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Fluorescent Probes

Straightforward Design of Fluorescent Receptors for Sulfate: Study of Non-Covalent Interactions Contributing to Host–Guest Formation

Tatiana A. Shumilova, Tobias Rüffer, Heinrich Lang, and Evgeny A. Kataev*[a]

Abstract: A straightforward design of receptors for binding and sensing of sulfate in aqueous medium was developed. The design involves the connection of two naphthalimide-based pH probes through a hydrogen-bonding motif. The structure of the receptor–sulfate complex, predicted by DFT calculations, was unambiguously confirmed by NMR measurements. There are three major interactions stabilizing the host–guest complex: electrostatic interactions, hydrogen bonding, and stacking interactions of the dyes. Study of two control receptors containing either one dye or methyl amide groups instead of amides, revealed that electrostatic and hydrogen bonding interactions contribute the most to affinity and selectivity of receptors. The receptors can detect sulfate in a 1:1 THF–buffer mixture in pH window 3.6–4.5 demonstrating up to 7-fold fluorescence enhancement. To the best of our knowledge, the reported PET (photoinduced electron transfer) anion probes possess the largest response for sulfate in aqueous solution yet described.

Anion recognition has received much attention over the last two decades due to its possible applications in biological, medical, and industrial areas.[1] Sensing of anions is a more challenging goal than sensing of cations of the same size, because anions have more complex geometries and higher solvation energies.[2] The last fact becomes even more important, if recognition process takes place in a highly competitive medium, such as water.[3] Among all tetrahedral oxyanions, sulfate is an interesting target for recognition and sensing, because sulfate is present in the environment[4] and in living organisms.[5] Several approaches for selective recognition of sulfate in aqueous solution already exist. They include the use of electrostatic interactions,[6] hydrogen bonding[7] or their combinations.[8,10] However, creating a simple receptor with strong fluorescent response for sulfate in water remains a challenge.[9]

Most of the known PET-based (PET = photoinduced electron transfer) probes for anions and for sulfate in particular undergo quenching of fluorescence upon interactions with anions.[10] Modern applications require either turn-on or ratiometric response.[11] Herein, we report the design, synthesis, and anion binding properties of novel sulfate-selective PET probes with a turn-on response. The developed probes are selective for sulfate in aqueous buffer–THF solutions and can be used for the determination of sulfate in the presence of other competing anions. The design approach suggested in this work represents a general strategy towards sulfate-selective probes working in water and will serve as a starting point to discover future probes for specific applications.

Our recent studies on cryptands functionalized with anthracene and naphthalimide dyes revealed that correctly positioned positive charges in the receptor structure are sufficient to achieve selectivity for sulfate in a buffered solution at pH 3.6–5.6.[11b] We proposed that two positively charged groups placed on an appropriate distance should be sufficient to bind and detect sulfate in aqueous solution. The pH-sensitive dyes should carry these groups as shown in Figure 1. As a linker between two dyes, we used a rigid fragment containing hydrogen bond donor groups, and serving as additional binding sites. The naphthalimide dye, utilized in this work, was recently published by Sessler and Kim demonstrating approximately 21-fold fluorescence enhancement upon lowering the pH of the solution from 11 to 2.[12] Probes based on this pH sensor have been reported to detect mercury,[13] aluminum,[14] chromium,[15] lead,[16] cysteine,[17] pyrophosphate,[18] and ATP.[19]

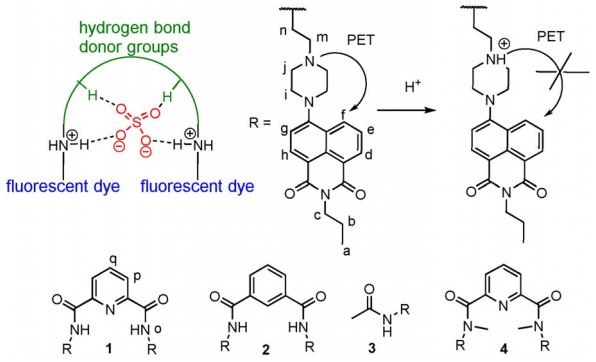

Figure 1. Design approach for a fluorescent receptor for sulfate and mechanism of the turn-on response implemented in this work. Structures of receptors **1**–**4**.

[a] *T. A. Shumilova, Dr. T. Rüffer, Prof. Dr. H. Lang, Prof. Dr. E. A. Kataev*
Institute of Chemistry
Technische Universität Chemnitz
09107 Chemnitz (Germany)
E-mail: evgeny.kataev@chemie.tu-chemnitz.de

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To link two pH-sensors by hydrogen bond donor groups, we chose pyridine-2,6-dicarboxamide and benzene-1,3-dicarboxamide,[6e] which were introduced by Hamilton, Crabtree, and others for the design of anion receptors.[20] Thus, the corresponding receptors **1** and **2** were prepared. Compounds **3** and **4** were prepared for the control studies to elucidate the roles of the second naphthalimide-piperazine fragment and NH-sites on the properties of receptors. Pyridine-2,6-dicarboxamide is known to form strong intramolecular hydrogen bonds with the pyridine nitrogen favoring a relatively rigid *syn,syn*-conformation of the NH-groups even in aqueous solution.[6c] To provide additional flexibility for receptors, we introduced an ethylamine linker between the pyridine and the dye. We expected that this flexibility should be an important requirement to allow two naphthalimide dyes to form π–π-interactions in the complex with sulfate and to isolate the anion from the solvent molecules.[21] To find out if such an arrangement of the naphthalimide rings is possible, we performed DFT calculations of $\mathbf{1}H_2^{2+}$ with sulfate. The conformational search yielded three minima with structures shown in Figure 2. Structure **I** has the

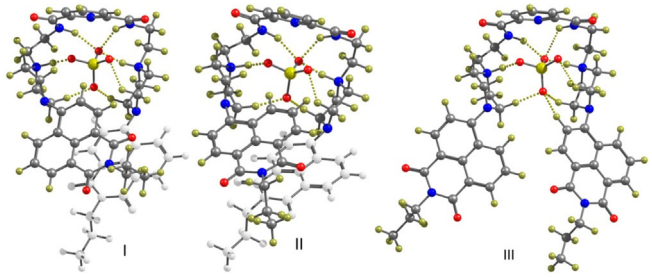

Figure 2. Optimized structures **I**–**III** of $\mathbf{1}{\cdot}H_2SO_4$. In structures **I** and **II**, the lower stacking naphthalimide ring is shown in pale colors for clarity.

lowest energy and displays stacking interactions between two naphthalimide rings. The sulfate anion is "wrapped" by the receptor and forms hydrogen bonds with amide-NH-, NH^+-, and CH-fragments. Structure **II** is 3 kcal mol^{-1} less stable and was generated by the rotation of the naphthalimide ring by 180°. In structure **III**, naphthalimide rings do not stack, which leads to 18 kcal mol^{-1} higher energy of the complex relative to **I**. Thus, DFT calculations provided an evidence of a good host–guest complementarity.

Receptors **1** and **2** were synthesized by acylation of the naphthalimide precursor bearing a free amine group with the corresponding diacids dichlorides (for details see the Supporting Information).[12] According to the single-crystal X-ray analysis, **1** has parallel oriented naphthalimide rings, which display CH–π interactions (Figure 3). Between the molecules π–π-stacking interactions were found. The receptor coordinates one methanol molecule through hydrogen bonds with amide-NHs.

To reach 0.1 mM concentration of receptors, which is suitable for NMR, fluorescence and UV/Vis spectroscopy, we used 1:1 THF–acetate buffer mixtures. UV/Vis dilution experiments confirmed that no aggregation is present for the receptors at 0.1 mM concentration (Figure S2 in the Supporting Information).

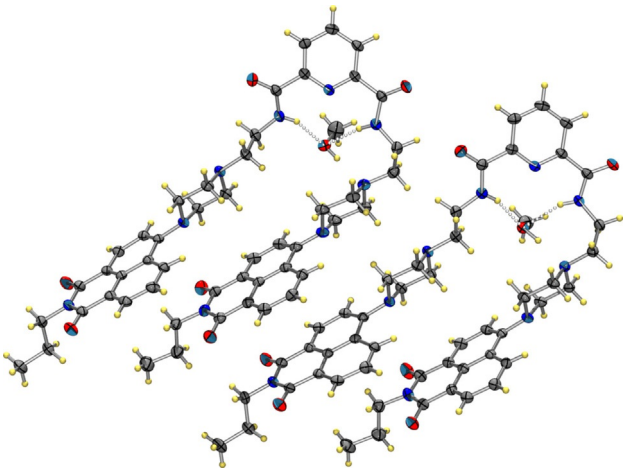

Figure 3. Single-crystal X-ray structure of receptor **1**.

To assess the working pH-window of the receptors we recorded fluorescence spectra in solutions with different pH values. The pH values in all our experiments reflect the pH of the aqueous phase and not of the solvent mixture. This simplification was used for the sake of fast screening of receptor properties even though the protonation state of the solution/receptor might differ in water and THF-water mixtures. The pH-dependent fluorescence was also recorded in the presence of an excess of sulfate and phosphate (Figure 4a). Interestingly, the presence of sulfate shifts the original curve (S1) towards basic pH values by almost one pH unit. Similar behavior was observed for receptor **2**, whereas **3** did not show any shifts (Figure S3). Thus, these results indicate a strong binding of sulfate by **1** and **2**, which in turn induces a pK_a shift of the piperazine amine groups. To provide an evidence that coordination of sulfate causes the protonation equilibrium to concomitantly shift to a higher protonated state of the receptor, we measured UV/Vis spectra of receptor **1** at different pH values. The observed spectral changes were compared with those detected by titration of **1** with sulfate at a constant pH value—pH 4.1. Lowering the pH of the solution induces a hypsochromic shift of the absorption maximum (Figure 4c). The titration of **1** with Na_2SO_4 resulted in similar changes, yielding $\log K = 3.58 \pm 0.01$. Based on Figure 4a, the fluorescence increase upon addition of an excess of sulfate to the receptors can be predicted. For instance, the fluorescence of the solution will be increased by approximately 7-fold (shown with an arrow) if 100 equiv of sulfate are added to **1**.

Next, we studied the interaction of receptors with common mono-negative anions present in the environmental water together with oxalate as a di-negative anion but having a geometry different from sulfate. Addition of sulfate to **1** and **2** induced the strongest fluorescence enhancement, while Iodide led to a decrease of fluorescence due to the dynamic quenching process.[22] Analysis of the obtained binding constants (Table 1) leads to the conclusion that the receptors bind sulfate about 100 times stronger than other anions. The binding stoichiometry was determined by the residual plot method as described previously in the literature.[23] which confirmed a major

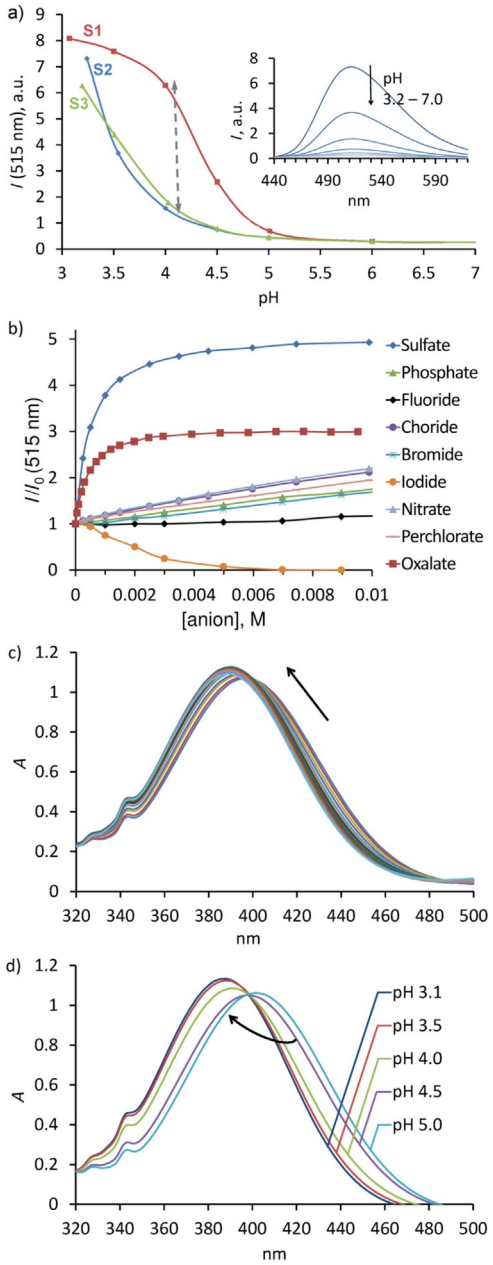

Figure 4. a) Fluorescence vs. pH for **1** (ex. 380 nm); spectra were recorded in a 1:1 water-THF, containing 50 mM of acetate buffer; S1 = without any additives, S2 = in the presence of 10 mM NaH_2PO_4, S3 = in the presence of 10 mM Na_2SO_4. b) Binding isotherms obtained by fluorescence titration of **1** in a 1:1 buffer-THF (pH 4.1) with anions as their sodium salts. c) Changes in the UV/Vis spectrum of **1** in a 1:1 buffer-THF (pH 4.1) induced by addition of Na_2SO_4. d) Dependence of the UV/Vis spectrum of **1** on pH.

1:1 binding mode. The affinity of receptor **1** for sulfate obtained by fluorescence measurements agrees with that calculated from UV/Vis titrations. Interestingly, oxalate induced lower enhancement of fluorescence, but had approximately the same binding constant as that for sulfate. This fact supports the conclusion that electrostatic interactions contribute the most to the sulfate binding. Some of the studied mononegative anions, especially perchlorate, caused fluorescence enhancement upon increasing its concentration, although calculated binding constants were very low ($<50\ M^{-1}$). An explanation to this phenomenon we found in the work of Dyson.[24] The authors reported that sterically large anions often suppress cation–anion interactions, which leads to increased fluorescence intensity.

Table 1. Binding constants of receptors **1**, **2** and **4** with anions determined in a 1:1 THF –50 mM acetate buffer (pH 4.1) at 23 °C.

Anion[a]	log*K*[b] 1	2	4
sulfate	3.30	3.36	2.03
oxalate	2.98	3.00	2.50
phosphate	1.28	1.89	1.45
fluoride	–[b]	1.82	–[b]
chloride	1.51	1.48	1.15
bromide	1.25	1.53	1.17
iodide	–[c]	1.27	–[c]
nitrate	1.45	1.27	<1
perchlorate	1.03	1.15	<1

[a] All anions were used as their respective sodium salts. [b] Measurement error ≤10%. [c] Slow binding kinetics.

To understand how the amount of charge on the receptor reflects its binding and emission properties, we carried out a series of titrations for **1** with sulfate at different pH values. The higher the pH values of the solution is, the less positive charge the receptor should carry. As can be inferred from Figure 5, increasing the pH of the solution leads to a decrease of binding constants. However, the overall fluorescence enhancement increases until the maximum (7-fold) at pH 4.4 and then slightly decrease, albeit keeping the saturation point the same. The competition fluorescence experiments with different anions showed that only oxalate increases the error of sulfate detection (Figure S7 in the Supporting Information). Sulfate can be detected in water with the detection limit of 1.7×10^{-6} M.

We questioned if 2,6-pyridinecarboxamide spacer between two naphthalimide dyes serves as a simple spacer or a hydrogen bond donor site. ^{1}H NMR titrations of **1** with sulfate revealed that the signals of methylene groups belonging to piperazine (i-l) and the ethylene linker (n, m) shifted towards low field upon addition of sulfate (Figure S2). These shifts are ob-

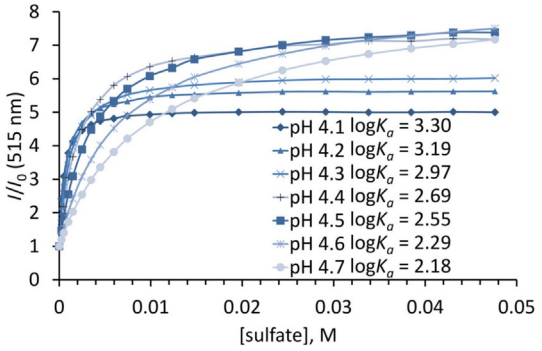

Figure 5. Binding isotherms for **1** (0.1 mM) with Na_2SO_4 at different pH values of the buffer obtained by fluorescence titrations in a 1:1 THF –50 mM acetate buffer. Errors for pH measurements are less than 0.05 pH units.

served because the ethylene protons are located directly near the protonation center. The naphthalimide protons (h, d, f) in the aromatic area were also shifted, which indicates that sulfate binding induces conformational changes in the structure. On the other hand, the pyridine protons (p, q) moved towards high field suggesting the formation of hydrogen bonds between NH-donor sites and the anion.

Next, we prepared control receptor **4** bearing methyl amide groups.[25] According to ^{1}H NMR, receptor **4** exists as a mixture of conformers with a dynamic exchange at room temperature, which disappears at 50 °C (Figure S1 in the Supporting Information). The pH scanning experiment revealed that addition of sulfate did induce a pK_a shift, though the shift was much smaller than that for **1** or **2** (Figure S4). Fluorescent titrations of **4** with anions revealed that the receptor binds both sulfate (log$K$ = 2.03 ± 0.01) and oxalate (log$K$ = 2.50 ± 0.01), but with approximately one order of magnitude lower constants than those determined for **1** and **2**. Thus, anion binding studies with **4** suggest that the NH-donor sites considerably stabilize the overall host–guest complex.

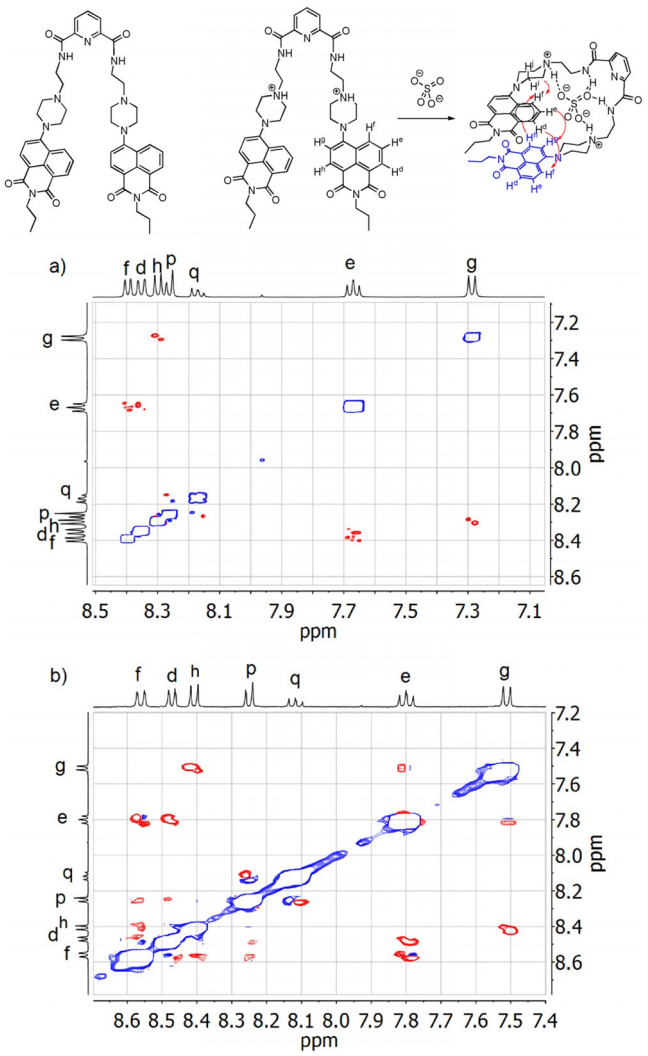

Figure 6. ^{1}H–^{1}H ROESY Spectra of receptor **1** a) in the absence and b) in the presence of sulfate in 3:2 THF –50 mM acetate buffer (pH 3.6).

To reveal if stacking interactions are present in solution, as predicted by DFT calculations, we carried out ROESY measurements with **1** in the absence and in the presence of sulfate in solution. Analysis of the spectra in Figure 6 leads to the conclusion that **1** exists in the acetate buffer presumably in the conformation, in which naphthalimide rings do not interact. CH–π interactions found in the X-ray structure were also not present. A number of new cross-signals appeared after addition of 20 equiv of $(NMe_4)_2SO_4$. These cross-signals unambiguously suggest π–π interactions between naphthalimide rings in the complex with sulfate. The proposed structure based on the ROESY experiment resembles the DFT predicted structure **II**. Thus, stacking interactions between two dyes additionally contribute to the formation of the complex with sulfate.[26]

In conclusion, a straightforward design of a fluorescent receptor for binding and sensing of sulfate in a 1:1 THF-buffer mixture was developed. The sulfate anion is bound to the receptor through electrostatic and hydrogen bonding interactions and at the same time, it induces conformational changes of the receptor structure that favor π–π-interactions between dyes. The mechanism of fluorescence enhancement is attributed to the pK_a shift of naphthalimide dyes induced by sulfate binding. To solve the problem of using solvent mixtures and achieve the solubility of receptors in pure water, the structure of dyes can be easily optimized by for example, attaching an oligoethylene glycol substituent in the naphthalimide core. Studies along these lines are in progress.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: anion recognition · fluorescence sensing · naphthalimide · selectivity · sulfate

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