Fluoride Sensing Hot Paper

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Test-Strip-Based Fluorometric Detection of Fluoride in Aqueous Media with a BODIPY-Linked Hydrogen-Bonding Receptor**

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Abstract: *The measurement of biologically relevant anions, such as fluoride, is an important task in analytical chemistry, in particular, for dental health and osteoporosis. Although a large number of fluoride probes are known, the applicability under relevant conditions is limited to a few examples. To improve this situation, BODIPY-amidothiourea dyes with varying hydrogen-bond donating strengths were developed, the most H-acidic of which (**1c**) could detect F^- from an inorganic source (NaF) in 50 % aqueous solution (DMSO/water 1:1, v/v) with 0.01 ppm sensitivity through selective fluorescence quenching by a photoinduced electron-transfer (PET) process. Use of the probe and a reference dye with a test-strip assay and a portable and rapidly recording lateral-flow fluorescence reader made determination of F^- in neat aqueous solutions, such as spiked water samples and toothpaste extracts, possible in a self-referenced manner, achieving a detection limit of 0.2 ppm.*

Over the past decade, the field of anion detection has grown exponentially because of the significant role of anions in biological systems and the environment.[1] Among the small inorganic anions, F^- is of paramount importance because of its duplicitous nature.[2] Water fluoridation or addition of fluoride to toothpaste has become a widespread practice in many countries owing to the beneficial effects for dental health and the treatment of osteoporosis.[3] High doses of F^- are, however, dangerous and can lead to dental or skeletal fluorosis;[4] they are also associated with kidney failure[5] and nephrolithiasis.[6] Only in 2011, the US Environmental Protection Agency (EPA) followed various other countries[7] and reduced the recommended F^- level in drinking water from 1 to 0.7 ppm.[8] Although considerable attention has been devoted to the development of molecular probes for F^- potentially suitable for rapid at-site analyses, the applicability of the probes as such under environmentally relevant conditions is limited to a very few examples.[9] This problem has been ascribed to the strong solvation of both, anion and binding site of the host in competitive solvents.[10] To achieve anion detection in aqueous solution, several design strategies have been proposed: Lewis acid[11] or metal–anion coordination,[12] cationic receptors,[13] anion–π interaction,[14] formation of Si–F bonds,[15] the construction of hydrophobic microenvironments around receptor moieties[16] and the use of highly acidic donor units.[17] However, most of these probes[11–14,16,17] accomplish only the detection of F^- from organic origin, that is, with tetrabutylammonium fluoride (TBAF) as the source, and in mixed organic–aqueous solution (typically with a content of 5–30 % of an organic co-solvent such as DMSO, CH_3CN or EtOH).[18] Moreover, for charged receptors not only do electrostatic forces govern and compromise directional binding but problems associated with the additional introduction of counterions into the system have to be faced. On the other hand, F^- dosimeters based on a desilylation reaction were reported to operate in aqueous solution.[15] A drawback of this irreversible reaction, however, is the rather slow response time from tens of minutes to even hours, which limits real-time and on-site applicability. The preferable binding units are thus neutral receptors with a directional hydrogen-bonding motif. However, what still remains to be overcome for these receptors is the competition of water.

Several analytical techniques have been demonstrated for quantitative fluoride determination,[19] mostly requiring costly instrumentation, experienced operators, and tedious procedures. Thus, the development of a simple and inexpensive method for F^- detection is still urgently needed. Herein, we report how newly designed boron dipyrromethene-(BODIPY-) amidothiourea probes (**1a–c**) can be used for the detection of F^- ions in aqueous media in a test-strip-based assay with a portable, handheld, and rapidly recording lateral-flow fluorescence reader. Several recent reports on such simple dipstick-based tests for metal ions[20] and small molecules[21] have demonstrated the enormous potential that lies with such assays. However, the application of such a system for the quantitative detection of anions has not been reported to date.

BODIPY–amidothiourea dyes (**1a–c**) were synthesized as shown in Scheme 1 and all the compounds have been characterized by standard analytical procedures. The hydrogen-bond donating ability of the thiourea group has been tuned by the incorporation of electron releasing (OCH_3) and withdrawing (NO_2) groups on the thiouredio phenyl group. As expected, the presence of the -NO_2 group increases the acidity of the thiourea protons, which is evident from a downfield shift ($\delta = 0.1$ and 0.3 ppm) of the ^1H NMR signals of the NH protons.

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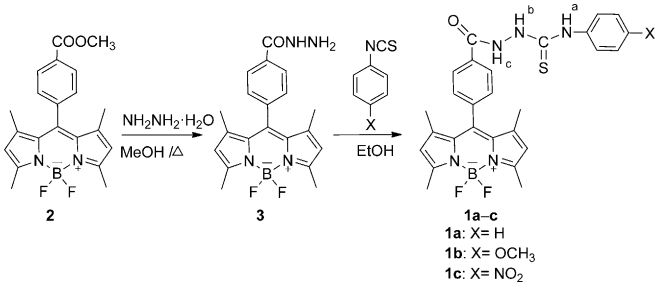

Scheme 1. Synthetic route to probes **1a–c**.

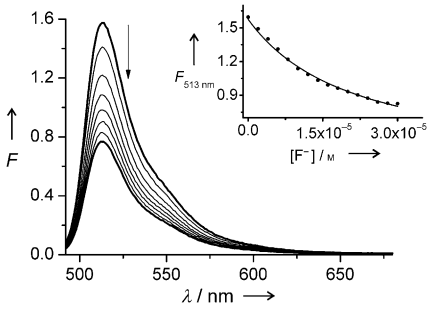

Figure 1. Fluorescence quenching of **1c** (2.0 μM) upon addition of F^- ions (0–0.8 ppm) in DMSO/water (1:1, v/v; 9 mM MES buffer, pH 6.8), $\lambda_{exc} = 482$ nm. Inset: changes in the fluorescence intensity at 513 nm and the calculated 1:1 binding isotherm.

Single crystals suitable for X-ray diffraction were obtained by slow evaporation of solutions of **1b** and **1c** in CH_2Cl_2. Crystallographic data and refinement parameters are given in Table S1 of the Supporting Information. Compounds **1b** and **1c** crystallize with four and two molecules in the unit cell, respectively (Figures S1–S4). The BODIPY core is virtually planar with a root-mean-square (rms) deviation of 0.0192 Å (C1, C9, N1, N2) in **1b** and 0.0532 Å in **1c**, that is, it shows a higher distortion in **1c** than in **1b**. In both compounds, the thiourea NHs are in *syn* conformation; the amide NH is positioned *anti* to it.

Compounds **1a–c** show absorption and emission bands centered at 500 and 510 nm, respectively, which is characteristic for the parent BODIPY dyes.[22] Table S2 shows the photophysical features of **1a–c** in MeCN, DMSO, and DMSO/water mixtures. All the three dyes show an increased fluorescence quantum yield and lifetime in DMSO/water mixtures, when compared to the neat organic solvent, which is an advantage for highly efficient sensing systems based on fluorescence quenching.

Initial anion-interaction studies were carried out in polar aprotic solvents, such as MeCN and DMSO. Addition of a source of F^- ions to **1a** in MeCN results in a blue shift of the absorption maximum of 2 nm (Figure S5) indicating an increase in electron density at the *meso*-phenyl group due to anion-receptor interaction. The corresponding emission shows a 7.1-fold decrease without any spectral shift (Figure S6). Fluorescence quenching was found to saturate with the addition of two equivalents of F^-, revealing a strong interaction between receptor and F^-. The fluorescence quenching data cannot be fitted to a 1:1 binding model which indicates that more than one interaction mode is present. Based on earlier observations for related receptors,[23] we tentatively ascribe deprotonation to the second competitive process besides H-bonding complexation. Among the other anions, addition of 10 equivalents of AcO^- and $H_2PO_4^-$ show a 4.8- and 1.4-fold reduction in fluorescence (Figure S6, S7), reflecting the order of basicity of the anions. In addition, the interactions between **1a** and AcO^- and $H_2PO_4^-$ obey to a stoichiometric model and fits of the fluorescence titration data yielded binding constants of $(1.05 \pm 0.41) \times 10^6$ and $(5.83 \pm 0.34) \times 10^5\ M^{-1}$, respectively.

For applicability in more realistic scenarios, the sensing performance of **1a–c** was investigated in various organic–aqueous mixtures, of which DMSO/water[24] yielded the best response and was used for further studies. Fluorescence titrations were carried out in aqueous DMSO with varying percentages of water using sodium fluoride (NaF) as the F^- source. Among the three dyes, **1c** showed the best response towards F^- in DMSO/water mixtures. Even at 50% water content (DMSO/water 1:1, v/v), **1c** showed significant fluorescence quenching (Figure 1) with a binding constant (1:1 model) of $(5.34 \pm 0.55) \times 10^4\ M^{-1}$. In contrast, dyes **1a** and **1b** show only a weak response towards F^- at ratios up to 30% water (detailed binding data in Table S3); beyond that, any response is significantly diminished. This clearly indicates that the presence of the electron withdrawing -NO_2 group is essential for increasing the acidity of the thiourea NH groups to successfully compete with water molecules for F^- binding in organic–aqueous media with a high water content. In fact, computational analysis of the ground-state charge distribution in **1a–c** and various receptor analogues from the literature revealed the importance of pushing the electron deficiency in the thiourea moiety to an extreme to enable H-bonding complexation in the presence of competitive media (Table S4). For **1c**, the limit of detection for F^- from an inorganic source in 1:1 DMSO/water was determined to 0.01 ppm, which is significantly lower than for most reported F^- indicators.[11–17] To explore the selectivity of the probe, we carried out titrations with all the other anions as shown in Figure S9. Addition of AcO^- and $H_2PO_4^-$ (only at very high concentration) also shows quenching, however, these changes are smaller in magnitude (ca. 50% and 20%, respectively) when compared to F^-. In the presence of the other anions no changes in fluorescence were observed. It is known that unidirectional H-bonding anion receptors cannot discriminate entirely between F^- and AcO^-. However, most of the F^--contaminated water samples and dental products lack AcO^-. The probe can hence be effectively used for the detection of F^- in organic–aqueous mixtures down to 0.01 ppm sensitivity.

The mechanism responsible for fluorescence quenching was assessed with fluorescence-lifetime measurements. Probe **1c** exhibits a bi-exponential decay with lifetimes of 0.04 (19%) and 2.62 ns (81%) in a DMSO/water (1:1, v/v) mixture. Upon addition of an F^- ion source, the shorter lifetime further decreases to 0.02 ns with a concomitant increase in amplitude to 64% (temporal resolution of the instrument is ±3 ps); the lifetime of the other component

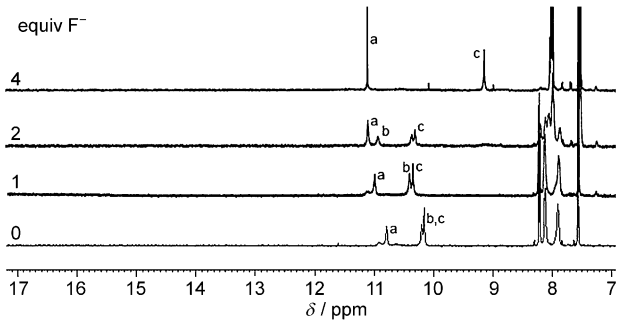

Figure 2. ^{1}H NMR titration spectra of **1c** (2 mM) with 0, 1, 2, and 4 equivalents of F^- ions in [D_6]DMSO/H_2O (95:5, v/v); proton labels are shown in Scheme 1.

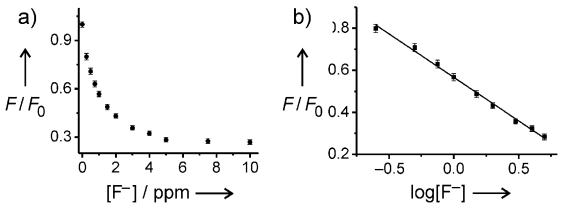

Figure 3. Plot of the fluorescence intensity ratio of **1c** (10.0 μM) versus a) concentration of F^- ions and b) logarithm of F^- ion concentration.

remains unchanged but shows a decrease in amplitude to 36%. The formation of a new, faster decaying component with an increased amplitude indicates the formation of an actual new species, the H-bound complex, involving a photo-induced electron transfer (PET) process[25] from the F^--bound receptor to the BODIPY fluorophore.

To understand the nature of the interaction between F^- ions and the receptor, we carried out ^{1}H NMR titration studies in a [D_6]DMSO/H_2O (95:5, v/v) mixture[26] (Figure 2). Addition of 1 equivalent of F^- ions results in the broadening and downfield shift of the thiourea proton peaks "a" ($\Delta\delta =$ 0.24 ppm) and "b" ($\Delta\delta = 0.21$ ppm), which indicated hydrogen-bonding interactions between the NH groups of **1c** with F^- ions. At higher concentration of F^- ions, peak "b" disappears, indicating deprotonation. This two-step process is in accordance with earlier reports.[27] However, in buffered solution with 50% water as used for the spectroscopic studies, F^- does not induce deprotonation and only H-bonding between the anion and receptor is detected, control experiments with OH^- support these findings (Figure S10). In contrast, in neat [D_6]DMSO (Figure S11), 1 equivalent of F^- results in the disappearance of peak "b", and at higher concentrations a new triplet at $\delta = 16.1$ ppm appears as expected for deprotonation of this kind of receptor.[27c]

Fluorescent probes for F^- ion detection are commonly used in cuvette-based tests employing conventional fluorometers. This approach is inconvenient and cannot be used for in situ/on-site detection. Thus, a test-strip-based assay was developed to achieve the detection of F^- ions in neat aqueous solution and enable the recording of the fluorescence intensity changes with a portable, handheld, lateral-flow reader. To date, dipstick-type F^- tests have only been reported and used for the qualitative detection of F^- at rather high concentrations.[15a,28] Hence, our goal was to develop a simple dip-and-detect method for the quantitative determination of F^- at lower ppm concentrations. The method that allowed us to achieve our goal is as follows. A drop of an ethanolic solution of **1c** was placed on the test strip (pipette or dispenser), dried, and the fluorescence intensity was measured using a lateral-flow reader. We used nitrocellulose strips in this case because this matrix ensures that, although only sterically embedded(i.e. "physisorbed" no leaching of the rather hydrophobic probe molecules occurs in water while ensuring that the full molecular PET response can unfold. The test strip was then immersed for 10 s in aqueous NaF solution of varying concentrations, dried (40 s with and 5 min without hair dryer in cold-air-blow mode at room temperature), and the fluorescence assessed with the reader. Figure 3 shows the fluorescence-intensity changes and the calibration curve of the test-strip assay with increasing concentration of F^- ions.

The test strip shows a detection limit of 0.2 ppm, which is significantly less than the recommended F^- levels mentioned above.[7] Moreover, the intensity change is proportional to the logarithm of F^- concentration enabling straightforward quantitative detection in aqueous media. To validate the applicability, we measured the content of F^- from two commercially available toothpastes. An aqueous extract was prepared with the necessary dilution and analyzed. By knowing the extent of fluorescence quenching for the test solutions and by extrapolating it to a calibration curve generated from standard NaF solutions, the amount of F^- in toothpastes A and B was determined to 1679 ± 72 and $1167 \pm$ 56 ppm, respectively, which is in reasonable agreement with the value given by the manufacturer ($A = 1450$ and $B =$ 1000 ppm). The observed positive error of approximately 16% is presumably due to the presence of an excess of $H_2PO_4^-$ in the toothpaste formulation, leading to a false positive signal at such high phosphate concentrations.

The test-strip method was then tested for analyzing various water samples, spiked with known concentrations of F^- in a range relevant for water fluoridation. Three water samples were tested: distilled water, tap water (Berlin, Germany) and simulated sea water.[29] The concentration of F^- was calculated from the calibration curve as discussed above and the results obtained from the test-strip method were compared to those assessed with an independent method employing an ion-selective electrode (ISE, Table 1). Both methods show a good agreement and the performance of the test strip is unaffected by any other ions present in tap

Table 1: Measurement of F^- ions in spiked water samples by ISE and test-strip methods

Water sample	Amount of F^- spiked [ppm]	F^- [ppm]/ ISE	F^- [ppm]/test-strip assay
Distilled water	0.58	0.57 ± 0.02	0.60 ± 0.03
Tap water	0.58	0.60 ± 0.02	0.62 ± 0.04
Simulated sea water	0.77	0.80 ± 0.02	0.84 ± 0.05

or saline water. To further simplify the method, a two-point referencing system was implemented on the strip by spotting two defined concentrations of 1,3,5,7-tetramethyl-8-phenyl BODIPY (**4**),[22] a dye that has almost the same spectroscopic properties as **1c** but does not respond to F^- under assay conditions. The concentrations of **4** were chosen to match 100% ($\mathbf{4}_{100}$) and 30% ($\mathbf{4}_{30}$) signal intensity of the initial spot of **1c**. Since the fluorescence of the reference spots does not change in the presence of F^-, a simple comparison of the intensity change at spot **1c** with spots $\mathbf{4}_{100}$ and $\mathbf{4}_{30}$ and the known F^- quenching data enables the determination of F^- content in a single-dip measurement (Figure 4, and Figure S13). Hence, this simple and user-friendly test-strip assay can be used to determine the concentration of F^- ions in water samples in the relevant, lower ppm concentration range. As would be expected for a supramolecular recognition scenario, the binding of F^- ions is reversible and the strips are thus reusable.

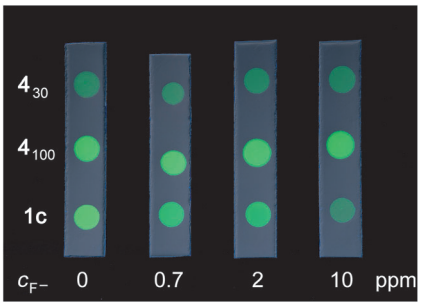

Figure 4. Photograph of four different internally referenced strips dipped into aqueous solutions containing 0, 0.7, 2, and 10 ppm of F^- ions (under UV lamp illumination; for details, see text and Section S18 in the Supporting Information).

In summary, we have demonstrated the potent binding of F^- ions from an entirely inorganic fluoride source to an amidothiourea receptor in aqueous DMSO solution, with as much as 50% water, which is unprecedented for neutral hydrogen-bond-based probes and shows the importance of tuning the electronic properties of such binding sites to extreme electron deficiency. Quantitative detection of inorganic F^- ions in 100% aqueous solution with a remarkable detection limit of 0.2 ppm was possible by using the probe in a straightforward manner with an internally referenced test-strip assay and a lateral-flow reader. This simple and cost-effective method can be applied to determine the F^--ion content of drinking water. Further work is directed toward customizing, miniaturizing, and further simplifying the detection device so that such tests might become a valuable tool for F^- analysis in concerned households.

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[1] a) S. Kubik, *Chem. Soc. Rev.* **2010**, *39*, 3648–3663; b) T. Gunnlaugsson, M. Glynn, G. M. Tocci, P. E. Kruger, F. M. Pfeffer, *Coord. Chem. Rev.* **2006**, *250*, 3094–3117.
[2] a) M. Cametti, K. Rissanen, *Chem. Commun.* **2009**, 2809–2829; b) E. Newbrun, *J. Public Health Dent.* **2010**, *70*, 227–233.
[3] a) K. L. Kirk in *Biochemistry of the Elemental Halogens and Inorganic Halides*, Plenum, New York, **1991**, pp. 19–68; b) H. S. Horowitz, *J. Public Health Dent.* **2003**, *63*, 3–8.
[4] a) M. A. Holland, L. M. Kozlowski, *Clin. Pharm.* **1986**, *5*, 737–741; b) E. T. Everett, *J. Dent. Res.* **2011**, *90*, 552–560.
[5] a) M.-L. Cittanova, B. Lelongt, M.-C. Verpont, M. Gèniteau-Legendre, F. Wahbè, D. Priè, P. Coriat, P. M. Ronco, *Anesthesiology* **1996**, *84*, 428–435; b) M. Ludlow, G. Luxton, T. Mathew, *Nephrol. Dial. Transplant.* **2007**, *22*, 2763–2767.
[6] P. P. Singh, M. K. Barjatiya, S. Dhing, R. Bhatnagar, S. Kothari, V. Dhar, *Urol. Res.* **2001**, *29*, 238–244.
[7] R. W. Evans, J. W. Stamm, *J. Public Health Dent.* **1991**, *51*, 91–98.
[8] a) K. Sebelius, *Fed. Regist.* **2011**, *76*, 2383–2388; b) EPA National Primary Drinking Water Standards **2003**, see also http://www.epa.gov/safewater/contaminants/index.html for more information.
[9] a) T. Gunnlaugsson, P. E. Kruger, P. Jensen, J. Tierney, H. D. P. Ali, G. M. Hussey, *J. Org. Chem.* **2005**, *70*, 10875–10878; b) C.-W. Chiu, F. P. Gabbaï, *J. Am. Chem. Soc.* **2006**, *128*, 14248–14249; c) I.-S. Ke, M. Myahkostupov, F. N. Castellano, F. P. Gabbaï, *J. Am. Chem. Soc.* **2012**, *134*, 15309–15311.
[10] S. Kubik, C. Reyheller, S, Stüwe, *J. Inclusion Phenom. Macrocyclic Chem.* **2005**, *52*, 137–187.
[11] T. W. Hudnall, C.-W. Chiu, F. P. Gabbaï, *Acc. Chem. Res.* **2009**, *42*, 388–397.
[12] a) P. D. Beer, D. P. Cormode, J. Davis, *Chem. Commun.* **2004**, 414–415; b) A. Labande, J. Ruiz, D. Astruc, *J. Am. Chem. Soc.* **2002**, *124*, 1782–1789.
[13] a) M. D. Best, S. L. Tobey, E. V. Anslyn, *Coord. Chem. Rev.* **2003**, *240*, 3–15; b) B.-G. Zhang, P. Cai, C. Y. Duan, R. Miao, L. G. Zhu, T. Niitsu, H. Inoue, *Chem. Commun.* **2004**, 2206–2207; c) M. S. Han, D. H. Kim, *Angew. Chem.* **2002**, *114*, 3963–3965; *Angew. Chem. Int. Ed.* **2002**, *41*, 3809–3811.
[14] S. Guha, S. Saha, *J. Am. Chem. Soc.* **2010**, *132*, 17674–17677.
[15] a) R. Hu, J. Feng, D. Hu, S. Wang, S. Li, Y. Li, G. Yang, *Angew. Chem.* **2010**, *122*, 5035–5038; *Angew. Chem. Int. Ed.* **2010**, *49*, 4915–4918; b) S. Y. Kim, J. Park, M. Koh, S. B. Park, J.-I. Hong, *Chem. Commun.* **2009**, 4735–4737; c) B. Zhu, F. Yuan, R. Li, Y. Li, Q. Wei, Z. Ma, B. Du, X. Zhang, *Chem. Commun.* **2011**, *47*, 7098–7100; d) B. Ke, W. Chen, N. Ni, Y. Cheng, C. Dai, H. Dinh, B. Wang, *Chem. Commun.* **2013**, *49*, 2494–2496.
[16] a) A. Metzger, V. M. Lynch, E. V. Anslyn, *Angew. Chem.* **1997**, *109*, 911–914; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 862–865; b) M. A. Palacios, R. Nishiyabu, M. Marquez, P. Anzenbacher, *J. Am. Chem. Soc.* **2007**, *129*, 7538–7544.
[17] a) P. Anzenbacher, Jr., M. A. Palacios, K. Jursíková, M. Marquez, *Org. Lett.* **2005**, *7*, 5027–5030; b) R. Nishiyabu, P. Anzenbacher, *Org. Lett.* **2006**, *8*, 359–362; c) Z. Li, F.-Y. Wu, L. Guo, A.-F. Li, Y.-B. Jiang, *J. Phys. Chem. B* **2008**, *112*, 7071–7079.
[18] Strategies relying on rather hydrophobic nanoparticle-based probes also require a TBA salt as phase-transfer catalyst, see F. Du, Y. Bao, B. Liu, J. Tian, Q. Li, R. Bai, *Chem. Commun.* **2013**, *49*, 4631–4633.
[19] a) M. C. Breadmore, A. S. Palmer, M. Curran, M. Macka, N. Avdalovic, P. R. Haddad, *Anal. Chem.* **2002**, *74*, 2112–2118; b) J. P. Hutchinson, C. J. Evenhuis, C. Johns, A. A. Kazarian, M. C. Breadmore, M. Macka, E. F. Hilder, R. M. Guijt, G. W. Dicinoski, P. R. Haddad, *Anal. Chem.* **2007**, *79*, 7005–7013.

[20] a) D. Mazumdar, J. Liu, G. Lu, J. Zhou, Y. Lu, *Chem. Commun.* **2010**, *46*, 1416 – 1418; b) J. Chen, X. Zhou, L. Zeng, *Chem. Commun.* **2013**, *49*, 984 – 986; c) J. V. Ros-Lis, M. D. Marcos, R. Martínez-Máñez, K. Rurack, J. Soto, *Angew. Chem.* **2005**, *117*, 4479 – 4482; *Angew. Chem. Int. Ed.* **2005**, *44*, 4405 – 4407.

[21] a) J. Liu, D. Mazumdar, Y. Lu, *Angew. Chem.* **2006**, *118*, 8123 – 8127; *Angew. Chem. Int. Ed.* **2006**, *45*, 7955 – 7959; b) J. Chen, Z. Fang, P. Lie, L. Zeng, *Anal. Chem.* **2012**, *84*, 6321 – 6325; c) E. Climent, D. Gröninger, M. Hecht, M. A. Walter, R. Martínez-Máñez, M. G. Weller, F. Sancenón, P. Amorós, K. Rurack, *Chem. Eur. J.* **2013**, *19*, 4117 – 4122.

[22] M. Kollmannsberger, K. Rurack, U. Resch-Genger, J. Daub, *J. Phys. Chem. A* **1998**, *102*, 10211 – 10220.

[23] a) W. X. Liu, Y.-B. Jiang, *Org. Biomol. Chem.* **2007**, *5*, 1771 – 1775; b) W.-X. Liu, R. Yang, A.-F. Li, Z. Li, Y.-F. Gao, X.-X. Luo, Y.-B. Ruan, Y.-B. Jiang, *Org. Biomol. Chem.* **2009**, *7*, 4021 – 4028; c) R. Koteeswari, P. Ashokkumar, V. T. Ramakrishnan, E. J. P. Malar, P. Ramamurthy, *Chem. Commun.* **2010**, *46*, 3268 – 3270.

[24] Water content has been restricted to 50% as a result of the aggregation of the probe at more than 60% water content. However, this aggregation behavior is circumvented by embedding the probe in a test strip.

[25] R. M. Duke, T. Gunnlaugsson, *Tetrahedron Lett.* **2007**, *48*, 8043 – 8047.

[26] The amount of H_2O has been restricted to 5% because of the broadening of ^1H NMR signals of **1c** at higher H_2O content.

[27] a) M. Boiocchi, L. Del Boca, D. Esteban-Gomez, L. Fabbrizzi, M. Licchelli, E. Monzani, *J. Am. Chem. Soc.* **2004**, *126*, 16507 – 16514; b) C. Pérez-Casas, A. K. Yatsimirsky, *J. Org. Chem.* **2008**, *73*, 2275 – 2284; c) P. Ashokkumar, V. T. Ramakrishnan, P. Ramamurthy, *Chem. Eur. J.* **2010**, *16*, 13271 – 13277.

[28] a) Z.-H. Lin, S.-J. Ou, C.-Y. Duan, B.-G. Zhang, Z.-P. Bai, *Chem. Commun.* **2006**, 624 – 626; b) A. K. Mahapatra, S. K. Manna, P. Sahoo, *Talanta* **2011**, *85*, 2673 – 2680; c) M. Varlan, B. A. Blight, S. Wang, *Chem. Commun.* **2012**, *48*, 12059 – 12061; d) X. Yong, M. Su, W. Wang, Y. Yan, J. Qu, R. Liu, *Org. Biomol. Chem.* **2013**, *11*, 2254 – 2257.

[29] M. Hecht, W. Kraus, K. Rurack, *Analyst* **2013**, *138*, 325 – 332.

