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PAPER

A ratiometric fluorescence sensor for caffeine†

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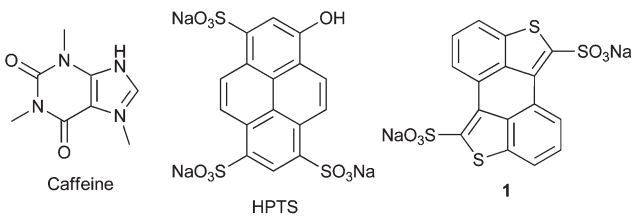
The dye disodium 3,4:3′,4′-bibenzo[*b*]thiophene-2,2′-disulfonate can be used as a molecular probe for the fluorimetric detection of caffeine in aqueous solution. The fluorescence response is attributed to non-covalent interactions of caffeine with the dye in the ground state and in the excited state. The bimodal interaction allows performing ratiometric measurements with very good selectivity over structurally related analytes. The dye was also used to develop a simple test strip for the visual differentiation of normal and decaffeinated coffee with a standard UV lamp.

Introduction

Caffeine is an important analyte for the food and pharmaceutical industry because of its wide diffusion in beverages and drugs.[1–4] In spite of the analytical interest, most of the methods developed so far are time consuming or require complex instrumentation.[5,6] Common quantification methods are based on high performance liquid chromatography (HPLC), mass spectrometry, or capillary electrophoresis.[7–10] More recently, voltammetric sensing methodologies have also been reported, mostly based on Nafion® polymers modified with graphene or carbon nanotubes.[11–15] Fluorescence-based sensors are of great interest because of their potentially high sensitivity and ease of use.[16] Examples of fluorescent sensors for caffeine have been reported in the literature, mostly based on hydrogen bonding in organic solvents.[17–21] A fluorimetric method for caffeine sensing in water, which exploits the interaction between trisodium 8-hydroxypyrene-1,3,6-trisulfonate (HPTS) and caffeine, has recently been reported by our group.[22] HPTS was successfully used to quantify caffeine in common beverages and drugs. However, HPTS displays only modest affinity for caffeine ($K_a \sim 250\ M^{-1}$). Furthermore, its selectivity over the structurally related alkaloids theobromine and theophylline was low. These shortcomings prompted us to investigate alternative molecular receptors that exhibit both enhanced sensitivity and selectivity. To achieve this goal, we aimed at increasing favorable van der Waals interactions by examining dyes possessing three criteria: (a) more polarizable atoms (*e.g.*, sulfur), (b) a conserved large π-stacking surface, and (c) a π-electron poor framework (*i.e.*, reducing the delocalized π-density on the central ring). The latter criterion is motivated by a design principle that will be discussed in detail elsewhere.[23] According to these criteria, the fluorescent dye disodium 3,4:3′,4′-bibenzo[*b*]thiophene-2,2′-disulfonate (**1**) appeared to be a promising candidate. Below, we describe the synthesis and the caffeine binding properties of dye **1**. It is demonstrated that **1** can be used as a sensitive and selective probe for caffeine in water. Furthermore, we report the development of a simple test strip for the differentiation of normal and decaffeinated coffee.



Results and discussion

The disulfonated bibenzo[*b*]thiophene **1** was synthesized by the reaction of 3,4:3′,4′-bibenzo[*b*]thiophene[24] with sulfuric acid in acetic anhydride[25] and subsequent cation exchange. The absorption spectrum of a solution of **1** in phosphate buffer (100 mM, pH = 7.0) is shown in Fig. 1.

In analogy to other polyaromatic molecules, the spectrum of **1** displays a well defined vibrational structure. In the emission spectrum, two main bands are observed. The higher energy emission band (located between 400 nm and 500 nm) features a vibrational structure which mirrors the lowest energy absorption band. It can be ascribed to an $S_1 \rightarrow S_0$ transition. The lower energy emission band (located between 500 nm and 650 nm) is structureless and likely due to the formation of an excimer.[26]

The emission spectrum of a solution of **1** (λ_{ex} = 350 nm) was strongly influenced by the presence of caffeine: upon addition of

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†Electronic supplementary information (ESI) available: Sensitivity tests; ^1H and ^{13}C NMR of the synthetic intermediates, and computational details. See DOI: 10.1039/c2ob26117k

the alkaloid (0–7.5 mM), the emission color changed from purple to yellow-green (Fig. 2). The two main emission bands showed opposite behavior: the intensity of the high-energy band decreased, whilst the intensity of the low-energy band increased. For the latter band, a slight blue shift was also observed (from 556 nm to 552 nm). The decrease of the high-energy band is compatible with the formation of a ground-state complex between dye **1** and caffeine (similar to what was observed for HPTS). On the other hand, the intensity increase and the blue shift observed for the low-energy band suggest the formation of a complex involving the excimer.

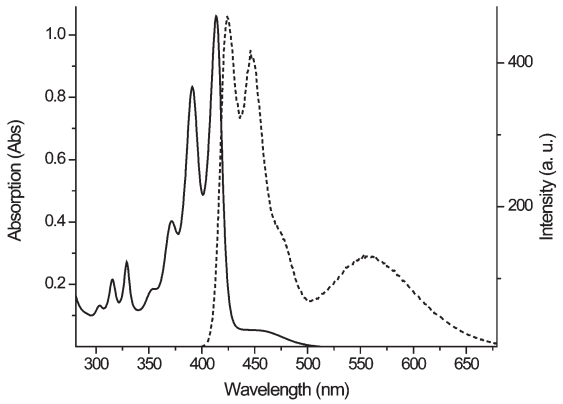

Fig. 1 Absorption (solid line) and emission spectra (dashed line) of dye **1** in buffered aqueous solution (100 mM phosphate buffer, pH = 7.0). λ_{ex} = 350 nm.

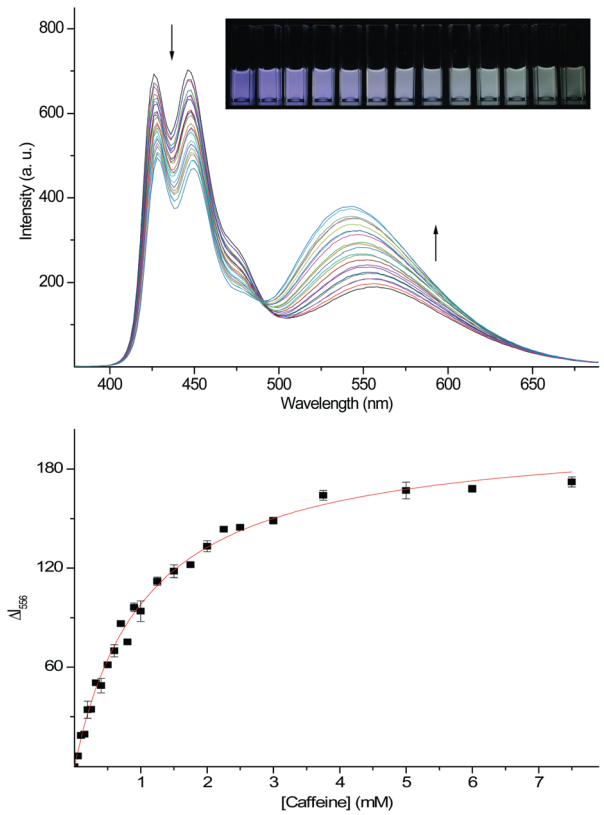

Fig. 2 Top: Fluorescence spectra of buffered aqueous solutions containing dye **1** and different amounts of caffeine ([**1**] = 50 μM, [caffeine] = 0–7.5 mM, λ_{ex} = 350 nm, 100 mM phosphate buffer, pH 7.0). The inset shows the emission colors at different caffeine concentrations. Bottom: changes of the fluorescence emission at 556 nm (average value of three independent measurements).

The fluorescence titration data could be fitted to a 1 : 1 binding model, resulting in an *apparent* binding constant of K_a = 930 ± 49 M^{-1}.[27] This value is about four-fold higher than the value obtained for HPTS,[22] and it allows sensing of caffeine in the sub-millimolar concentration range.

A number of biologically relevant analytes were examined in order to determine the selectivity of the sensor **1**. The measurements were performed with a dye concentration of 150 μM and analyte concentrations of 750 μM. The selectivity was evaluated by using a ratiometric approach (the ratio of the fluorescence intensity at 558 nm and 428 nm was employed). The results obtained for some of the analytes are depicted in Fig. 3; the data for other analytes are given in ESI.†

All analytes that were tested gave only modest fluorescence changes compared to what was observed for caffeine. Small signals were found for the structurally related analytes adenine and theophylline, but the majority of analytes gave only a minor emission change (<10%). It is noteworthy that only purine derivatives induced an increase of the low-energy emission band at ~550 nm, whilst a decrease of both emission bands was observed in the case of the other analytes. A comparison with the selectivity observed for HPTS shows that **1** is at least two-fold more selective than the sulfonated pyrene.[22] For example, the selectivity for caffeine over the related theophylline was 1.6 : 1 in the case of HPTS, whereas a selectivity of 4 : 1 was observed for **1**. Moreover, a ratiometric sensing approach was not applicable in the case of HPTS.

The dual emission change of **1** in the presence of caffeine prompted us to explore the possibility of using **1** for developing a visual, qualitative test for caffeine. Such a test would ideally consist of a strip which is dipped in coffee. The presence of caffeine would then be visualized with a common UV lamp.

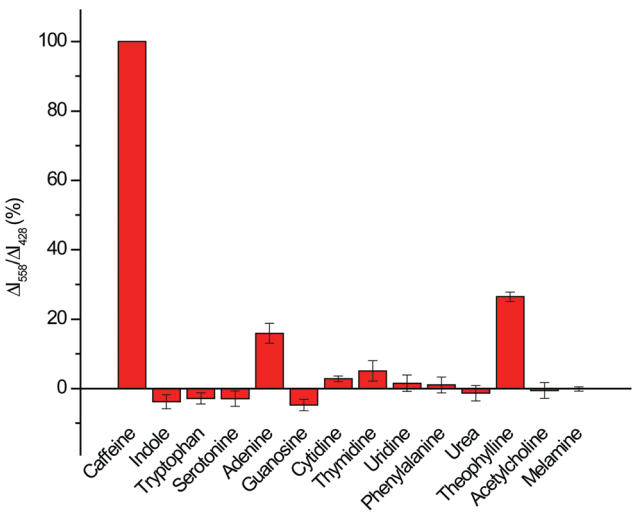

Fig. 3 Normalized ratiometric change of the emission of **1** in the presence of different analytes ([**1**] = 150 μM, [analyte] = 750 μM, λ_{ex} = 350 nm, 100 mM phosphate buffer, pH 7.0, average value of three independent measurements).

To develop such a test strip, two main obstacles had to be overcome: (a) the immobilization of the dye on a surface, and (b) the necessity of removing the colored/heterogeneous materials which are present in coffee and which hamper the visualization of the fluorescence.

The immobilization of **1** was successfully achieved by exploiting the presence of the sulfonate groups, which can interact with a positively charged surface. A standard silica TLC strip on an aluminum sheet was first functionalized with (3-aminopropyl)trimethoxysilane and then treated with iodomethane to give a silica surface functionalized with tetraalkylammonium groups. This surface functionalisation was achieved by dipping the TLC strips into solutions of the respective reagents. The modified strips were then immersed in an aqueous solution of **1** and dried. The resulting strips showed a pale blue emission under irradiation with a standard UV lamp (λ_{ex} = 355 nm). When treated with a drop of water containing caffeine, a green spot was clearly visible on the blue background. Caffeine detection in coffee can be achieved with the following simple procedure. The lower part of the functionalized TLC strip was dipped into the beverage for approx. 10 min. The strips were briefly dried and then dipped into plain water for another 10 min. The caffeine migrated with the solvent front giving a green fluorescent band under UV irradiation (Fig. 4, left). The dark components of coffee remained at the bottom of the strip, and the material migrating with the front of the solvent did not interfere with the detection of caffeine. A control experiment with decaffeinated coffee did not show any fluorescent band (Fig. 4, middle), but the green band reappeared when caffeine was added to decaffeinated coffee prior to testing (Fig. 4, right). Test strips for caffeine based on an antibodies technology have been developed.[28] The simplicity and the robustness of our method could be an advantage (no biological material is involved). It is clear, however, that further optimization from an engineering/technical point would be needed for a 'real-world' application.

To validate the formation of a ground state complex between **1** and caffeine and to identify the nature of the excimer, the energies and geometries of the relevant species were computed at the ωB97X-D/def2-SVP level using an implicit solvent model (see ESI† for more details). The computations predict a stable π-stacked complex between caffeine and **1** in the ground state (Fig. 5, A). The formation of such a complex can explain the observed quenching of the high-energy fluorescence band. The computations also reveal a stable complex between the excited state of **1** and caffeine (Fig. 5, B), but this aggregate does not

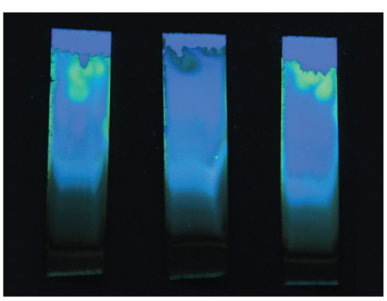

Fig. 4 Fluorescence response of functionalized TLC test strips which were treated with coffee (left), decaffeinated coffee (middle), or decaffeinated coffee spiked with caffeine (right).

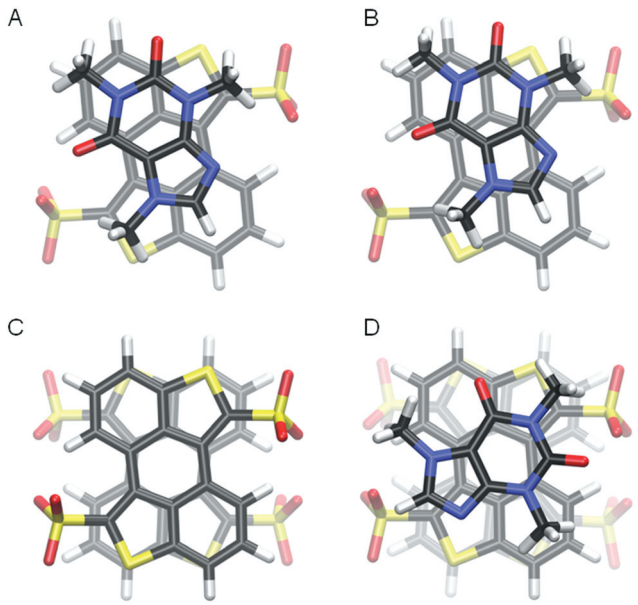

Fig. 5 (A) Ground state complex between caffeine and dye **1**, (B) excited state complex between caffeine and **1**, (C) excimer of $\mathbf{1}_2$, and (D) complex between the excimer of $\mathbf{1}_2$ and caffeine optimized at the ωB97X-D/def2-SVP in implicit water.

generate an exciplex band. A low-energy band was found to arise from excimer formation ($\mathbf{1}_2$), but it could only be obtained for one specific relative orientation of the two dyes **1** (Fig. 5, C). The vertical fluorescence peak is at 507 nm compared to 436 nm for the monomer. The π-stacked complex between the excimer and caffeine (Fig. 5, D) is characterized by a similar low-energy transition, blue shifted by ~6 nm (0.04 eV) in comparison to C, in good agreement with experiment.[29] The change of the emission frequency is probably related to the fact that the presence of caffeine alters slightly the preferred relative orientation of the stacked dyes (see ESI Fig. S4†). Our computations indicate that a hypothetical sandwich complex dye **1**–caffeine–dye **1** does not contribute to the optical response as the energy of the excited sandwich complex is 11 kcal mol^{-1} higher than that of caffeine bound to the excimer of dye **1**. In addition, the sandwich complex does not give rise to the excimer band, which critically depends on the interaction between two dyes. Finally, classical molecular dynamics simulations have been performed to estimate binding free energies in aqueous solution at 300 K. In the gas phase, dye **1** has a higher affinity for theophylline than for caffeine, which can be rationalized by an N–H···sulfonate hydrogen bond. However, when accounting explicitly for the water molecules and therefore for the solvation of the N–H groups, the affinity for caffeine is about 1 kcal mol^{-1} higher than for theophylline and indole is barely bound at all. These free energy simulations demonstrate that explicit solvent effects are important for reproducing the experimental selectivity (see details in ESI†).

Conclusions

We have shown that dye **1** can be used as a molecular probe for the fluorimetric detection of caffeine in aqueous solution. The

sensitivity and selectivity of the system is significantly better than that of the previously reported caffeine sensor HPTS.[22] Another advantage is the fact that dye **1** can be used as a ratiometric fluorescence sensor. The change in emission color upon addition of caffeine is easily detected by the naked eye. This allowed preparing a simple test strip for the differentiation of normal and decaffeinated coffee. A computational study has revealed that the interaction of **1** with caffeine occurs *via* π-stacking, which is particularly favorable for the bibenzo[*b*]thiophene core.

Experimental

General

All the chemicals and solvents were purchased from usual suppliers and used without further purifications. Silica gel TLC 60 F_{254} plates (Merck) on aluminum sheets were used for the realization of the caffeine-sensitive strips. 3,4:3′,4′-Bibenzo[*b*]-thiophene was prepared according to the literature procedure.[24] Absorption spectra were measured on a Lambda 40 UV-Vis spectrometer (Perkin Elmer). Fluorescence emission spectra were recorded on a Varian Cary Eclipse spectrofluorimeter in 10 mm path polymethylmethacrylate cuvettes (Semadeni) with an excitation wavelength of 350 nm. ^{1}H and ^{13}C NMR were recorded on a Bruker Advance DPX 400. Multiplicities of the ^{1}H NMR signals are assigned as follows: d (doublet), t (triplet). Chemical shifts (δ) are reported in parts per million (ppm) relative to the residual peak of the solvent. All solutions used for fluorimetric titrations and sensitivity tests were prepared in 100 mM phosphate buffer (pH = 7.0). Fitting of the fluorimetric titration data was done by using a non-linear regression algorithm implemented in the software Origin 8.5 using the following equation:

$$\Delta I = \frac{AK_a[c]}{1 + K_a[c]}$$

where ΔI is the absolute value of the difference between the emission intensity of the sample in the absence and in the presence of quencher, A is a proportionality constant, K_a is the association constant and $[c]$ is the molar concentration of quencher.

Synthesis of disodium 3,4:3′,4′-bibenzo[*b*]thiophene-2,2′-disulfonate (1). A solution of 3,4:3′,4′-bibenzo[*b*]thiophene (100 mg, 0.38 mmol) in acetic anhydride (15 mL) was cooled in an ice bath. H_2SO_4 (40 μL) was added slowly to the mixture, which was then stirred at room temperature for 6 h. The orange precipitate was filtered and washed with a saturated NaCl solution. The solid was dried under vacuum to obtain 125 mg of **1** (0.27 mmol, 71%).

^{1}H-NMR (400 MHz, D_2O) δ: 8.67 (d, 2H, 7.9 Hz), 7.85 (d, 2H, 8.0 Hz), 7.56 (t, 2H, 7.9 Hz). ^{13}C-NMR (100 MHz, D_2O) δ: 137.9, 137.3, 128.2, 127.5, 127.0, 123.8, 122.4, 120.3. Elemental analysis calc. for **1** × H_2O ($C_{16}H_8Na_2O_7S_4$): C 39.50, H 1.66; found: C 39.74, H 1.56.

Preparation of caffeine-sensitive test strips. A silica TLC plate was first dipped for 10 min in a 25% solution of (3-aminopropyl)trimethoxysilane in acetone containing a catalytic amount of water. After drying at 100 °C for 1 min, the TLC plate was then dipped for 10 min in a 17% solution of iodomethane in CH_2Cl_2 and dried again. The resulting TLC was then placed for 5 min into a solution of **1** in H_2O (250 μM) and dried. The last step was repeated four times in order to obtain a bright and homogeneous coating, providing a good contrast for measurements.

Acknowledgements

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References

1 A. Nehlig, *Coffee, Tea, Chocolate, and the Brain*, CRC Press, Boca Raton, 2004.
2 C. J. Reissig, E. C. Strain and R. R. Griffiths, *Drug Alcohol Depend.*, 2009, **99**, 1–10.
3 P. Nawrot, S. Jordan, J. Eastwood, J. Rotstein, A. Hugenholtz and M. Feeley, *Food Addit. Contam.*, 2003, **20**, 1–30.
4 A. P. Winston, E. Hardwick and N. Jaberi, *Adv. Psychiatr. Treat.*, 2005, **11**, 432–439.
5 A. Campean, M. Tertis and R. Sandulescu, *Cent. Eur. J. Chem.*, 2011, **9**, 688–700.
6 I. C. Chung, C.-C. Chang, H.-S. Chiu, S.-F. Jiang, M.-H. Lee, C.-L. Chung, B.-D. Lu, C.-Y. Huang and H.-Y. Lin, *J. Nanosci. Nanotechnol.*, 2011, **11**, 10633–10638.
7 M. T. Jafari, B. Rezaei and M. Javaheri, *Food Chem.*, 2011, **126**, 1964–1970.
8 A. Serra, A. Macia, M.-P. Romero, C. Pinol and M.-J. Motilva, *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.*, 2011, **879**, 1519–1528.
9 S. N. Alvi and M. M. Hammami, *J. Chromatogr. Sci.*, 2011, **49**, 292–296.
10 A. R. De Sena, S. A. De Assis and A. Branco, *Food Technol. Biotechnol.*, 2011, **49**, 413–423.
11 J.-Y. Sun, K.-J. Huang, S.-Y. Wei, Z.-W. Wu and F.-P. Ren, *Colloids Surf., B*, 2011, **84**, 421–426.
12 F. Zhao, F. Wang, W. Zhao, J. Zhou, Y. Liu, L. Zou and B. Ye, *Microchim. Acta*, 2011, **174**, 383–390.
13 S. Yang, R. Yang, G. Li, J. Li and L. Qu, *J. Chem. Sci.*, 2010, **122**, 919–926.
14 S. Yang, R. Yang, G. Li, L. Qu, J. Li and L. Yu, *J. Electroanal. Chem.*, 2010, **639**, 77–82.
15 J. M. Zen, Y. S. Ting and Y. Shih, *Analyst*, 1998, **123**, 1145–1147.
16 A. P. Demchenko, *Introduction to Fluorescence Sensing*, Springer, 2008.
17 P. Ballester, M. A. Barcelo, A. Costa, P. M. Deya, J. Morey, M. Orell and C. A. Hunter, *Tetrahedron Lett.*, 2000, **41**, 3849–3853.
18 C. Siering, B. Beermann and S. R. Waldvogel, *Supramol. Chem.*, 2006, **18**, 23–27.
19 A. K. Mahapatra, P. Sahoo, S. Goswami, H.-K. Fun and C. S. Yeap, *J. Inclusion Phenom.*, 2010, **67**, 99–108.
20 C. Siering, H. Kerschbaumer, M. Nieger and S. R. Waldvogel, *Org. Lett.*, 2006, **8**, 1471–1474.
21 Y. L. Wei, L. H. Ding, C. Dong, W. P. Niu and S. M. Shuang, *Spectrochim. Acta, Part A*, 2003, **59**, 2697–2703.
22 S. Rochat, S. N. Steinmann, C. Corminboeuf and K. Severin, *Chem. Commun.*, 2011, **47**, 10584–10586.
23 J. F. Gonthier, S. N. Steinmann, L. Roch, A. Ruggi, N. Luisier, K. Severin and C. Corminboeuf, *Chem. Commun.*, 2012, DOI: 10.1039/c2cc33886f.
24 F. Wudl, R. C. Haddon, E. T. Zellers and F. B. Bramwell, *J. Org. Chem.*, 1979, **44**, 2491–2493.
25 M. Pailer and E. Romberger, *Monatsh. Chem.*, 1961, **92**, 677–683.
26 J. R. Lackowicz, *Principles of Fluorescence Spectroscopy*, Springer, 2006.
27 The 1 : 1 model is a simplification as it neglects homo-aggregation and ternary complexes.
28 For instance the D+caf® kit developed by Silver Lake Research (http://www.silverlakeresearch.com/).
29 Computations show that a side-on complexation of caffeine to the dimer of dye **1** is less favorable. For an example of side-on coordination of an analyte to a π-stacked sensor see: S. Nishizawa, Y. Kato and N. Teramae, *J. Am. Chem. Soc.*, 1999, **121**, 9463–9464.

