Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Fluorescence sensing of caffeine in water with polysulfonated pyrenes

Sébastien Rochat, Stephan N. Steinmann, Clémence Corminboeuf and Kay Severin*

Institut des Sciences et Ingénierie Chimiques,
École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.
Fax: +41(0)21 6939305; Tel: +41(0)21 6939302; E-mail: kay.severin@epfl.ch

1. General: The chemicals were purchased from the usual suppliers and used as obtained. All solutions were prepared in bidistilled water and stock solutions were stored at 4 °C. Buffer solutions (100 mM phosphate buffer, pH 6.5) were prepared by dissolving appropriate amounts of K_2HPO_4 and KH_2PO_4 in water. Fluorescence measurements were performed at room temperature on a Varian Cary Eclipse spectrofluorimeter. Absorbance spectra were recorded on a Perkin-Elmer Lambda 40 spectrometer. ^{1}H NMR spectra were recorded on a Bruker Advance DPX 400 instrument using the residual protonated solvents as internal standard. HPLC analyses were performed with a system consisting of a Waters 600 controller unit, a Waters Delta 600 pump and a Waters 2487 dual wavelength absorbance detector. An Atlantis C_{18} analytical column (5 µm, 4.6 x 150 mm) was used as stationary phase, and Millipore water (50 mM phosphate pH 3.0, the pH was adjusted by adding concentrated HCl to a solution of Na_2HPO_4) and HPLC-grade methanol as mobile phase.

2. Fluorescence titration experiments with caffeine and HPTS: An aliquot (5 µL) of a stock solution of HPTS (10 mM in pH 6.5 phosphate buffer) was added to an appropriate amount of a stock solution of caffeine (50 mM in pH 6.5 phosphate buffer) and the volume was completed to 1.0 mL. The final concentrations were: [HPTS] = 50 µM, [caffeine] = 0 – 50 mM, [phosphate] = 100 mM. The equilibrium was established rapidly, and the fluorescence could be measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm). The fluorescence of HPTS was quenched upon binding to caffeine. Fitting of the binding isotherm to a 1:1 binding model (WinEQNMR 2)[1] gave an association constant of 245(5) M^{-1}. A Stern-Volmer plot for sub- to low millimolar concentrations of caffeine displayed a linear relationship (I_0/I *vs*. [caffeine]) and provided an association constant of 212(5) M^{-1}. This value was used for the determination of caffeine concentrations in the ‘real-world‘ samples (see below). A detection limit of 0.17 mM could be deduced (detection limit = 3 x σ_0).

3. UV-vis titration experiments with caffeine and HPTS: The same solutions were analyzed by UV-vis spectroscopy. The absorbance at 460 nm is reduced upon addition of caffeine, whereas the main band (403 nm) undergoes a slight bathochromic shift (Fig. S1)

[1] M. J. Hynes, *J. Chem. Soc., Dalton Trans.*, 1993, 311-312.

Fitting of the binding isotherm obtained by plotting the absorbance at 460 nm vs. [caffeine] to a 1:1 binding model (WinEQNMR 2) afforded an association constant of 318(13) M^{-1} (Fig. S2).

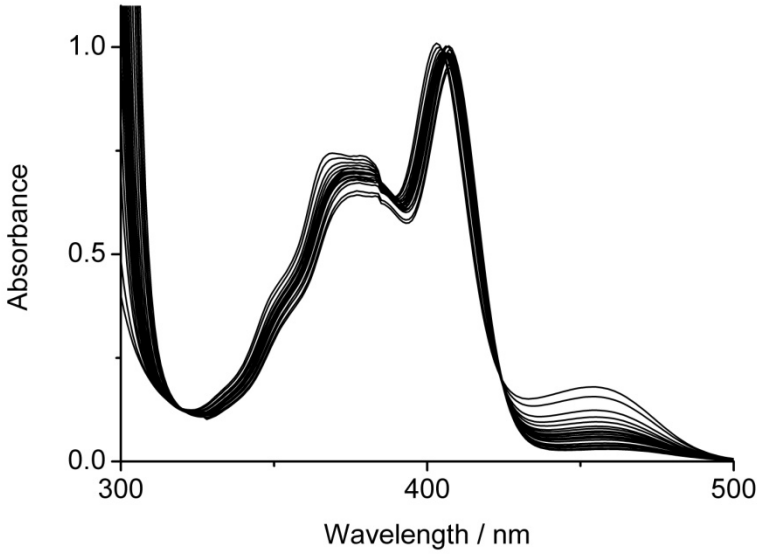

Fig. S1 UV-vis spectra of buffered aqueous solutions (pH 6.5, 100 mM phosphate) containing HPTS (50 μM) and different amounts of caffeine (0 – 50 mM). The spectra were recorded at room temperature.

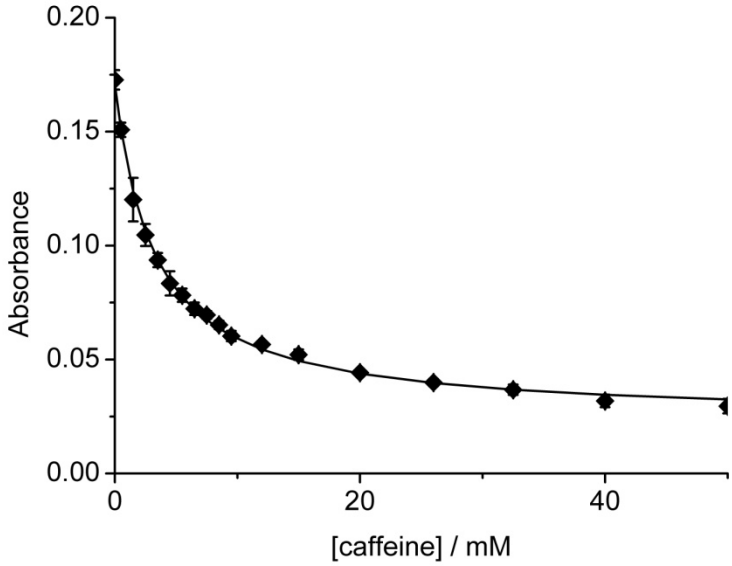

Fig. S2 Absorbance at 460 nm of buffered aqueous solutions (pH 6.5, 100 mM phosphate) containing HPTS (50 μM) and different amounts of caffeine (0 – 50 mM). The spectra were recorded at room temperature. The curve was obtained by fitting the data to a 1 :1 binding model. The data points represent averages of three independent measurements.

4. ^{1}H NMR titration experiments with caffeine and HPTS: NMR titration experiments were performed with higher concentrations of HPTS: The final concentrations were:[HPTS] = 2.0 mM, [caffeine] = 0 – 40 mM, [phosphate] = 100 mM in D_2O. The signals labeled with an asterisk were used to derive a binding constant assuming a 1 :1 complexation. An association constant of 151(17)M^{-1} was obtained.

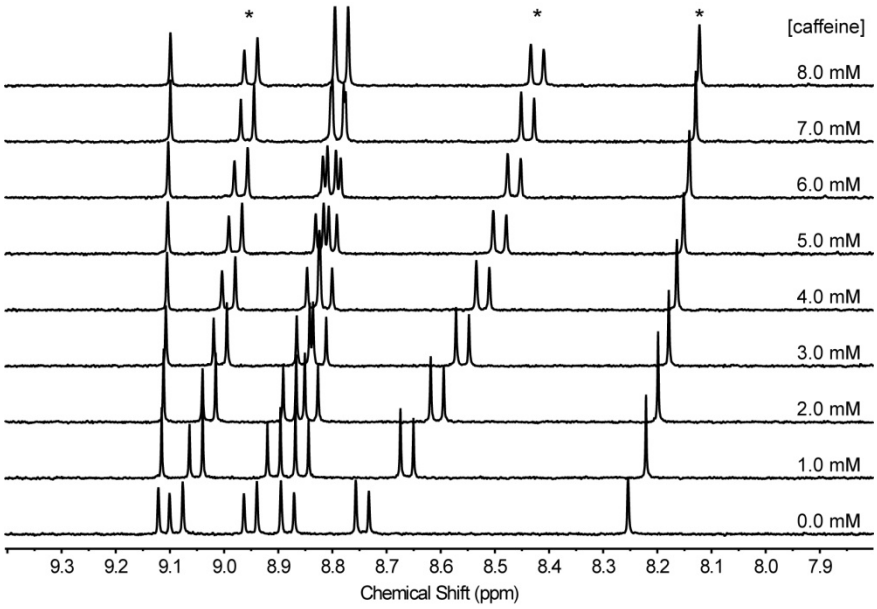

Fig. S3 Part of the ^{1}H NMR spectra of solutions containing HPTS (2.0 mM) and different amounts of caffeine in D_2O (pH 6.5, 100 mM phosphate).

5. Fluorescence titration experiments with theophylline and HPTS: An aliquot (5 μL) of a stock solution of HPTS (10 mM in pH 6.5 phosphate buffer) was added to an appropriate amount of a stock solution of theophylline (40 mM in pH 6.5 phosphate buffer) and the volume was completed to 1.0 mL. The final concentrations were: [HPTS] = 50 μM, [theophylline] = 0 – 40 mM, [phosphate] = 100 mM. The equilibrium was established

rapidly, and the fluorescence could be measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm). The fluorescence of HPTS was quenched upon binding to theophylline (Fig. S4). Fitting of the binding isotherm (Fig. S5) to a 1:1 binding model (WinEQNMR 2) gave an association constant of 148(6) M^{-1}. A Stern-Volmer plot (Fig. S5, insert) for sub- to low millimolar concentrations of theophylline displayed a linear relationship (I_0/I *vs.* [theophylline]) and provided an association constant of 121(3) M^{-1}.

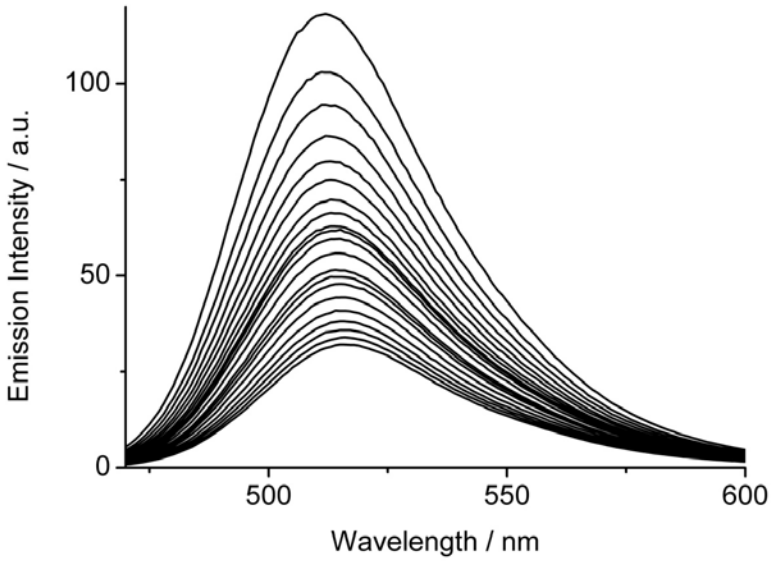

Fig. S4 : Fluorescence spectra (λ_{ex} = 460 nm, RT) of aqueous solutions (pH 6.5, 100 mM phosphate) containing HPTS (50 μM) and different amounts of theophylline (0 – 40 mM).

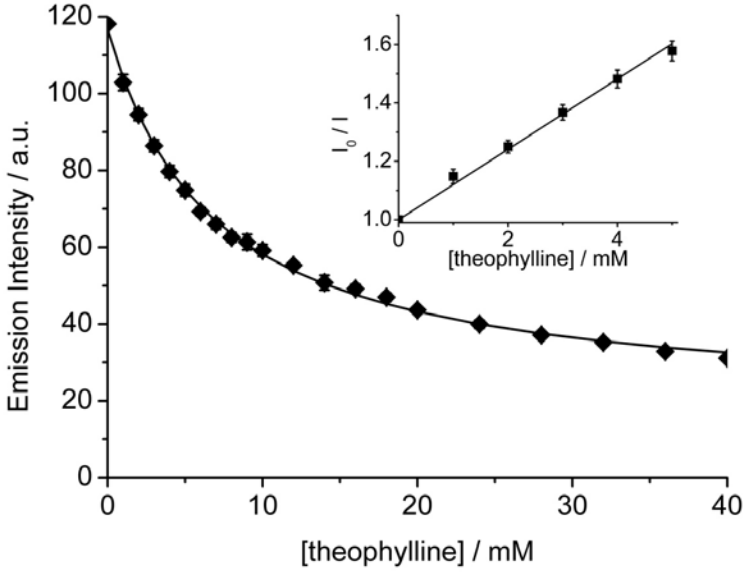

Fig. S5 The corresponding binding isotherm at λ_{em} = 512 nm. The curve was obtained by fitting the data to a 1:1 binding model. The inset shows a Stern-Volmer plot (I_0/I) obtained for low concentrations of theophylline. The line was obtained by fitting to a linear model. The data points represent averages of three independent measurements.

6. Fluorescence titration experiments with theobromine and HPTS: owing to the lower solubility of theobromine (compared to that of caffeine and theophylline), titration could only be performed in the low millimolar concentration range (Fig. S6). As a consequence, a 'fittable' binding isotherm was not obtained. The association constant obtained from the Stern-Volmer plot (Fig. S7) was 113(2) M^{-1}. An aliquot (5 µL) of a stock solution of HPTS (10 mM in pH 6.5 phosphate buffer) was added to an appropriate amount of a stock solution of theobromine (5.0 mM in pH 6.5 phosphate buffer) and the volume was completed to 1.0 mL. The final concentrations were: [HPTS] = 50 µM, [theobromine] = 0 – 5 mM, [phosphate] = 100 mM. The equilibrium was established rapidly, and the fluorescence could be measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm). The fluorescence of HPTS was quenched upon binding to theobromine (Fig. S6).

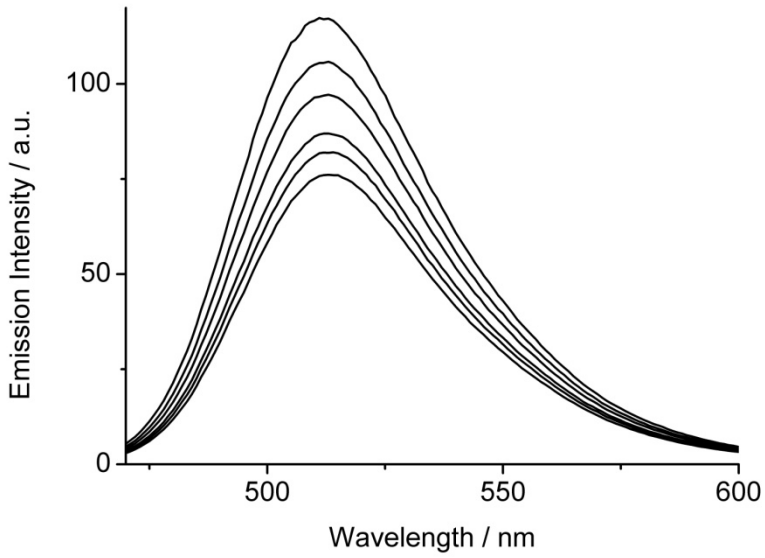

Fig. S6 : Fluorescence spectra (λ_{ex} = 460 nm, RT) of aqueous solutions (pH 6.5, 100 mM phosphate) containing HPTS (50 µM) and different amounts of theobromine (0 – 5 mM).

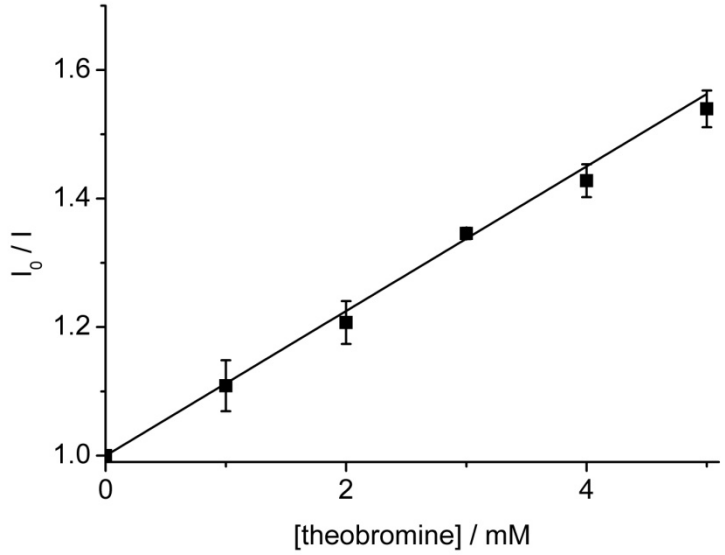

Fig. S7 Stern-Volmer plot (I_0/I) obtained for aqueous solutions (pH 6.5, 100 mM phosphate) containing HPTS (50 μM) and different amounts of theobromine (0 – 5 mM). The line was obtained by fitting to a linear model. The data points represent averages of three independent measurements.

7. Effect of pH: The fluorescence intensity of HPTS is known to increase with the pH. However, the quenching induced by caffeine was found to be more pronounced at lower pH values (Fig. S8). No quenching was induced by caffeine at pH 8.0. At pH 7.0, the emission intensity was reduced by *ca*. 7 %, and at pH 6.5, the reduction was 18%.

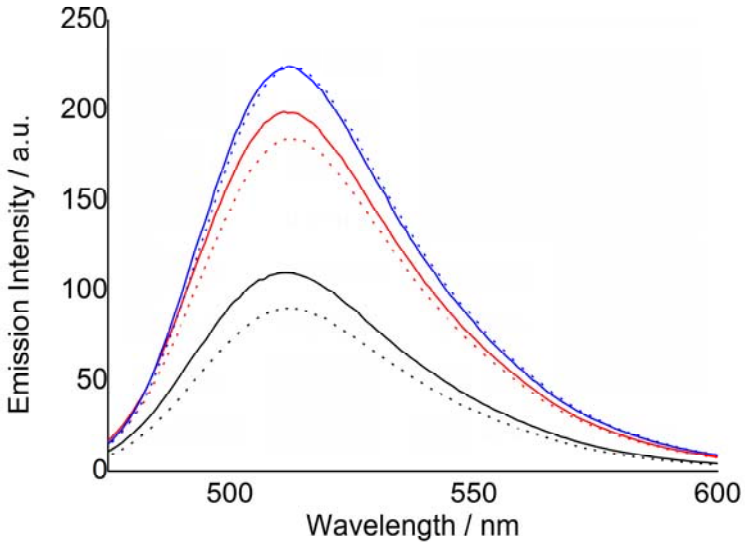

Fig. S8 Fluorescence spectra of solutions of HPTS (50 μM) at various pH values (black: pH 6.5; red: pH 7.0; blue: pH 8.0) in absence (solid lines) and in the presence of 1.0 mM caffeine (dotted lines). The spectra were recorded at RT in the presence of 100 mM phosphate buffer.

8. Selectivity experiments with HPTS: An aliquot (5 μL) of a stock solution of HPTS (10 mM in pH 6.5 phosphate buffer) was added to an appropriate amount of the desired analyte. The final concentrations were: [HPTS] = 50 μM, [analyte] = 1.0 mM, [phosphate] = 100 mM. The equilibrium was established rapidly, and the fluorescence could be measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm). The most pronounced quenching was observed for caffeine, significant quenching was also obtained for theophylline and theobromine (see detailed titrations above). A variety of aromatic analytes were tested and gave no or negligible responses.

9. Fluorescence titration experiments with caffeine and PTS: An aliquot (5 μL) of a stock solution of PTS (10 mM in pH 7.0 phosphate buffer) was added to an appropriate amount of a stock solution of caffeine (50 mM in pH 7.0 phosphate buffer) and the volume was completed to 1.0 mL. The final concentrations were: [PTS] = 50 μM, [caffeine] = 0 – 50 mM, [phosphate] = 100 mM. The equilibrium was established rapidly, and the fluorescence could

be measured immediately (λ_{ex} = 364 nm, λ_{em} = 404 nm). The fluorescence of PTS was quenched upon binding to caffeine (Fig. S9). Fitting of the binding isotherm (Fig. S10) to a 1:1 binding model (WinEQNMR 2) gave an association constant of 304(47) M^{-1}. A Stern-Volmer plot (Fig. S10, insert) for low millimolar concentrations of caffeine displayed a linear relationship (I_0/I *vs*. [caffeine]) and provided an association constant of 314(4) M^{-1}. A detection limit of 0.05 mM could be deduced (detection limit = 3 x σ_0).

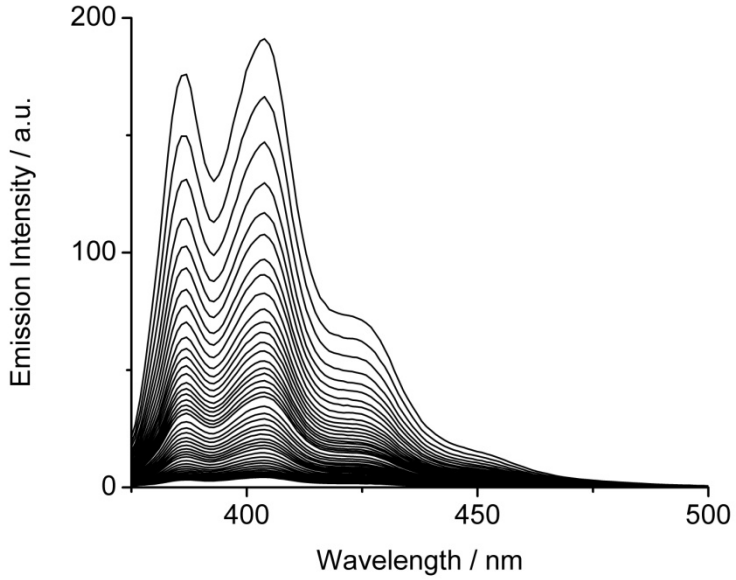

Fig. S9 Fluorescence spectra (λ_{ex} = 364 nm, RT) of aqueous solutions (pH 7.0, 100 mM phosphate) containing PTS (50 μM) and different amounts of caffeine (0 – 50 mM).

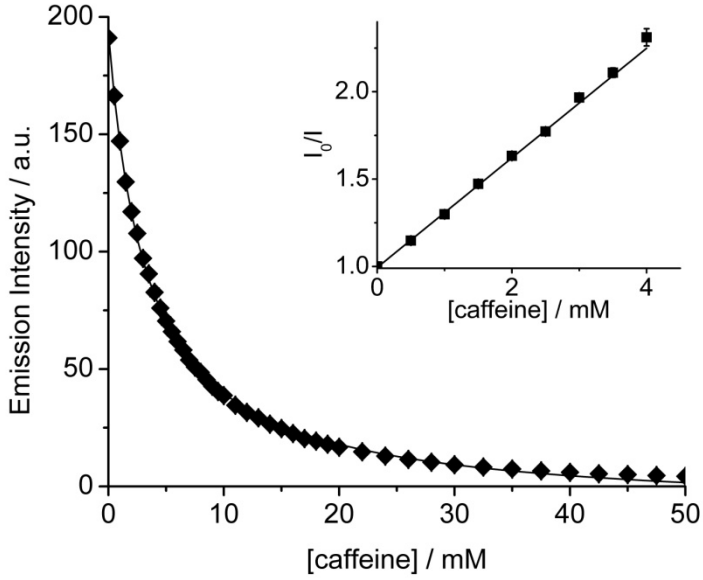

Fig. S10 The corresponding binding isotherm at λ_{em} = 404 nm. The curve was obtained by fitting the data to a 1:1 binding model. The insert shows a Stern-Volmer plot (I_0/I) obtained for low concentrations of caffeine. The line was obtained by fitting to a linear model. The data points represent averages of three independent measurements.

10. Selectivity experiments with PTS: An aliquot (5 μL) of a stock solution of PTS (10 mM in pH 7.0 phosphate buffer) was added to an appropriate amount of the desired analyte. The final concentrations were: [PTS] = 50 μM, [analyte] = 1.0 mM, [phosphate] = 100 mM. The equilibrium was established rapidly, and the fluorescence could be measured immediately (λ_{ex} = 364 nm, λ_{em} = 404 nm). The most pronounced quenching was observed for caffeine, but significant quenching was also observed for other analytes (Fig. S11).

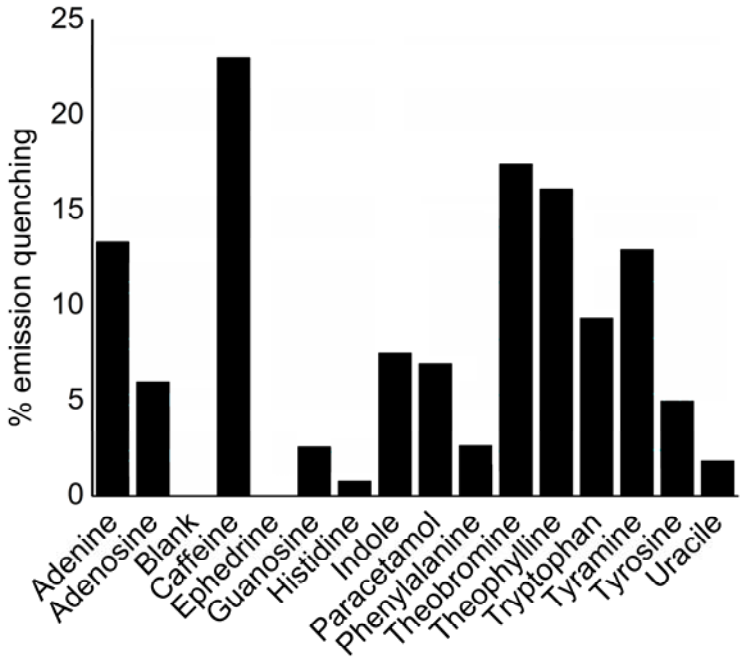

Fig. S11 Analyte-induced fluorescence quenching of PTS in buffered aqueous solutions ([PTS] = 50 μM, [analyte] = 1.0 mM, pH 7.0, 100 mM phosphate).

11. Computational study: Two levels of theory were considered: B3LYP-dDsC[2] and ωB97X-D.[3] Ten unique relative orientations were obtained by sampling the potential energy surface starting from different geometries using the def2-SVP basis set. The sulfonate groups were microsolvated with two water molecules each (i.e. a total of 6). The explicit solvent molecules account for the most important solvent effects on these negatively charged functional groups. Spurious electrostatic interactions, which are screened to a large extent in solution, would otherwise lead to artifacts in the gas-phase structures. The lowest energy structures were identified based on single-point energy computations with the def2-TZVP basis set and an implicit solvent with settings corresponding to water (the C-PCM model[4] as implemented in Q-Chem[5] with a dielectric constant of 78.39 and a cavity constructed from

[2] (*a*) A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648-5652; (*b*) P. J. Stephens, F. J. Devlin, C. F. Chabalowski and M. J. Frisch, *J. Phys. Chem.*, 1994, **98**, 11623-11627; (*c*) S. N. Steinmann and C. Corminboeuf, *J. Chem. Theory Comput.*, 2010, **6**, 1990-2001; (*d*) S. N. Steinmann and C. Corminboeuf, *J. Chem. Phys.*, 2011, **134**, 044117.
[3] J.-D. Chai and M. Head-Gordon, *Phys. Chem. Chem. Phys.*, 2008, **10**, 6615-6620.
[4] (*a*) M. Cossi, N. Rega, G. Scalmani and V. Barone, *J. Comput. Chem.*, 2003, **24**, 669-681; (*b*) A. W. Lange and J. M. Herbert, *J. Chem. Phys.*, 2010, *133*, 244111.
[5] Y. Shao, L. F. Molnar, Y. Jung, J. Kussmann, C. Ochsenfeld, S. T. Brown, A. T. B. Gilbert, L. V. Slipchenko, S. V. Levchenko, D. P. O'Neill, R. A. DiStasio Jr, R. C. Lochan, T. Wang, G. J. O. Beran, N. A.

Bondi radii[6] scaled by 1.2, discretized by a 194-Lebedev grid[7]). The binding energies were computed using strict integration settings (integral threshold of 10^{-12} and a 75/302 Euler-Maclaurin-Lebedev[7,8] grid) and corrected for basis set superposition error. The two levels of theory give similar geometries and the binding energies (Table 1). Caffeine is bound stronger by 3 kcal mol^{-1} than indole and 0.3-0.5 kcal mol^{-1} stronger than theobromine. These differences essentially arise from the long-range dispersion contribution given separately in Table 1.

Single-point computations were also performed on the next two lowest (at the C-PCM-B3LYP-dDsC/def2-TZVP level) lying isomers (see Figure S13) to confirm the reliability of the adopted procedure. Note that the energy differences between the isomers are relatively small (0.2-3.5 kcal mol^{-1}, see Table 2).

Table 1: Binding energies (with respect to relaxed monomers) in kcal mol^{-1}:

Analyte	Long-range dispersion contribution (dDsC)	B3LYP-dDsC (C-PCM)	ωB97X-D (C-PCM)
Caffeine	-25.5	-13.7	-13.4
Theobromine	-25.9	-13.2	-13.1
Indole	-21.1	-10.7	-10.4

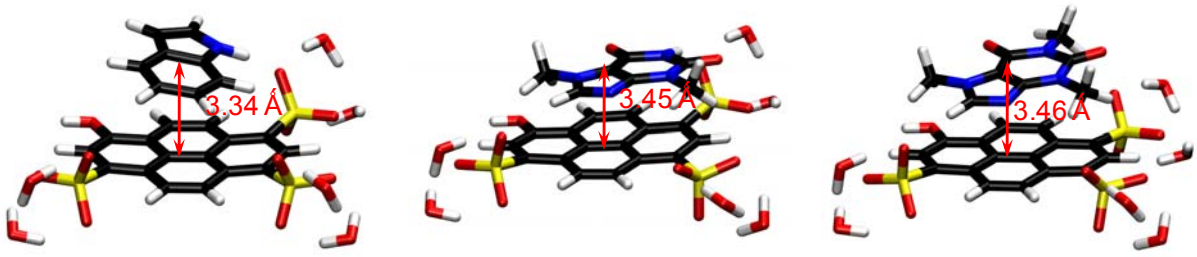

Fig. S12 Geometries and inter-monomer distances optimized at the B3LYP-dDsC/def2-SVP level for HPTS complexes with indole, theobromine and caffeine (from left to right).

Besley, J. M. Herbert, C. Y. Lin, T. Van Voorhis, S. H. Chien, A. Sodt, R. P. Steele, V. A. Rassolov, P. E. Maslen, P. P. Korambath, R. D. Adamson, B. Austin, J. Baker, E. F. C. Byrd, H. Dachsel, R. J. Doerksen, A. Dreuw, B. D. Dunietz, A. D. Dutoi, T. R. Furlani, S. R. Gwaltney, A. Heyden, S. Hirata, C.-P. Hsu, G. Kedziora, R. Z. Khalliulin, P. Klunzinger, A. M. Lee, M. S. Lee, W. Liang, I. Lotan, N. Nair, B. Peters, E. I. Proynov, P. A. Pieniazek, Y. M. Rhee, J. Ritchie, E. Rosta, C. D. Sherrill, A. C. Simmonett, J. E. Subotnik, H. L. Woodcock III, W. Zhang, A. T. Bell, A. K. Chakraborty, D. M. Chipman, F. J. Keil, A. Warshel, W. J. Hehre, H. F. Schaefer III, J. Kong, A. I. Krylov, P. M. W. Gill and M. Head-Gordon, *Phys. Chem. Chem. Phys.*, 2006, **8**, 3172-3191.
[6] A. Bondi, *J. Phys. Chem.*, 1964, **68**, 441-451.
[7] V. I. Lebedev and D. N. Laikov, *Doklady Mathematics*, 1999, **59**, 477-481.
[8] C. W. Murray, N. C. Handy and G. L. Laming, *Mol. Phys.*, 1993, **78**, 997-1014.

Table 2: Binding energies for the three lowest lying isomers at the BSSE corrected B3LYP-dDsC level (with respect to the relaxed monomers) in kcal mol^{-1}:

Analyte	**1**st	**2**nd	**3**rd
Caffeine	-13.7	-13.5	-11.5
Theobromine	-13.2	-11.5	-9.7
Indole	-10.7	-10.1	-9.4

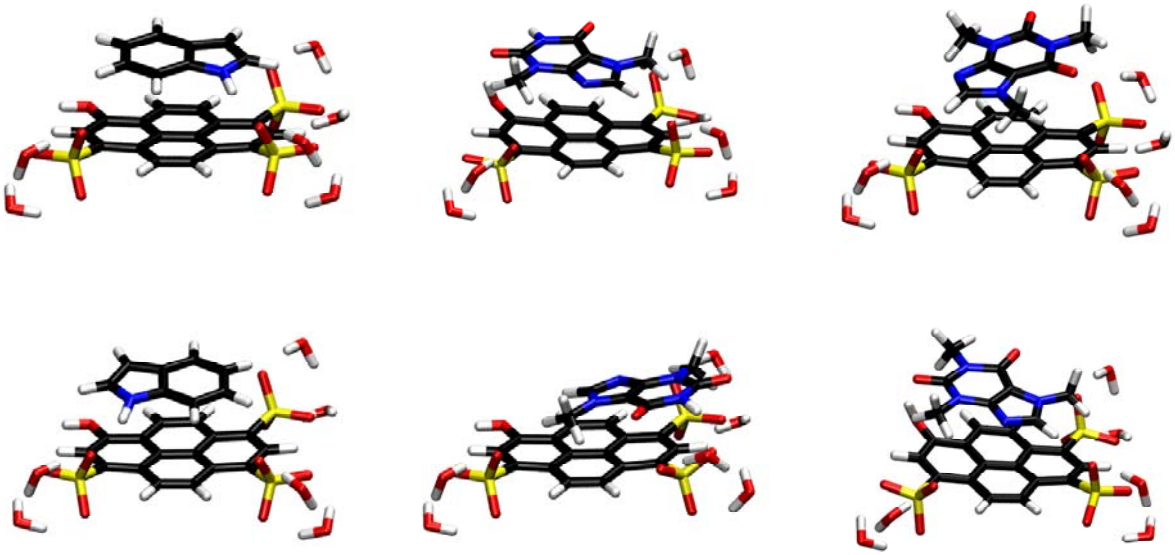

Fig. S13 Geometries optimized at the B3LYP-dDsC/def2-SVP level for HPTS complexes with indole, theobromine and caffeine (from left to right) of the 2nd (1st row) and 3rd lowest isomer (2nd row).

XYZ-Structures:

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70
HPTS-Indole (1st)
C     1.915867   -1.498055    0.023258
C     1.179448   -2.718657    0.100717
C    -0.181051   -2.721390    0.093384
C    -0.919646   -1.496142    0.000000
C    -0.209307   -0.255265    0.000000
C     1.220837   -0.255265   -0.000000
C    -0.914891    0.984394   -0.072635
C    -2.320318    0.949817   -0.175038
C    -3.009967   -0.257291   -0.216036
C    -2.323062   -1.463141   -0.126730
H     1.735716   -3.655093    0.136903
H    -0.736184   -3.655669    0.138281
H    -4.092613   -0.252705   -0.332631
C    -0.164652    2.207320   -0.078329
C     1.197195    2.204016   -0.089550
C     1.938063    0.978077   -0.086642
C     3.341610    0.923856   -0.183053
C     4.015742   -0.291634   -0.173129
C     3.317621   -1.487718   -0.063294
O     3.976803   -2.688862   -0.053687
H     5.104803   -0.273693   -0.258170
H    -0.716501    3.144584   -0.090027
H     1.753103    3.139867   -0.108793
H     4.913192   -2.498783   -0.189144
C     0.101386    0.876964   -3.223557
C     1.493356    0.777254   -3.438254
C     2.105969   -0.462370   -3.574667
C     1.326823   -1.629898   -3.476954
C    -0.691703   -0.257091   -3.112916
C    -0.068782   -1.505002   -3.224957
C     1.609584   -3.037250   -3.492368
C     0.418490   -3.680563   -3.236152
H    -0.363637    1.854706   -3.083923
H     2.107024    1.681075   -3.434350
H     3.189466   -0.527165   -3.707069
H    -1.755875   -0.172050   -2.889183
H     2.579575   -3.508746   -3.639206
H     0.209961   -4.741932   -3.113540
N    -0.594612   -2.768666   -3.094522
H    -1.502400   -2.994841   -2.657728
S    -3.272081   -2.980071   -0.308160
O    -4.703782   -2.586426   -0.397159
O    -2.972367   -3.834702    0.881901
O    -2.765855   -3.609670   -1.581089
H    -4.568417   -4.887457    1.050934
O    -5.487111   -5.037841    0.744631
H    -5.609678   -4.180866    0.287802
H    -3.705594   -5.366060   -1.726441
O    -4.279740   -6.144987   -1.607675
H    -4.791184   -5.884934   -0.816422
S    -3.279431    2.461101   -0.401386
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O	-2.934925	3.347055	0.756563
O	-4.711486	2.037322	-0.387165
O	-2.830163	3.010490	-1.712542
H	-5.602617	3.604922	0.326659
O	-5.474594	4.481737	0.743634
H	-4.528314	4.367029	0.975784
H	-3.968886	4.654031	-1.955745
O	-4.611142	5.380981	-1.852764
H	-4.978589	5.192875	-0.967213
S	4.362142	2.394411	-0.420196
O	5.775081	1.891711	-0.378369
O	4.056340	3.309160	0.722371
O	3.973591	2.948198	-1.745582
H	5.730237	4.256134	0.957934
O	6.683079	4.277400	0.730055
H	6.728174	3.388639	0.319114
H	5.284386	4.462542	-2.029322
O	5.997792	5.120940	-1.937731
H	6.298702	4.959347	-1.022762

75
HPTS-Theobromine (1st)

C	2.266020	1.940070	-0.019151
C	1.529279	3.162335	-0.023632
C	0.170071	3.164354	0.020147
C	-0.569888	1.935945	0.037754
C	0.139187	0.695701	0.000000
C	1.570444	0.695701	0.000000
C	-0.567260	-0.548586	0.000000
C	-1.976688	-0.517171	0.029903
C	-2.666424	0.691365	0.100506
C	-1.977417	1.897609	0.110823
H	2.086142	4.099249	-0.027866
H	-0.387345	4.097543	0.039396
H	-3.752896	0.688383	0.173813
C	0.188781	-1.769350	0.003986
C	1.549393	-1.765266	0.052718
C	2.289903	-0.538916	0.068270
C	3.694034	-0.483882	0.163365
C	4.371110	0.731096	0.114645
C	3.672538	1.926347	0.004634
O	4.332528	3.126134	-0.048932
H	5.460884	0.716141	0.190637
H	-0.362472	-2.706996	0.012745
H	2.105491	-2.701163	0.074825
H	5.274493	2.937049	0.041819
N	-1.569297	1.621183	3.266843
C	-2.508008	0.598527	3.401300
N	-2.000478	-0.691198	3.527047
C	-0.175666	1.535671	3.281021
C	0.245333	0.171613	3.427450
C	-0.640875	-0.887570	3.527946
N	1.498851	-0.406237	3.407756
C	1.295915	-1.741689	3.499250
N	0.012510	-2.072254	3.593558
C	-2.936091	-1.803500	3.569250

O	-3.704138	0.818677	3.412247
O	0.544583	2.520629	3.170110
C	2.767847	0.284685	3.312010
H	-1.968247	2.520880	2.946143
H	2.134035	-2.434970	3.434555
H	-3.725511	-1.638962	2.826627
H	-2.389194	-2.714478	3.310390
H	-3.391082	-1.890164	4.571600
H	3.042980	0.719335	4.287655
H	3.528084	-0.433708	2.981287
H	2.674004	1.104319	2.591122
S	-2.936815	3.403018	0.369721
O	-4.367609	3.029888	0.225071
O	-2.491058	4.383680	-0.671827
O	-2.561311	3.847490	1.753637
H	-4.028818	5.447358	-0.951950
O	-4.980980	5.598336	-0.771216
H	-5.182815	4.707811	-0.420223
H	-3.540600	5.625362	1.965784
O	-4.090644	6.419191	1.834920
H	-4.483092	6.244725	0.957087
S	-2.982555	-2.014663	-0.120640
O	-2.898663	-2.381934	-1.565684
O	-4.358201	-1.609176	0.299174
O	-2.383467	-3.044933	0.776604
H	-5.484536	-2.791696	-0.764214
O	-5.490928	-3.445949	-1.492632
H	-4.599514	-3.246860	-1.848520
H	-3.588138	-4.654215	0.608712
O	-4.292408	-5.279914	0.358496
H	-4.785003	-4.759972	-0.306634
S	4.700390	-1.926160	0.563219
O	6.117040	-1.448924	0.468879
O	4.392212	-2.996915	-0.428388
O	4.303182	-2.287005	1.961077
H	6.076414	-4.006692	-0.479951
O	7.022647	-3.990967	-0.230437
H	7.069670	-3.047517	0.028479
H	5.644421	-3.700942	2.506928
O	6.365332	-4.355423	2.542349
H	6.660382	-4.371486	1.611434

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HPTS-Caffeine (1st)

C	-0.692787	-1.245939	0.001003
C	0.043941	-2.469141	0.013207
C	1.404113	-2.469765	0.006389
C	2.142630	-1.239934	0.000000
C	1.430823	0.000000	0.000000
C	0.000000	0.000000	0.000000
C	2.138833	1.241741	0.002546
C	3.547292	1.206931	0.041160
C	4.238424	-0.001769	0.029625
C	3.550742	-1.209210	-0.007126
H	-0.513360	-3.404963	0.040176
H	1.962945	-3.402564	0.024300

H	5.327265	-0.001979	0.043119
C	1.385875	2.462745	-0.028416
C	0.024139	2.461250	-0.017810
C	-0.721376	1.237327	0.012686
C	-2.130134	1.178056	0.050707
C	-2.800010	-0.041532	0.027371
C	-2.097746	-1.237775	-0.000506
O	-2.753495	-2.439529	-0.005064
H	-3.891760	-0.027135	0.051357
H	1.934462	3.401699	-0.036072
H	-0.529041	3.398752	-0.039538
H	-3.698009	-2.251079	0.052650
N	1.837899	-1.868873	3.557343
C	2.920007	-0.974754	3.632763
N	2.632010	0.373606	3.496136
C	0.468796	-1.540223	3.519652
C	0.280530	-0.125712	3.461893
C	1.325975	0.782149	3.437572
N	-0.862556	0.645054	3.355870
C	-0.441393	1.930123	3.274213
N	0.878775	2.053246	3.339693
C	3.730359	1.328264	3.460955
O	4.054213	-1.377054	3.812130
O	-0.412641	-2.396130	3.539670
C	-2.231818	0.176776	3.353348
C	2.214800	-3.277558	3.532719
H	-1.152663	2.737000	3.099974
H	4.574817	0.874241	2.931090
H	3.412510	2.229272	2.925230
H	4.054156	1.589209	4.483168
H	-2.558624	-0.067726	4.378355
H	-2.861801	0.969521	2.930901
H	-2.300270	-0.732423	2.746530
H	2.962700	-3.440153	2.743969
H	2.648050	-3.576115	4.501119
H	1.303750	-3.851659	3.337042
S	4.510746	-2.728086	-0.198796
O	5.942096	-2.325426	-0.123237
O	4.116578	-3.244642	-1.547732
O	4.090424	-3.655505	0.897188
H	5.680996	-4.168227	-2.095808
O	6.619975	-4.392177	-1.919146
H	6.792373	-3.688140	-1.261566
H	5.063512	-5.374032	0.524919
O	5.625963	-6.083545	0.162687
H	6.056694	-5.621210	-0.583526
S	4.531956	2.723801	0.049600
O	4.318874	3.341368	-1.295099
O	5.938968	2.281211	0.276658
O	3.995611	3.567291	1.159554
H	6.946553	3.670799	-0.656620
O	6.879795	4.446571	-1.249820
H	5.960545	4.291639	-1.554427
H	5.167989	5.202483	1.190892
O	5.842419	5.881844	1.005710
H	6.277686	5.509275	0.213863

S	-3.190263	2.617558	0.303929
O	-4.578873	2.127038	0.030292
O	-2.763917	3.685804	-0.642987
O	-2.996532	2.989751	1.743159
H	-4.445516	4.664483	-0.982249
O	-5.416583	4.620313	-0.873277
H	-5.472270	3.679022	-0.607117
H	-4.409656	4.423616	2.035811
O	-5.136302	5.066686	1.950631
H	-5.310854	5.040946	0.989790

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HPTS-Indole 2nd

C	0.79614100	-2.49651100	0.87931800
C	-0.43914800	-2.90446500	1.46925700
C	-1.59850900	-2.23928200	1.21272800
C	-1.61499500	-1.09424100	0.34919000
C	-0.39325400	-0.66620900	-0.25735400
C	0.82236500	-1.37337200	0.00413700
C	-0.36536700	0.49939300	-1.08179300
C	-1.56254000	1.22571800	-1.24482400
C	-2.75699400	0.79246400	-0.67814000
C	-2.78962400	-0.36016100	0.09663300
H	-0.42942300	-3.76432700	2.13984200
H	-2.53524700	-2.55324300	1.66684600
H	-3.66447500	1.38000300	-0.80791800
C	0.87782600	0.89504700	-1.67897700
C	2.03054400	0.20926300	-1.44015000
C	2.05061500	-0.93353700	-0.57802800
C	3.22825600	-1.63451700	-0.25647800
C	3.19917900	-2.73008400	0.59748600
C	2.00324000	-3.15633500	1.16190600
O	1.97414400	-4.22466600	2.01985100
H	4.14346000	-3.23234400	0.82067000
H	0.87924300	1.76289700	-2.33543400
H	2.96582100	0.52213400	-1.90133300
H	2.88694900	-4.51372300	2.14072400
C	2.94049200	0.60954000	2.29647900
C	2.31538700	-0.19230700	3.27315600
C	0.96365200	-0.04805200	3.56665600
C	0.21066100	0.91745500	2.87772300
C	2.22290800	1.58137900	1.60799800
C	0.86400300	1.73173900	1.90347000
C	-1.17818300	1.27657000	2.85261700
C	-1.30540400	2.25635800	1.89760100
H	3.98177100	0.43692300	2.01904800
H	2.89867900	-0.96658900	3.78030500
H	0.47811700	-0.70146600	4.29678900
H	2.70002200	2.16546600	0.82007900
H	-2.00038400	0.80494300	3.38554400
H	-2.19929800	2.74816700	1.52168100
N	-0.08297100	2.55529100	1.34520800
H	-0.01443000	3.06990200	0.45802200
S	4.85775100	-1.12578300	-0.84294800
O	5.79667100	-2.17492900	-0.32802100
O	4.78501000	-1.11293800	-2.33595100

O	5.10162100	0.22126800	-0.25399900
H	6.59628000	-1.54335000	-2.84383600
O	7.46743800	-1.87562400	-2.54116100
H	7.17753100	-2.18920000	-1.65950600
H	7.00920900	0.64069500	-0.79396400
O	7.90873900	0.61942000	-1.16926500
H	7.87782500	-0.19424200	-1.70878900
S	-4.34607200	-0.80528900	0.89057400
O	-5.35483000	0.18006200	0.40312000
O	-4.64698500	-2.20551100	0.44567800
O	-4.08389200	-0.71940600	2.35843300
H	-6.54069300	-2.25404300	0.56297200
O	-7.37977600	-1.75859800	0.68059500
H	-7.01105500	-0.86229000	0.54141800
H	-5.76426600	-1.28163100	3.24588000
O	-6.66905700	-1.58170900	3.45629200
H	-7.04911600	-1.67668000	2.56091500
S	-1.55435600	2.84731000	-2.02449000
O	-1.05943800	2.67781100	-3.42324400
O	-2.95625100	3.34789500	-1.95749900
O	-0.60725500	3.66420500	-1.18658300
H	-3.07978900	4.51348100	-3.49764000
O	-2.62823500	4.83353600	-4.30540200
H	-1.94053800	4.13774500	-4.35075200
H	-0.68134500	5.51020100	-2.01108200
O	-0.87860300	6.29264300	-2.55743600
H	-1.48692400	5.91790300	-3.22384300

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HPTS-Indole 3rd

C	0.88118900	-2.43273800	-1.14828600
C	-0.34201700	-3.16968300	-1.14781500
C	-1.54039000	-2.54698100	-0.97860600
C	-1.61615800	-1.12482200	-0.80928300
C	-0.41053900	-0.35823400	-0.83592800
C	0.84978900	-1.01792100	-0.98340700
C	-0.45003300	1.05818200	-0.65888700
C	-1.70126100	1.67236000	-0.45850500
C	-2.87416400	0.92457900	-0.42678400
C	-2.83604500	-0.45419100	-0.59595700
H	-0.29060600	-4.25193500	-1.27016300
H	-2.46853000	-3.11389100	-0.97473600
H	-3.82604800	1.42175500	-0.24648900
C	0.78480800	1.78912600	-0.66400500
C	1.98528100	1.15602500	-0.77773400
C	2.06413800	-0.26706600	-0.92275500
C	3.28011600	-0.97328900	-0.98087900
C	3.30861000	-2.35005000	-1.17370800
C	2.12656700	-3.07145500	-1.26829900
O	2.14405600	-4.43285600	-1.43252300
H	4.28119000	-2.84640400	-1.20409500
H	0.73929300	2.87144800	-0.56340200
H	2.91302400	1.72598200	-0.76825400
H	3.06782500	-4.70973100	-1.40105600
C	-0.94054200	0.56326100	2.57787800
C	-1.56812800	-0.69930800	2.61273200

C	-0.81980700	-1.86944500	2.57836700
C	0.58068400	-1.78609100	2.48805000
C	0.44139800	0.67734300	2.48117300
C	1.19313600	-0.50138700	2.42588000
C	1.63714500	-2.74852500	2.33749800
C	2.80078800	-2.03518400	2.17082300
H	-1.53509500	1.47794900	2.55420700
H	-2.65724100	-0.77106800	2.59686000
H	-1.32479800	-2.83810900	2.55702400
H	0.90466400	1.66042300	2.39041500
H	1.53362600	-3.83127200	2.30424200
H	3.81229300	-2.37262700	1.95586500
N	2.54762400	-0.68830000	2.25212900
H	3.22329500	-0.00799700	1.89414500
S	-4.37733200	-1.37253800	-0.40461200
O	-5.45992800	-0.35278300	-0.29662900
O	-4.51110600	-2.21878600	-1.63748800
O	-4.19249800	-2.18970200	0.83079900
H	-6.37699700	-2.39906300	-1.80982300
O	-7.27483900	-2.12917400	-1.51791400
H	-7.00455900	-1.32454300	-1.03028600
H	-5.86260300	-3.27633800	0.97103100
O	-6.73856000	-3.68563200	0.83728000
H	-7.04989500	-3.21670600	0.03814800
S	-1.82837100	3.43489700	-0.09503000
O	-1.19005400	4.14795400	-1.24736600
O	-3.28822900	3.72327100	0.01681900
O	-1.08247100	3.62258600	1.18485100
H	-3.37108700	5.58807900	-0.50823500
O	-2.87984700	6.34044600	-0.89823300
H	-2.12182900	5.82400700	-1.24547600
H	-1.25376000	5.57721800	1.60605200
O	-1.46747900	6.52894700	1.59567100
H	-1.96597500	6.60495800	0.75866900
S	4.86075700	-0.18285300	-0.64455700
O	5.88024200	-1.27432600	-0.75082300
O	5.05833200	0.89218500	-1.66008400
O	4.74344600	0.35580500	0.75088000
H	6.98067500	0.94587900	-1.86316700
O	7.82799700	0.49123400	-1.67622600
H	7.44802900	-0.35505200	-1.36270900
H	6.65203300	0.99628200	1.14847200
O	7.59761900	1.22371300	1.09524400
H	7.79173200	1.05165200	0.15355800

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HPTS-Theobromine 2nd

C	1.03421900	-2.06398700	-1.14474500
C	-0.14303400	-2.87469200	-1.16612200
C	-1.38618200	-2.32112500	-1.15291800
C	-1.55597300	-0.89913800	-1.05841100
C	-0.39717800	-0.06168500	-1.01142700
C	0.90702700	-0.64379500	-1.10146000
C	-0.52539100	1.34709300	-0.79940700
C	-1.82106500	1.86979600	-0.59386500
C	-2.94890600	1.06424900	-0.71047100

C	-2.82486100	-0.29552600	-0.96272500
H	-0.01150300	-3.95647900	-1.19209500
H	-2.27607900	-2.94540800	-1.18036100
H	-3.93743700	1.49912100	-0.57147500
C	0.66130200	2.15252600	-0.78474300
C	1.89809000	1.60083000	-0.93556500
C	2.07018100	0.18490800	-1.06702800
C	3.33243700	-0.44271500	-1.11060100
C	3.44966900	-1.82702800	-1.12107500
C	2.31816800	-2.63789700	-1.09619800
O	2.44547200	-3.99747900	-0.97127000
H	4.44477300	-2.26961900	-1.10729600
H	0.54200200	3.22669200	-0.65433400
H	2.78680400	2.22870300	-0.92237900
H	2.87298300	-4.14338200	-0.09982300
N	0.80929700	-3.49394900	2.12517200
C	2.09824000	-3.01073700	1.98932000
N	2.25344700	-1.66187300	2.17514500
C	-0.41227100	-2.80429100	2.33033100
C	-0.15930800	-1.39757300	2.34816500
C	1.12592800	-0.87122700	2.30586300
N	-0.99056100	-0.29698200	2.40230200
C	-0.18056800	0.78833100	2.38447500
N	1.11262600	0.47252200	2.34940200
C	3.57970900	-1.05753300	2.14321500
O	3.03333600	-3.77502300	1.72646600
O	-1.45470200	-3.41889600	2.46484500
C	-2.44397400	-0.29338500	2.44193500
H	0.71711300	-4.49222200	1.97170500
H	-0.58875800	1.80152300	2.32354000
H	4.23860900	-1.68714100	1.53957100
H	3.98534300	-0.97841800	3.16591000
H	3.52407400	-0.05862000	1.69931700
H	-2.86047100	-1.00158800	1.71303600
H	-2.78142700	0.72388400	2.20677100
H	-2.79619400	-0.57911200	3.44569200
S	-4.33507200	-1.27765200	-1.00253500
O	-5.46096000	-0.29985400	-1.00070100
O	-4.27423100	-2.08648500	-2.26071300
O	-4.27234000	-2.12188300	0.22920000
H	-6.12601300	-2.34098600	-2.66248500
O	-7.05415200	-2.09793600	-2.45992000
H	-6.85912700	-1.30140300	-1.92537000
H	-5.94325100	-3.24237800	0.17780600
O	-6.79521600	-3.65318600	-0.05961500
H	-7.00570100	-3.19391600	-0.89593400
S	-2.10161400	3.52794500	0.06077800
O	-1.29507000	4.49121400	-0.74773300
O	-3.56832700	3.77036200	-0.05695000
O	-1.64244800	3.42867600	1.48474000
H	-3.67909600	5.68549800	-0.19575000
O	-3.18672900	6.52578600	-0.30009600
H	-2.33417800	6.13853200	-0.58510800
H	-2.09885400	5.23140300	2.27265500
O	-2.38650300	6.14919400	2.42999100
H	-2.68229800	6.41640400	1.53796800

S	4.87287900	0.49824400	-1.04445100
O	5.96133500	-0.52728200	-1.11103600
O	4.86165400	1.40538600	-2.23075200
O	4.84193500	1.23166900	0.25530000
H	6.75184700	1.61541800	-2.58596700
O	7.65590100	1.28720800	-2.39784500
H	7.39509900	0.46488000	-1.93429300
H	6.62457200	2.14994900	0.38042300
O	7.52852500	2.47728800	0.21726400
H	7.69072000	2.15866800	-0.69170200

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HPTS-Theobromine 3rd

C	1.58731400	-3.19001000	-0.04354100
C	0.41643700	-3.98893600	0.13729100
C	-0.82445800	-3.47504500	-0.08288400
C	-0.99355400	-2.11655400	-0.50895900
C	0.15879000	-1.30456900	-0.73576400
C	1.46235000	-1.84292700	-0.49602300
C	0.02136900	0.05831400	-1.15293000
C	-1.27891800	0.58399900	-1.28218100
C	-2.39951200	-0.21781600	-1.07261700
C	-2.26617300	-1.54439400	-0.69605600
H	0.54174700	-5.01943700	0.47069900
H	-1.71603300	-4.08009900	0.06454700
H	-3.39395900	0.21356200	-1.16766600
C	1.21179700	0.82717900	-1.38743700
C	2.45140700	0.31230900	-1.15784200
C	2.62252300	-1.02346500	-0.66660300
C	3.87641700	-1.57045800	-0.33151300
C	3.99462100	-2.88539400	0.10731400
C	2.87107400	-3.69200400	0.23546000
O	2.98042100	-4.99035500	0.66127600
H	4.99277300	-3.25400800	0.35531500
H	1.09282800	1.85540100	-1.72138900
H	3.34245200	0.91338100	-1.33213100
H	3.91519600	-5.15347200	0.83729900
N	-1.75796500	3.78751500	1.71371200
C	-2.80218600	2.87891600	1.87335300
N	-2.43716300	1.55095200	2.07748100
C	-0.37997800	3.55254100	1.73439600
C	-0.10890000	2.16026500	1.95153900
C	-1.10532000	1.21236700	2.10197800
N	1.07586600	1.45389300	1.97794000
C	0.72802300	0.15787200	2.15217300
N	-0.58284600	-0.02968100	2.24580500
C	-3.48278400	0.54060900	2.09529000
O	-3.96856400	3.22895200	1.87593800
O	0.43854200	4.45256200	1.60170400
C	2.40082700	1.99998200	1.76262000
H	-2.02469700	4.66155100	1.23242400
H	1.47810100	-0.63060500	2.17607800
H	-4.08880500	0.62298000	3.01213900
H	-3.02378500	-0.45231400	2.04115000
H	-4.14462700	0.68052900	1.23178700
H	3.12532100	1.19058300	1.60711500

H	2.70320500	2.62025100	2.62038800
H	2.38381100	2.63836400	0.87176600
S	-3.76808200	-2.48186600	-0.35695400
O	-4.90496900	-1.56929200	-0.67107700
O	-3.71021900	-3.67171000	-1.26806800
O	-3.68052200	-2.85928200	1.08442400
H	-5.53831000	-4.06932200	-1.51527300
O	-6.47810000	-3.81237400	-1.39467200
H	-6.32237200	-2.87312100	-1.17036300
H	-5.27256300	-4.02091000	1.42265200
O	-6.09339400	-4.54369400	1.35467500
H	-6.35795000	-4.36339500	0.43132300
S	5.39600300	-0.59842700	-0.34025000
O	6.47482000	-1.56910000	0.03450000
O	5.56273600	-0.06916300	-1.72684100
O	5.19020500	0.46805100	0.68128200
H	7.50091300	0.01626300	-1.92357700
O	8.35709500	-0.26363300	-1.53947100
H	7.99832300	-0.90934100	-0.89574300
H	7.02467300	1.36730700	0.74290600
O	7.95427700	1.62286700	0.60265400
H	8.20664200	1.05431300	-0.15023700
S	-1.64334500	2.31701900	-1.65966900
O	-1.69923000	2.45612200	-3.14095000
O	-2.97216700	2.53166600	-1.00493300
O	-0.55740700	3.13562900	-1.04301600
H	-3.67316600	4.00526600	-1.84916900
O	-3.51600300	4.72741600	-2.49802900
H	-2.85922600	4.26575600	-3.05295300
H	-1.13118200	4.95586100	-0.59228500
O	-1.78651600	5.66294000	-0.42734000
H	-2.45224800	5.46826900	-1.12083900

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HPTS-Caffeine 2nd

C	0.27291300	-1.97084700	1.73026300
C	-0.65457900	-1.39234500	2.65088100
C	-1.54790600	-0.44390300	2.25302000
C	-1.57596700	0.01880400	0.89522500
C	-0.66201000	-0.54712200	-0.04763600
C	0.25378700	-1.56628400	0.36467100
C	-0.63332900	-0.08114800	-1.39686600
C	-1.47635700	0.98938100	-1.75235800
C	-2.38223900	1.52550100	-0.84312200
C	-2.45602300	1.02809600	0.45399700
H	-0.61972600	-1.71696300	3.69110700
H	-2.23528600	0.01197300	2.96265300
H	-3.03809200	2.33881900	-1.15084100
C	0.25432900	-0.71552200	-2.32965000
C	1.11301100	-1.69669500	-1.93826200
C	1.17459700	-2.12696000	-0.57296300
C	2.12328500	-3.05230400	-0.09800800
C	2.15394400	-3.42987100	1.23808200
C	1.24045900	-2.90261000	2.13937700
O	1.29582200	-3.23542200	3.46947200
H	2.93930100	-4.11730600	1.56006400

H	0.22558200	-0.38812300	-3.36665200
H	1.77718500	-2.17289100	-2.65729000
H	2.07954000	-3.78589400	3.59071500
N	0.44911400	2.81392200	2.37982700
C	0.79005600	2.06664200	3.50730100
N	1.67401100	1.01199000	3.31572800
C	0.95723200	2.66088700	1.06325000
C	1.86759000	1.55974900	0.98350800
C	2.20662900	0.78128000	2.07088800
N	2.57585600	1.02512000	-0.07460900
C	3.27174000	-0.02070300	0.43074300
N	3.08102000	-0.19189600	1.73969700
C	2.00117200	0.14028400	4.42301100
O	0.34634600	2.33444400	4.61368600
O	0.63468000	3.40914400	0.15506000
C	2.57395000	1.49978900	-1.44866300
C	-0.54979400	3.85832900	2.59624300
H	3.86948200	-0.67731100	-0.20583900
H	2.98284200	0.39852200	4.85901000
H	2.03074000	-0.89803500	4.07071400
H	1.22626400	0.26385500	5.18706400
H	1.56079500	1.77029000	-1.77341100
H	2.95883500	0.69679300	-2.08820800
H	3.21539900	2.39068600	-1.54169200
H	-0.24765600	4.48805000	3.44465000
H	-1.52698200	3.39909700	2.80495600
H	-0.60548200	4.44340000	1.67320500
S	-3.80260100	1.62049400	1.50282600
O	-4.46341100	2.71836700	0.74322100
O	-4.67508100	0.42075900	1.69181000
O	-3.19338700	2.07841700	2.79174700
H	-6.41248000	1.19191500	1.83586300
O	-6.96907500	1.99804600	1.78602400
H	-6.32942900	2.55892700	1.30200600
H	-4.80735400	2.57376200	3.91127400
O	-5.70272300	2.77158900	4.24187600
H	-6.25078700	2.54934200	3.46368300
S	-1.36820000	1.73609500	-3.39189700
O	-1.68573900	0.63950900	-4.36283300
O	-2.37849200	2.83031300	-3.41443100
O	0.04484000	2.20604000	-3.50518300
H	-2.79993900	2.93306700	-5.33666500
O	-2.67706600	2.48840200	-6.19947700
H	-2.35469800	1.62940700	-5.85246700
H	0.20328700	3.09713100	-5.28020200
O	0.03904200	3.44746500	-6.17581100
H	-0.88639800	3.17070200	-6.32250300
S	3.47709300	-3.64903600	-1.12662700
O	4.23184700	-4.60972200	-0.26132900
O	2.87337800	-4.30860300	-2.32169700
O	4.26425300	-2.42530400	-1.47107400
H	4.21827800	-5.64961000	-2.78410500
O	5.02346800	-6.11417300	-2.47657900
H	4.99073300	-5.81818600	-1.54358800
H	5.88800500	-3.14819800	-2.44105900
O	6.55954100	-3.71378000	-2.86406100

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   H      6.12902900     -4.58983800     -2.83150000

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HPTS-Caffeine 3rd
   C      0.95949200     -1.77365700     -1.46875000
   C     -0.25332200     -2.49269600     -1.70471200
   C     -1.46506500     -1.87545300     -1.65468700
   C     -1.56700000     -0.48528600     -1.31367700
   C     -0.37248300      0.25683400     -1.05180000
   C      0.90401300     -0.37871400     -1.17406200
   C     -0.44286700      1.61014100     -0.59210100
   C     -1.72131100      2.16633300     -0.36622400
   C     -2.87849300      1.46794600     -0.69584300
   C     -2.80742800      0.16844200     -1.18083200
   H     -0.17822200     -3.55717400     -1.92604200
   H     -2.38297200     -2.42754600     -1.84192100
   H     -3.85131100      1.93026100     -0.53514100
   C      0.77949300      2.32515500     -0.36367800
   C      1.99394900      1.73472200     -0.54438600
   C      2.10243100      0.35735000     -0.92229000
   C      3.33150700     -0.32955100     -1.00473300
   C      3.37680400     -1.69302700     -1.26571300
   C      2.20738900     -2.42267600     -1.45374000
   O      2.26200400     -3.78966700     -1.56746400
   H      4.34370000     -2.19440900     -1.27846000
   H      0.70509400      3.36301200     -0.04338000
   H      2.91072200      2.29144600     -0.36040800
   H      2.68651200     -4.10489400     -0.74077900
   N      0.56162600     -3.72145500      1.45225800
   C      1.88200300     -3.28573000      1.49243100
   N      2.11549800     -2.00964600      1.93919400
   C     -0.60995500     -2.96198100      1.76349700
   C     -0.26626000     -1.60941200      2.05144400
   C      1.04377700     -1.17123200      2.16291600
   N     -1.03627200     -0.48254600      2.26423700
   C     -0.16489500      0.53175500      2.47770500
   N      1.10834300      0.14158100      2.44649200
   C      3.48030500     -1.51366500      2.07246800
   O      2.79655800     -4.04167600      1.14290500
   O     -1.71561200     -3.47671100      1.75747900
   C     -2.48558600     -0.37784900      2.21260000
   C      0.36071600     -5.07784100      0.96857000
   H     -0.51657100      1.56266800      2.57632900
   H      4.12160500     -2.03620700      1.35814700
   H      3.85192900     -1.69369600      3.09532200
   H      3.50373000     -0.43968900      1.85936300
   H     -2.88750400     -0.92594300      1.35048700
   H     -2.73703800      0.68738600      2.13185700
   H     -2.93029700     -0.79963400      3.12749800
   H      0.94323500     -5.78929800      1.57191500
   H      0.69396900     -5.15438400     -0.07577500
   H     -0.71146000     -5.28747100      1.04211300
   S     -4.36280800     -0.70470400     -1.44149000
   O     -5.43296400      0.32919200     -1.35180600
   O     -4.27488600     -1.32635100     -2.80133800
   O     -4.41043200     -1.71725900     -0.34537300
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H	-6.09730800	-1.39255700	-3.33102000
O	-7.02430900	-1.12079100	-3.16006600
H	-6.82792100	-0.41579300	-2.51026400
H	-6.13885000	-2.71586200	-0.65401500
O	-6.99666700	-3.02547200	-0.99950000
H	-7.12753300	-2.42982500	-1.76290200
S	-1.96279100	3.67941900	0.58722900
O	-1.03638100	4.72493800	0.05626700
O	-3.39685100	4.04737400	0.41895700
O	-1.63189800	3.26174200	1.98975900
H	-3.38340400	5.97583500	0.67192800
O	-2.81893400	6.76769300	0.78308900
H	-1.97548400	6.36605800	0.48922900
H	-2.01398400	4.90367200	3.10083700
O	-2.25340100	5.79061700	3.42607600
H	-2.46841100	6.25255000	2.59255100
S	4.91736200	0.48598400	-0.71894900
O	5.93485100	-0.61017800	-0.79771000
O	5.06177100	1.49176900	-1.81324300
O	4.83302300	1.10235600	0.63715900
H	6.96908600	1.55903400	-2.05387200
O	7.83415000	1.14103400	-1.85892100
H	7.48553300	0.30495500	-1.48692100
H	6.70791600	1.82443200	0.94686400
O	7.64311900	2.08280600	0.85346100
H	7.82600800	1.83431700	-0.07340900

12. Analysis of caffeine-containing samples: As samples, we chose caffeinated drinks (Coca Cola, Red Bull, Caffe Latte), caffeinated powders (soluble coffee Nescafé Gold, pain killer Panadol Extra) and a dietary supplement used by body builders (Superpump 250). The simple sample preparation was to extract the caffeine of the samples into an organic phase (chloroform), evaporate the organic solvent, and redissolve the residue in a buffer (water pH 6.5, 100 mM phosphate) containing HPTS (50 μM). The caffeine concentration was derived from fluorescence measurement (λ_{ex} = 460 nm, λ_{ex} = 512 nm). The linear relationship shown in Fig. 1, inset (see main text) was employed to calculate the caffeine concentration

In order to check the accuracy of our assay, the values obtained by fluorescence measurements were compared to the reported values (when available) or to the values obtained by HPLC measurements.

HPLC procedures: A mixture of HPLC-grade methanol (40 %) and acidified Millipore water (60 %, containing 50 mM phosphate, acidified to pH 3.0 with HCl) was used as elution

solvent.[9] A calibration curve was obtained by preparing samples with concentrations of caffeine between 0 and 100 µM. The area of the caffeine peaks (detection at 292 nm) was found to correspond linearly to the concentration of caffeine in the calibration samples. The original samples were diluted such as to have a caffeine concentration in this range. The obtained results are shown in Table 1. The caffeine concentrations determined by HPLC are in good agreement with the reported values (when available). The Caffe Latte sample (milk shake) was difficult to analyze due to the presence of numerous interfering species. In this case, the value of the caffeine concentration suffers from a large uncertainty.

Table 3: caffeine contents determined by HPLC for a series of products, compared with the reported values.

	Dilution factor	*Measured [caffeine] / uM*	*Deduced caffeine in sample*	*Reported caffeine in sample*
Coca Cola	*10*	*46.6*	*0.47 mM*	*0.51 mM*[a]
Red Bull	*100*	*69.3*	*6.93 mM*	*6.85 mM*[b]
Caffe Latte	*50*	*62.1*	*3.11 mM*	
	150	*24.5*	*3.68 mM*	
		Average value :	*3.40 mM*	*2.69 mM*[b]
Nescafé Gold	*3.33 mg / 25 mL*	*35.7*	*5.21 weight %*	*Not available*
Panadol Extra	*5.0 mg / 100 mL*	*25.3*	*9.83 weight %*	*9.29 weight %*[b]
Superpump 250	*20 mg / 10 mL*	*77.6*	*0.75 weight %*	*Not available*

[a] indicated as an average value on: https://secure.coca-cola.ch/content/ch/corporate/fr/inhaltsstoffe.aspx#4D38F3F7-C5D0-4EB6-BB89-62A715B77E70 (retrieved 30.03.2011).

[b] indicated on the bottle.

Fluorescence Assays: The general sample preparation procedure was adapted to the different analytes. Details are given below:

[9] R. E. Leacock, J. J. Stankus and J. M. Davis, *J. Chem. Ed.*, 2011, **88**, 232-234.

Coca Cola: 10 mL were extracted with 5 x 10 mL $CHCl_3$; after evaporation of the organic phase, the residue was dissolved in 5.0 mL H_2O (pH 6.5, 100 mM phosphate). 500 µL were added to a solution of HPTS in buffered water (final concentrations: [HPTS] = 50 µM, [phosphate] = 100 mM ; final volume = 1.0 mL). The fluorescence was measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm).

Red Bull: 2.5 mL were extracted with 5 x 2.5 mL $CHCl_3$; after evaporation of the organic phase, the residue was dissolved in 5.0 mL H_2O (pH 6.5, 100 mM phosphate). 200 µL were added to a solution of HPTS in buffered water (final concentrations : [HPTS] = 50 µM, [phosphate] = 100 mM; final volume = 1.0 mL). The fluorescence was measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm).

Caffe Latte: 5.0 mL were extracted with 5 x 10 mL $CHCl_3$ (difficult !); after evaporation of the organic phase, the residue was dissolved in 5.0 mL H_2O (pH 6.5, 100 mM phosphate). 400 µL were filtered and added to a solution of HPTS in buffered water (final concentrations : [HPTS] = 50 µM, [phosphate] = 100 mM; final volume = 1.0 mL). The fluorescence was measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm).

Nescafé Gold: 100 mg of coffee were dissolved in 5.0 mL water. The aqueous phase was extracted with 5 x 10 mL $CHCl_3$ (difficult !); after evaporation of the organic phase, the residue was dissolved in 5.0 mL H_2O (pH 6.5, 100 mM phosphate). 100 µL were added to a solution of HPTS in buffered water (final concentrations : [HPTS] = 50 µM, [phosphate] = 100 mM; final volume = 1.0 mL). The fluorescence was measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm).

Panadol Extra: 35 mg of crunched pill were dissolved in 10 mL water. The aqueous phase was extracted with 5 x 10 mL $CHCl_3$; after evaporation of the organic phase, the residue was dissolved in 5.0 mL H_2O (pH 6.5, 100 mM phosphate). 200 µL were added to a solution of HPTS in buffered water (final concentrations : [HPTS] = 50 µM, [phosphate] = 100 mM; final volume = 1.0 mL). The fluorescence was measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm).

Superpump 250: 200 mg of powder were dissolved in 10 mL water. The aqueous phase was extracted with 5 x 10 mL $CHCl_3$; after evaporation of the organic phase, the residue was dissolved in 5.0 mL H_2O (pH 6.5, 100 mM phosphate). 995 µL were filtered and added to a solution of HPTS in buffered water (final concentrations : [HPTS] = 50 µM, [phosphate] =

100 mM; final volume = 1.0 mL). The fluorescence was measured immediately (λ_{ex} = 460 nm, λ_{em} = 512 nm).