) **4**; (b) **4** + 500 μM $KClO_4$ in 20% MeCN/PhMe; (c) b washed with solvent; (d) **4** + 500 μM $KClO_4$ in MeOH.

process was verified by thoroughly washing the K^+-treated beads with the same solvent. As expected, the beads became less fluorescent due to the re-establishment of the PET process (Figure 1c).

Sensor bead **4** worked efficiently in nonpolar solvents, such as 20% MeCN/PhMe, but the detection of K^+ in a polar solvent remained a challenge, as the sensor bead **4** after treatment with $KClO_4$/MeOH did not show any fluorescence enhancement (Figure 1d). This is not unexpected since Merrifield resin does not swell in MeOH, and thus K^+ is not able to penetrate into the resin to be complexed by the sensor.

To get around this problem, sensor **3** was attached to Tentagel to explore the possibility of detecting K^+ in water, as Tentagel is known to swell in polar solvents.[5] Accordingly, a DMF solution of **3** was stirred with Tentagel macrobeads (∼0.4 mmol Br/g) in the presence of DBU at 55 °C (50% loading), followed by the acetylation of the remaining unreacted −CH_2Br groups to generate **5** (Scheme 1). As anticipated, sensor bead **5** showed fluorescence enhancement as depicted in Figure 2.
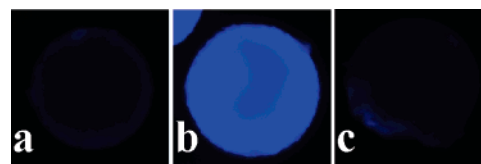
Figure 2. Fluorescence microscope image of (a) **5**; (b) **5** + 500 mM KCl in water; (c) b washed with water.

A fine-tuning of this experiment showed that the fluorescence intensity of **5** increased in the concentration range of 75−90 mM of KCl in water (see Supporting Information S3 and S4).

The binding specificity of the Tentagel-attached sensor bead **5** for K$^+$/Na$^+$ was also demonstrated (Figure 3), which
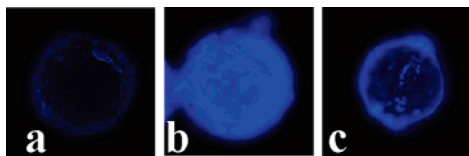

Figure 3. Fluorescence image of (a) **5**; (b) **5** + 500 mM KCl; (c) **5** + 500 mM NaCl in water.

proved that the sensor after being attached to the Tentagel beads retained its specificity toward K$^+$ in water.

A series of control experiments were performed to check the efficiency of the sensor beads in the detection of K$^+$. Two different control beads were prepared in which the fluorophore pyrene and the cation receptor 1-aza-18-crown-6 were separately and randomly attached to Merrifield resin (**6**) and Tentagel beads (**7**). As in the case of **4**, control bead **6** was treated with 500 μM KClO$_4$ solution in 20% MeCN/PhMe, which showed a very small increase in the fluorescence intensity compared to **4** (see Supporting Information S5). A similar experiment with control bead **7** (Tentagel-based) was also carried out. It was clear that sensor bead **5** was a much better sensor for K$^+$ (see Supporting Information S6).

Since the sensor design is modular, by altering only the cation receptor module, one can design sensors for the recognition of different cations. Thus, the receptor unit of sensor molecule **5** was changed from aza-18-crown-6 to aza-15-crown-5[6] to design molecule **5a** for greater Na$^+$ selectivity.

A 0.1 μM methanolic solution of **2a**, when titrated with the Na$^+$/K$^+$ salts, showed a slightly higher specificity for Na$^+$ over K$^+$ (Figure 4).

Compound **2a** was converted to the side chain carboxylic acid **3a** (by hydrogenolysis) which was used to prepare sensor bead **5a** (Scheme 1).

The fluorescence microscope images taken with bead **5a** showed that, at 100 mM concentration, **5a** could not detect either of the cations, but at 500 mM concentration, both the KCl- and NaCl-treated beads showed high fluorescence (Figure 5, and Supporting Information S7 and S8). **5a** can
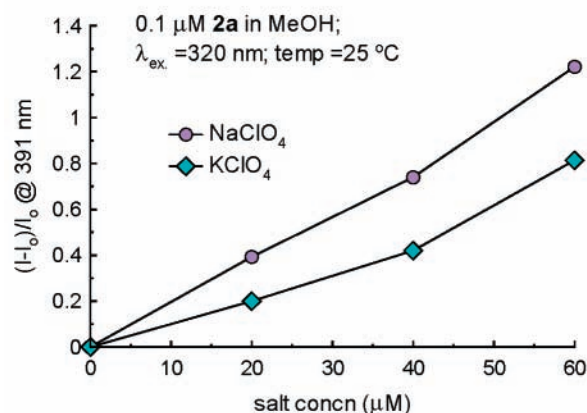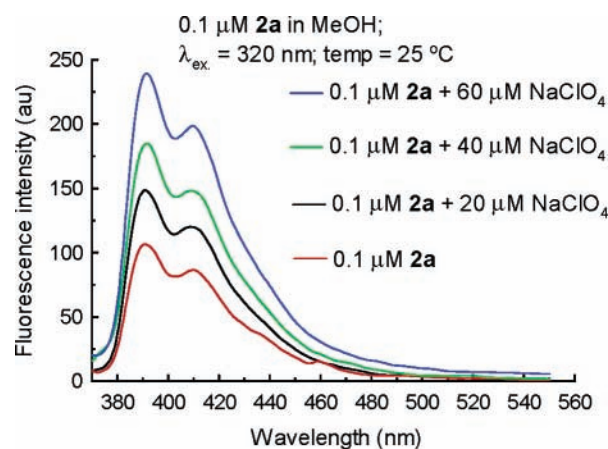
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(6) Binding constants of 1-aza-18-crown-6 in MeOH at 25 °C are log K (K$^+$) = 3.98; log K (Na$^+$) = 2.69. In the case of 1-aza-15-crown-5, binding constants are log K (K$^+$) = 1.60; log K (Na$^+$) = 1.70. 1-Aza-15-crown-5 is more selective to Na$^+$ over K$^+$, but the absolute value of the binding constant for Na$^+$ in the case of 1-aza-18-crown-6 is more than that with 1-aza-15-crown-5.

Figure 4. Fluorescence spectra of **2a** in MeOH (top). Plot of the relative enhancement in the fluorescence intensity of **2a** (bottom).

bind to Na$^+$ and K$^+$ only at relatively higher concentrations, and the difference in the fluorescence intensities between Na$^+$ and K$^+$ is difficult to distinguish. This is consistent with the binding constants of Na$^+$ and K$^+$ with 1-aza-15-crown-5 unit in methanol.[6,7]

Figure 5. Fluorescence microscope image of (a) **5a**; (b) **5a** + 100 mM NaCl; (c) **5a** + 100 mM KCl; (d) **5a** + 500 mM NaCl; (e) **5a** + 500 mM KCl in water.

As an alternative procedure[8] to detect alkali metal ions in water, water insoluble **3** was solubilized (8 μM) with Triton

(7) Since these crown ethers bind other cations, we have also carried out preliminary experiments with Ca^{2+} and Ba^{2+} (see Supporting Information S9 and S10).

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X-100 (1.6 mM), and fluorescence titration was carried out with KCl (and NaCl). The relative increase in the fluorescence intensity of sensor **3** with the added salt indicated that the sensor **3** was selective for K$^+$ over Na$^+$ in this medium also (Figure 6) (see the Supporting Information for the control experiment).
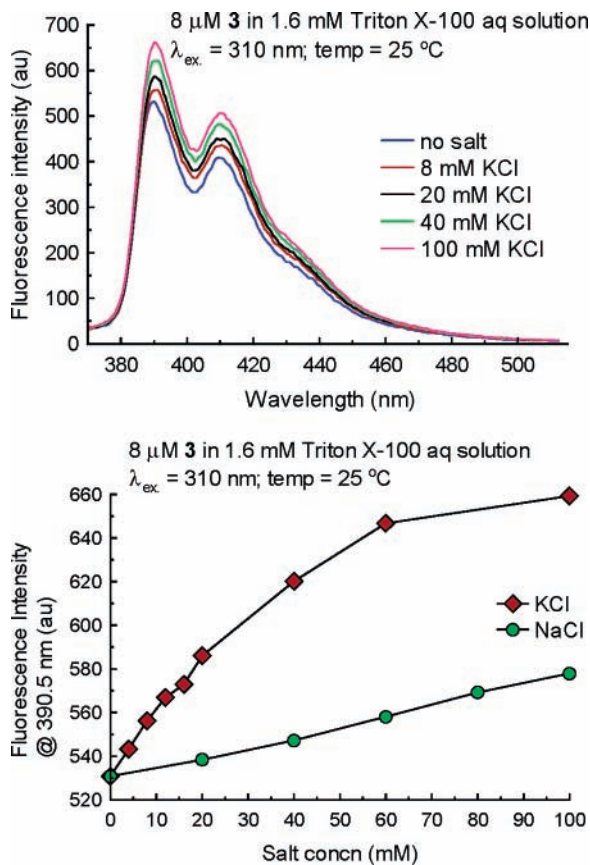

Figure 6. Fluorescence spectra (top). Plot of the enhancement in the fluorescence intensity (bottom).

A similar fluorescence titration was carried out with **2a** (2 μM) in aqueous Triton X-100 (1.2 mM), which showed a comparable difference in the fluorescence enhancement for Na$^+$ and K$^+$ (Figure 7), but it is less sensitive to K$^+$/Na$^+$ compared to the case of **3**.[6] The titration results in aqueous solution are in excellent agreement with the results obtained from the bead-attached sensors.
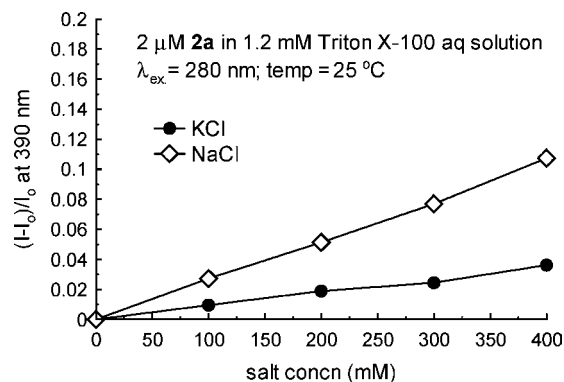

Figure 7. Relative increase in the fluorescence intensity ($I =$ fluorescence intensity in the presence of salt; $I_o =$ fluorescence intensity of **2a** in the absence of salt).

In conclusion, we have designed a PET sensor which can be easily prepared by a modular approach. An immobilized sensor on Merrifield resin showed fluorescence enhancement upon binding K$^+$ (0.5 mM) in 20% acetonitrile/toluene. Sensors attached to Tentagel beads can detect K$^+$ in water at ~90 mM. The modular concept was demonstrated by changing the receptor unit of the sensor to aza-15-crown-5. With appropriate developmental work, it should be possible to prepare reusable sensor beads for selective and quantitative determination of alkali metal ions. An array of the sensor beads can have practical applications, such as in fabricating sensing cartridges/artificial tongue.[9] Some of these possibilities are being explored in our laboratory and will be reported in due course.

Supporting Information Available: Synthesis of control beads, control experiment in Triton X-100 aqueous solution, fluorescence micrographs of beads for control experiments, and ^{1}H and ^{13}C NMR spectra of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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