Electronic Supplementary Information

Fluorescence Sensing of Spermine with a Frustrated Amphiphile

Ziya Köstereli and Kay Severin*

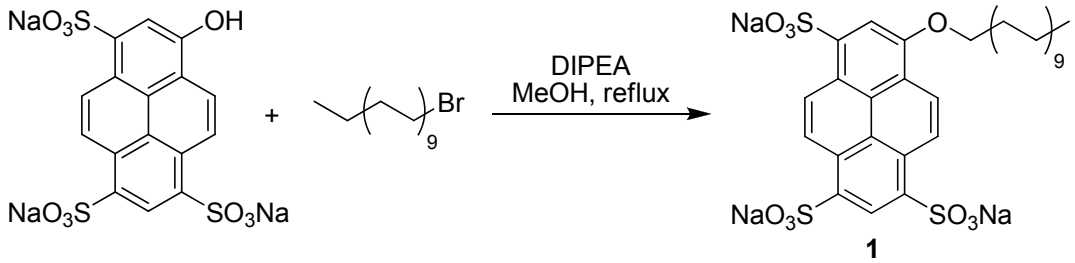
Institut des Sciences et Ingénierie Chimiques,
École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.
Fax: +41(0)21 6939305; Tel: +41(0)21 6939302; E-mail: kay.severin@epfl.ch

TABLE OF CONTENTS

1. General

All chemicals and solvents were purchased from standard suppliers and used without further purification. Stock solutions were prepared with bidistilled water and were stored at 4 °C. For phosphate buffer solutions (100 mM phosphate buffer, pD 7.0), appropriate amounts of K_2HPO_4 and KH_2PO_4 were dissolved in D_2O. MOPS buffer (0.8 mM MOPS buffer, pH 7.0) was prepared by dissolving 3-(N-morpholino)propanesulfonic acid in water. HCl, DCl and NaOH solutions were used to adjust the pH of the buffers. Fluorescence measurements were performed on a Varian Cary Eclipse fluorescence spectrophotometer at room temperature. ^{1}H NMR spectra were recorded on a Bruker Advance DPX 400 instrument or a Bruker Advance DRX 600 instrument at 25 ^{o}C. TEM imaging was performed with a FEI Tecnai G2 F30. High resolution mass spectra were recorded with a waters Q - TOF Ultima (ESI-TOF) instrument.

2. Synthesis



1

Trisodium 8-eicosyloxypyrene-1,3,6-trisulfonate (**1**) was synthesized in a similar fashion as described in the literature:[1] 1-bromoeicosane (2.21 mg, 6.11 mmol) and N,N-diisopropylethylamine (630 µL, 3.81 mmol) were added to a refluxing solution of trisodium 8-hydroxypyrene-1,3,6-trisulfonate (400 mg, 763 µmol) in MeOH (50.0 mL). The reaction mixture was heated under reflux with stirring for 6 days. The mixture was then cooled to RT, filtered, and concentrated under reduced pressure. The resulting solid was washed several times with diethyl ether. Further purification was achieved by column chromatography (SiO_2; eluent: NH_4OH:IPA - 1:2) to give dye **1** as a yellow solid (184 mg, 0.229 mmol, 30%).
^{1}H-NMR (400 MHz, CD_3OD): δ = 0.92 (t, J = 6.8 Hz, 3 H), 1.20-1.48 (m, 30 H), 1.52 (m, 2 H), 1.70 (p, J = 7.5 Hz, 2 H), 2.083 (p, J = 7.0 Hz, 2 H), 4.47 (t, J = 6.4 Hz, 2 H), 8.42 (s, 1 H), 8.68 (d, J = 9.6 Hz, 1 H), 9.12 (d, J = 9.6 Hz, 1 H), 9.20 (d, J = 9.6 Hz, 1 H), 9.26 (d, J = 9.6 Hz, 1 H), 9.40 (s, 1 H); ESI-MS calcd. for $C_{36}H_{48}S_3O_{10}$ $[(M-3Na+H)^{-2}]$ m/z = 368.1210 found 368.1205.

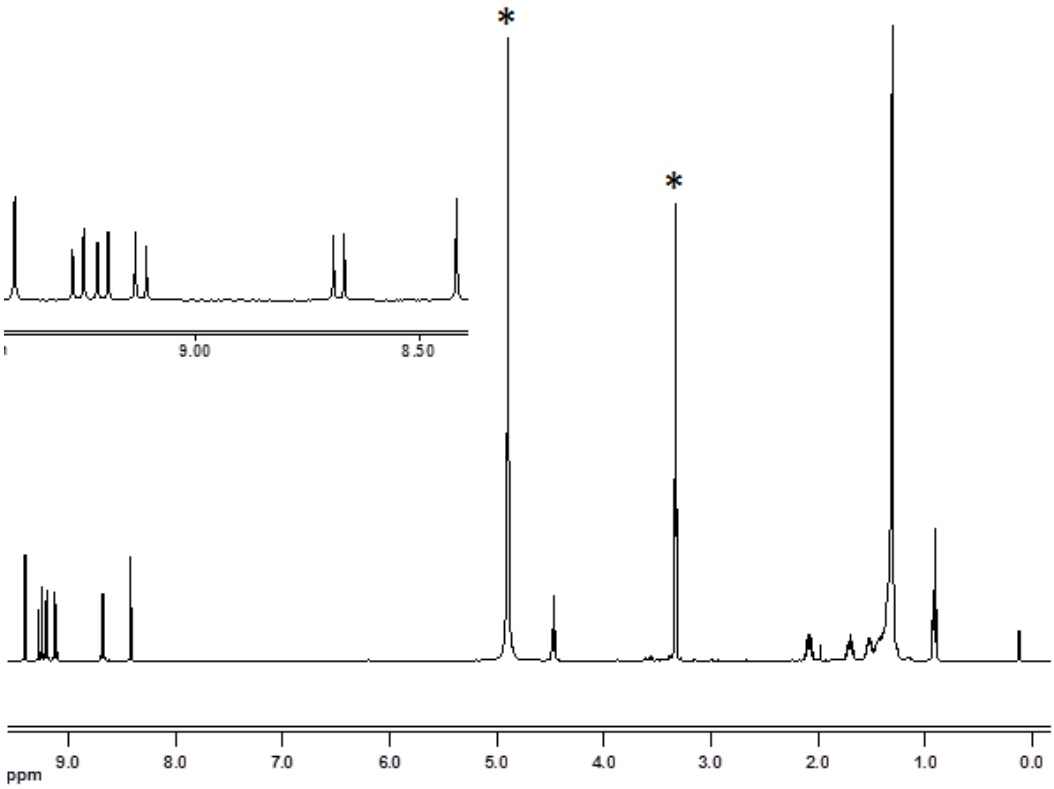


Figure S1. ^{1}H NMR spectrum of dye **1** in CD_3OD (solvent peaks are labeled with *).

3. Fluorescence measurements in buffered solution:

Stock solutions of dye **1** (trisodium 8-eicosyloxypyrene-1,3,6-trisulfonate), dye **2** (trisodium 8-methoxypyrene-1,3,6-trisulfonate) and the analytes were prepared in MOPS buffer (0.8 mM, pH 7) and kept at 4 °C. For the fluorescence titration experiments, solutions containing different concentration of dye **1** (0.25, 2, 5, 8 µM) were prepared in quartz cuvettes by mixing aliquots of a stock solution of dye **1** with buffer (final volume of 1.5 mL). The solutions were equilibrated for 2 h before starting the titration. For each titration, different stock solutions of the analyte spermine were used (10 µM for 0.250 nM dye, 45 µM for 2 µM dye, 120 µM for 5 µM dye, 201 µM for 8 µM dye). Appropriate amounts of analyte were added to the solution of dye **1** and a fluorescence spectrum was recorded after 5 min (λ_{ex}: 350 nm, λ_{em}: 430 nm). Dilution effects were neglected as the total volume of analyte solution that was added did not exceed 50 µL. It should be noted that plastic cuvettes should be avoided when using low dye concentrations. We encountered problems of reproducibility when using plastic cuvettes, probably because the dye sticks to the walls-

For selectivity tests, stock solutions (10 µM) of the analytes were prepared in MOPS buffer (0.8 mM, pH 7). An aliquot of analyte stock solution (30 µL) was added to a solution of dye **1** (200 nM, V_{total} = 1.5 mL) in a quartz cuvette. A fluorescence spectrum was recorded 5 min after addition of the analyte (λ_{ex}: 350 nm, λ_{em}: 430 nm).

The effect of high salt concentrations was studies using buffered aqueous solution (0.8 mM MOPS, pH 7) of dye **1** (8.0 μM) with and without spermine in the presence of NaCl (50 or 100 mM). Spermine-induced fluorescence quenching at pH 8 was investigated by using MOPS buffer (0.8 mM) adjusted to this pH. The results are summarized in Table S1.

Table S1. Fluorescence emission quenching (λ_{ex} = 350 nm, λ_{em} = 430 nm) under different conditions

Solutions	***I* [a.u.] (no spermine/plus spermine)**	**E.Q. [%]**
dye **1** (8 μM) + spermine (0 or 3.75 μM)	436/52	88
dye **1** (8 μM) + NaCl (50mM) + spermine (0 or 3.75 μM)	318/11	97
dye **1** (8 μM) + NaCl (100mM) + spermine (0 or 3.75 μM)	161/8	95
dye **1** (8 μM) + spermine (0 or 3.75 μM), pH 8	461/48	90

Control experiments with trisodium 8-methoxypyrene-1,3,6-trisulfonate (dye **2**): an aliquot of a stock solution of spermine was added to a solution of dye **2** in MOPS buffer (0.8 mM MOPS, pH 7, $[\text{spermine}]_{final}$ = 80 μM, $[\mathbf{2}]_{final}$ = 8 μM). A fluorescence spectrum was recorded after 5 min (λ_{ex}: 370 nm, λ_{em}: 430 nm).

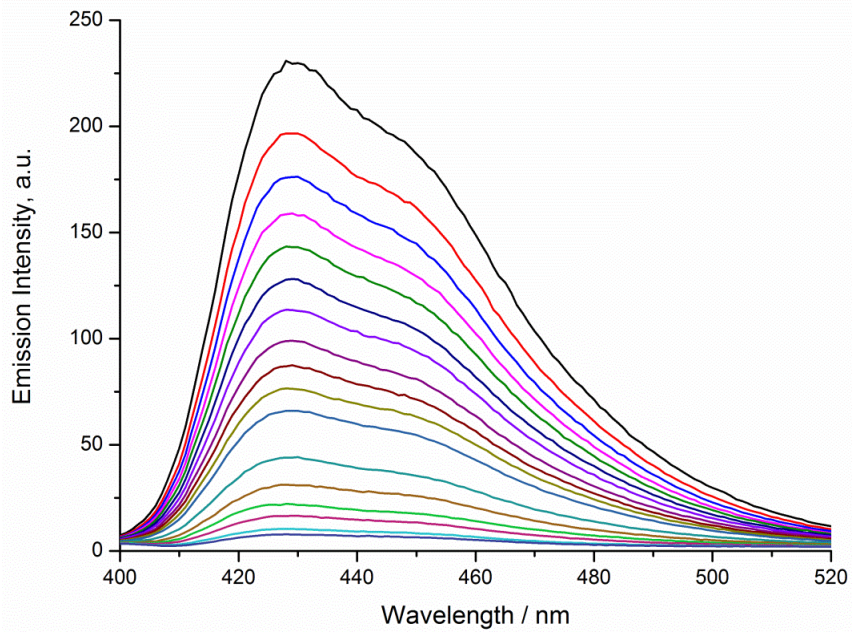


Figure S2. Fluorescence emission spectra (λ_{ex}: 350 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7) containing dye **1** (250 nM) and different amounts of spermine (0-400 nM).

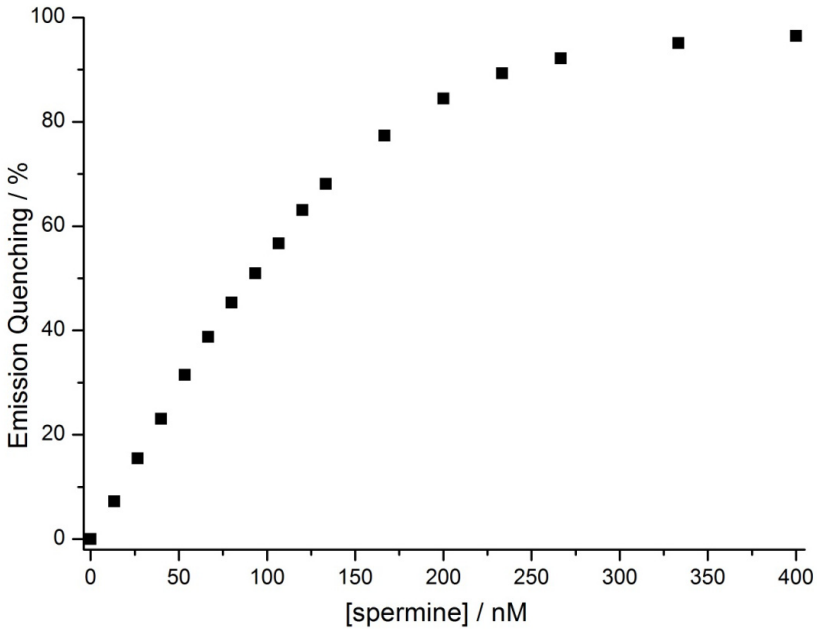


Figure S3. Fluorescence emission quenching (λ_{ex} = 350 nm, λ_{em} = 430 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7.0) containing dye **1** (250 nM) and different amounts of spermine (0-400 nM).

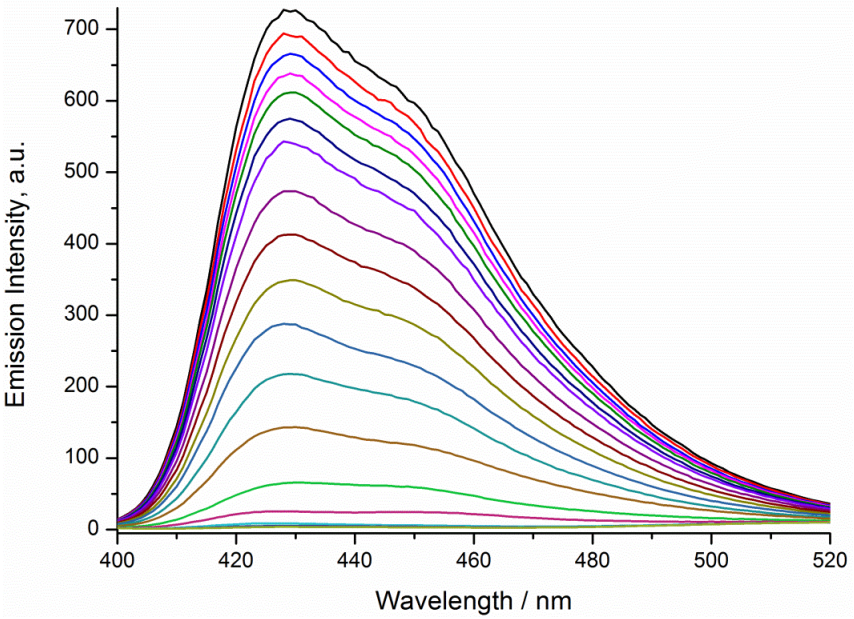


Figure S4. Fluorescence emission spectra (λ_{ex}: 350 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7) containing dye **1** (2.0 μM) and different amounts of spermine (0-1.44 μM).

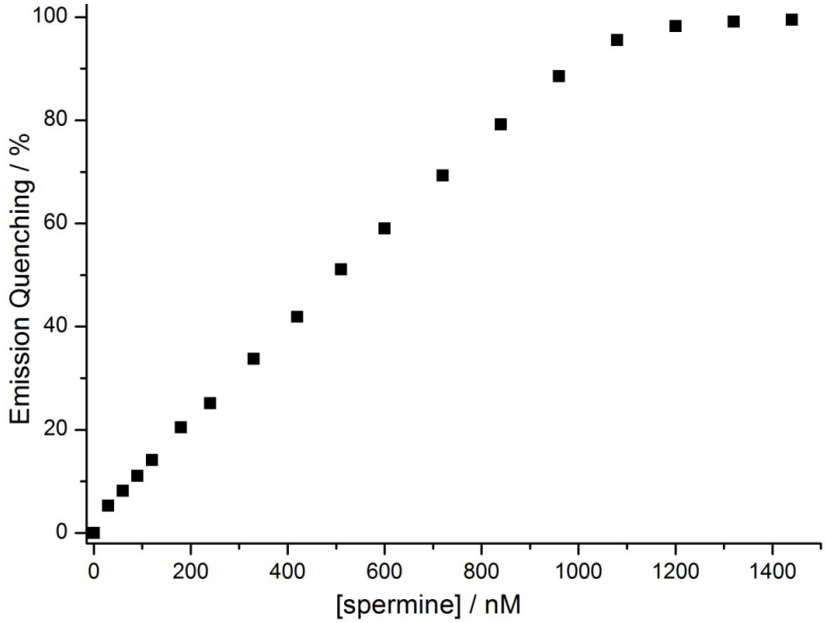


Figure S5. Fluorescence emission quenching (λ_{ex} = 350 nm, λ_{em} = 430 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7.0) containing dye **1** (2.0 μM) and different amounts of spermine (0-1.44 μM).

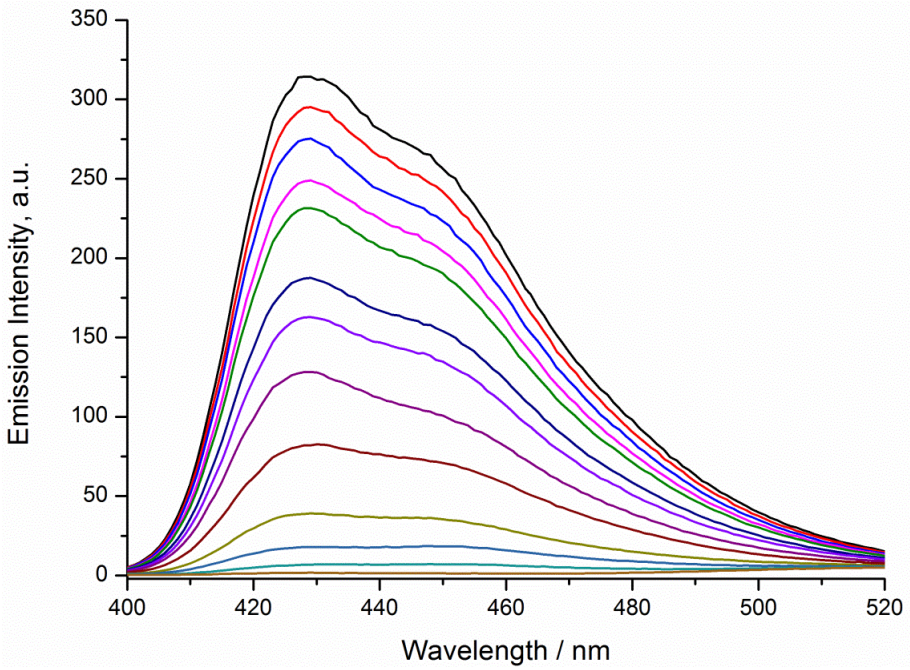


Figure S6. Fluorescence emission spectra (λ_{ex}: 350 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7) containing dye **1** (5.0 μM) and different amounts of spermine (0-3.20 μM).

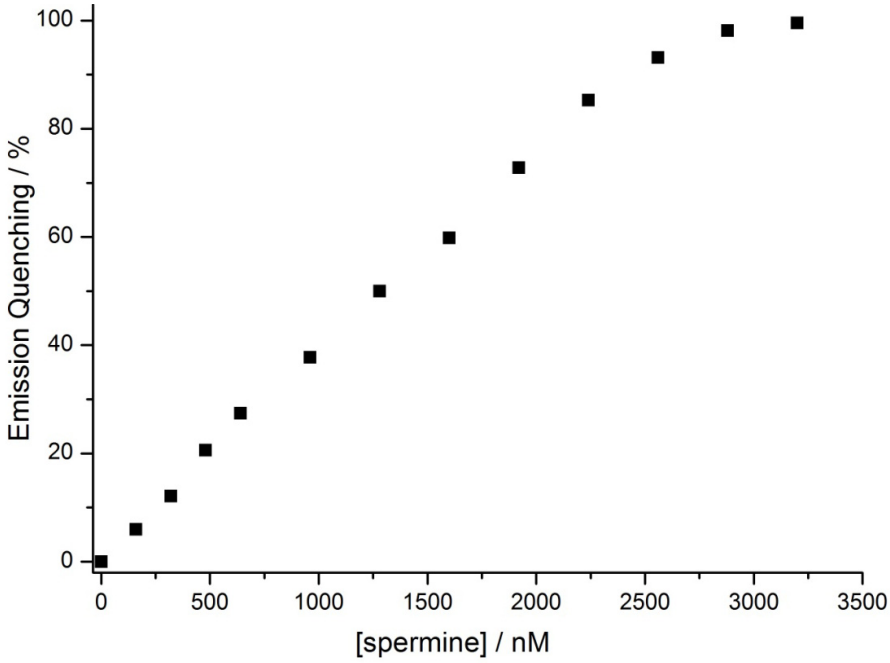


Figure S7. Fluorescence emission quenching (λ_{ex} = 350 nm, λ_{em} = 430 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7.0) containing dye **1** (5.0 μM) and different amounts of spermine (0-3.20 μM).

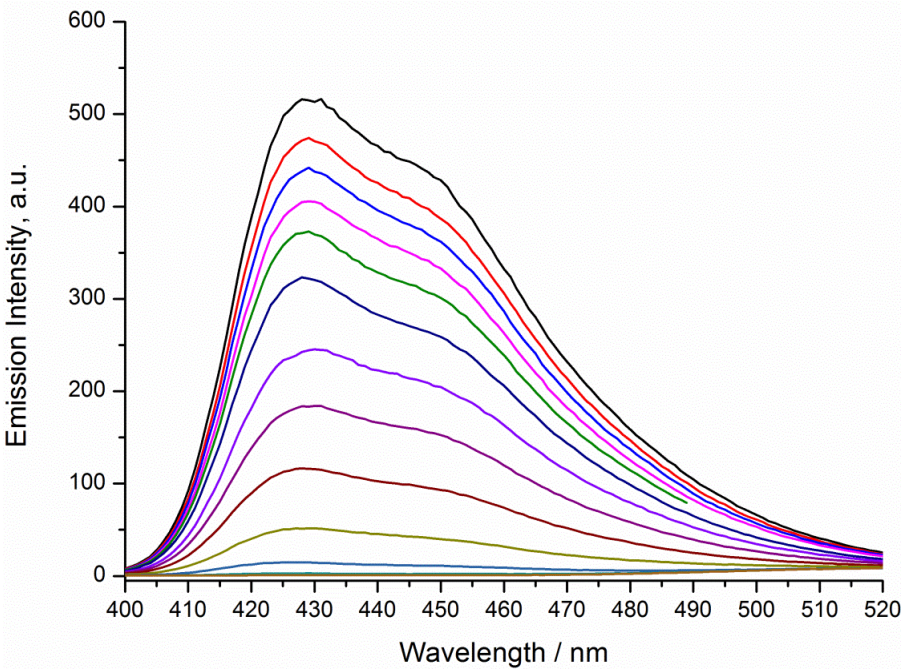


Figure S8. Fluorescence emission spectra (λ_{ex}: 350 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7) containing dye **1** (8.0 μM) and different amounts of spermine (0-5.36 μM).

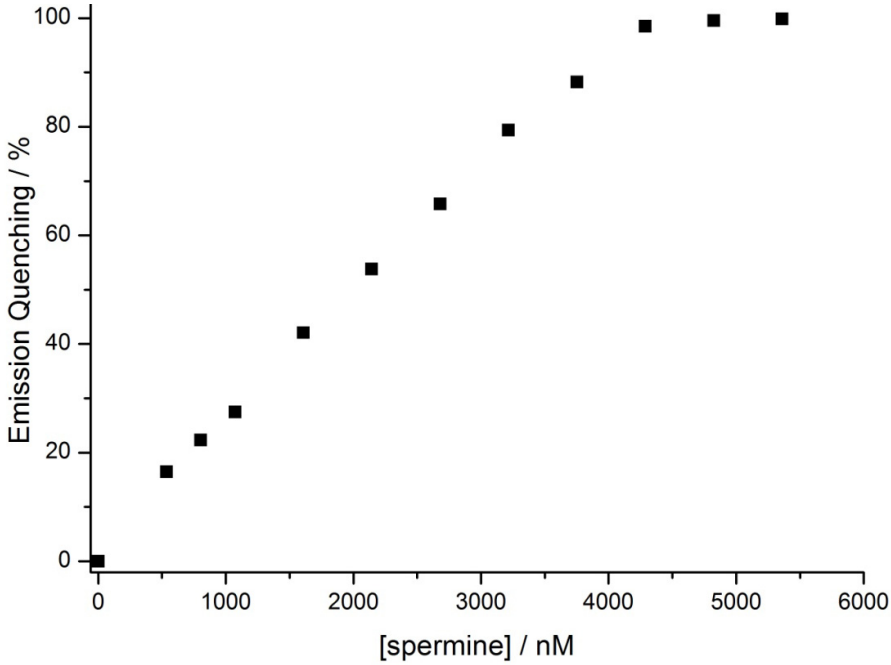


Figure S9. Fluorescence emission quenching (λ_{ex} = 350 nm, λ_{em} = 430 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7.0) containing dye **1** (8.0 μM) and different amounts of spermine (0-5.36 μM).

4. Binding Constants

Binding constants were calculated using fluorescence titration data obtained with dye **1** (0.25 μM) and different amounts of spermine. The data was fitted to an equation describing a 2.1 binding model (Figure S10).[2] The resulting binding constants were K_1 = 2.1x10^6 M^{-1} and K_2 = 5.1x10^6 M^{-1}.

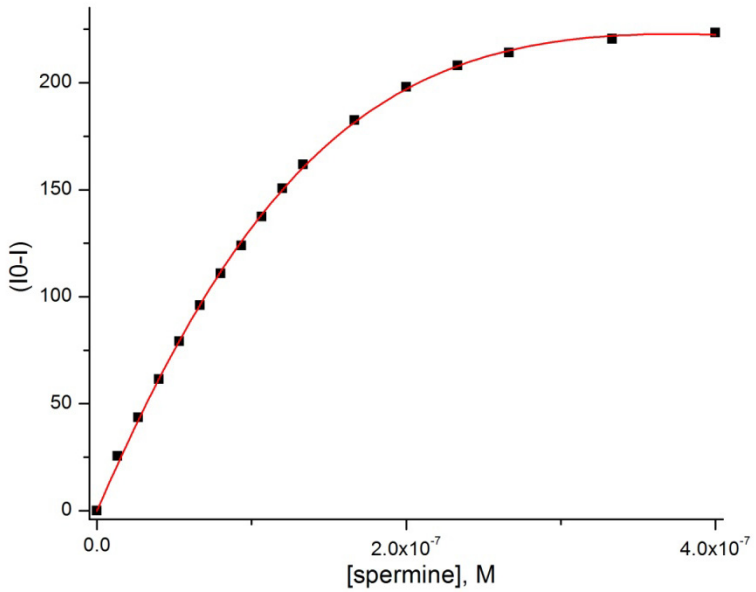


Figure S10. Fluorescence titration data (black) and the calculated binding isotherm (red)**.** **(**I0-I) is the difference in emission intensity.

.

5. Selectivity test at a higher dye concentration

For selectivity tests, stock solutions of the analytes were prepared in MOPS buffer (0.8 mM, pH 7). An aliquot of analyte stock solution was added to a solution of dye **1** (2.0 μM, V_{total} = 1.5 mL) in a quartz cuvette. A fluorescence spectrum was recorded 5 min after addition of the analyte (λ_{ex}: 350 nm, λ_{em}: 430 nm).

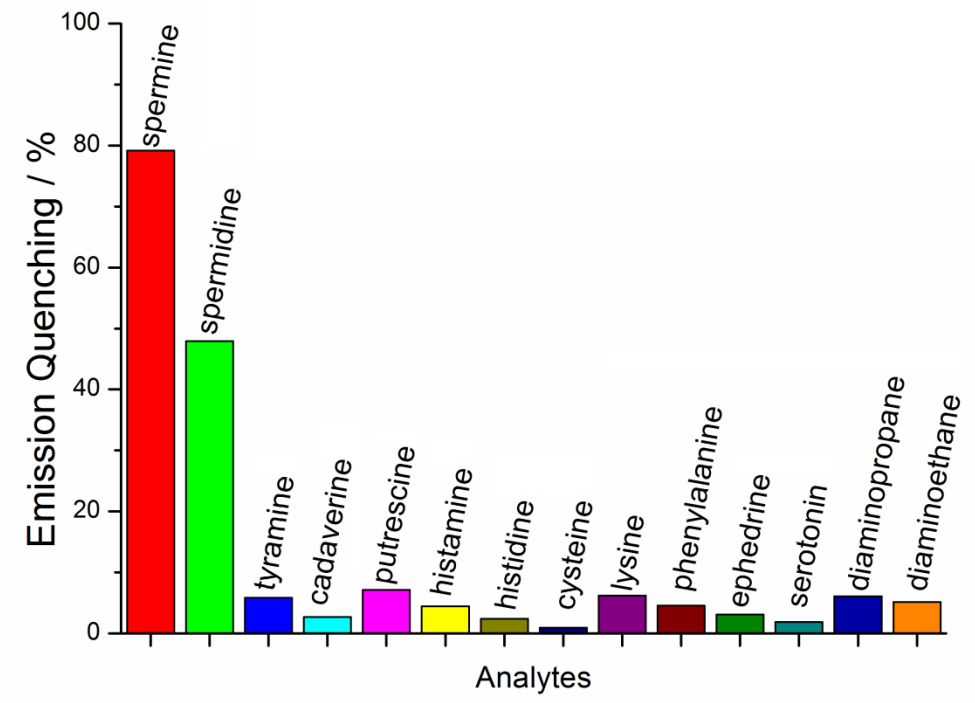


Figure S11. % Fluorescence emission quenching (λ_{ex} = 350 nm, λ_{em} = 430 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7.0) containing dye **1** (2.0 μM) and different amines (840 nM).

6. Fluorescence measurements in artificial urine:

Artificial urine was prepared as described in the literature.[3] It contained potassium dihydrogen phosphate (7.0 mM), dipotassium hydrogen phosphate (7.0 mM), sodium sulfate (10 mM), magnesium sulfate (2.0 mM), lactic acid (1.1 mM), citric acid (2.0 mM), calcium chloride (2.5 mM), sodium chloride (90 mM), urea (170 mM), sodium bicarbonate (25 mM), and ammonium chloride (25 mM). All compounds were dissolved in bi-distilled water. The pH of the solution was 6.8. Stock solutions of dye **1** (50 µM) and spermine (120 µM) in MOPS buffer (0.8 mM, pH 7) were employed. An aliquot of the dye stock solution (60 µL) was added to artificial urine ($[\mathbf{1}]_{final}$ = 2.0 µM, V_{total} = 1.5 mL) in a quartz cuvette, and a fluorescence spectrum was recorded after equilibration for 20min. Subsequently, an aliquot of the spermine stock solution was added (28 µL, $[spermine]_{final}$ = 2.24 µM), and a fluorescence spectrum was recorded after equilibration for 5 min (λ_{ex}: 350 nm, λ_{em}: 430 nm). For measurements with a total dye concentration of 5µM, the amounts of added dye and spermine were increased accordingly. For comparison, measurements with identical dye and spermine concentrations were performed in MOPS buffer instead of artificial urine. The results are depicted in Figure S10.

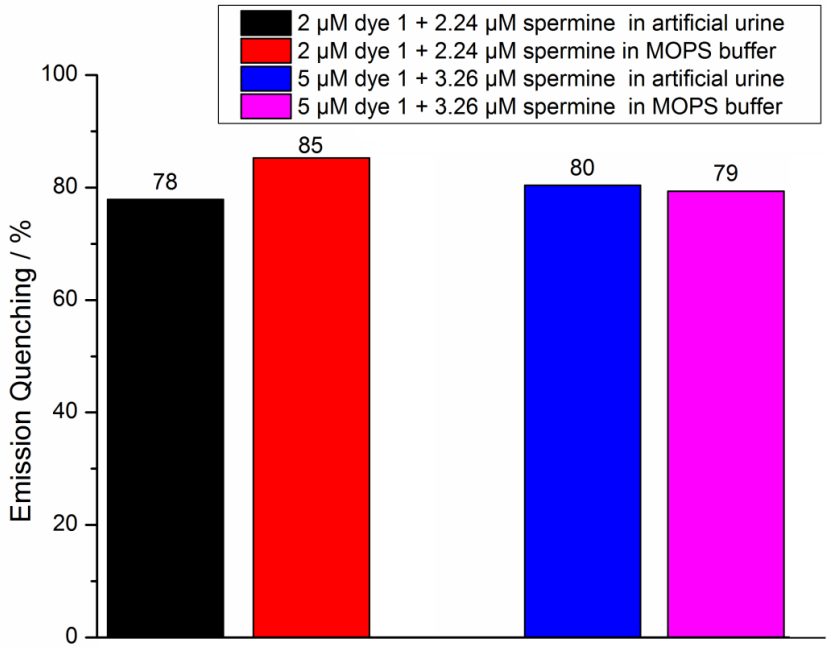


Figure S12. Fluorescence emission quenching (λ_{ex} = 350 nm, λ_{em} = 430 nm) of solutions containing dye **1** (2.0 µM and 5.0 µM) and spermine (2.24 µM and 3.26 mM) in artificial urine or buffer (0.8 mM MOPS, pH 7.0).

7. Fluorescence measurements in the presence of Nile Red

A stock solution of the dye Nile Red (1.0 mM) was prepared in ethanol. An aliquot of this stock solution ($[\text{Nike Red}]_{final}$ = ~ 8 µM, 12 µL) was pipetted in a vial. The ethanol was removed by evaporation. Subsequently, aliquots of stocks solutions of dye **1** ($[\mathbf{1}]_{final}$ = 8 µM, 240 µL in 0.8 mM MOPS, pH 7) and spermine ($[\text{spermine}]_{final}$ = 0 or 3.75 µM, o or 28 µL in 0.8 mM MOPS, pH 7) were added and the volume was completed to 1.5 mL with MOPS buffer (0.8mM, pH 7). The vial was sonicated for 10 min and the resulting mixture was filtered. A fluorescence spectrum of the clear solution was recorded (λ_{ex}: 520 nm). The results are depicted in Figure S13. The increased fluorescence emission in the presence of spermine is indicative of micelle formation.

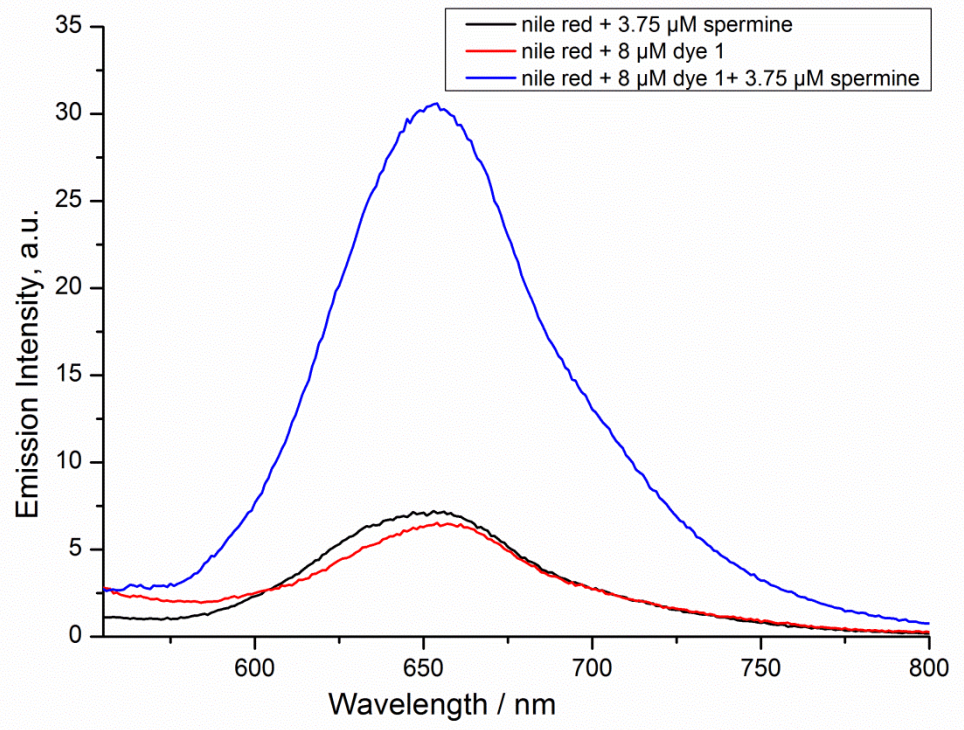


Figure S13. Fluorescence emission spectra (λ_{ex}: 520 nm) of buffered aqueous solutions (0.8 mM MOPS, pH 7) of Nile Red in the presence of dye **1** (8.0 µM) and spermine (0 or 3.75 µM).

8. Transmission electron microscopy

Diluted sample were placed on a Lacey carbon coated 300 mesh copper grid. Uranyl acetate solution (2 wt%) was used for staining. 20 μL of a solution containing dye **1** and spermine ([**1**] = [spermine] = 66 μM, 0.8mM MOPS, pH 7) was put on a parafilm sheet. The grid was placed on the top of the drop with its upper side down and kept there for 2 min. Then, 20 μL of a uranyl acetate solution (2 wt%) was put on a parafilm sheet. The grid was placed on the top of the drop with its upper side down and kept there for 5 min. Stained grids were dried in the fume hood at room temperature.

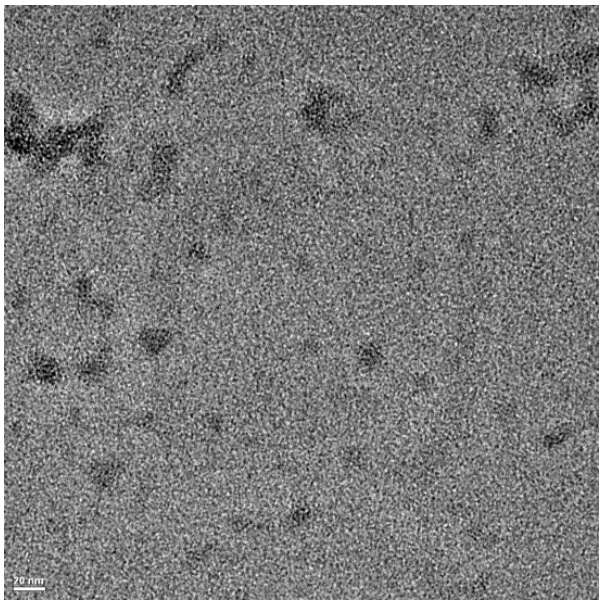


Figure S14. TEM image of a sample containing amphiphile **1** and spermine (for sample preparation see text above). The image reveals aggregates with a size of ~ 15 nm and larger (scale bar = 20 nm).

9. NMR studies

A ^{1} NMR spectrum (600 MHz) of a solution of dye **1** (66 μM) in D_2O was recorded. A second spectrum was recorded shortly after addition of an aliquot of a spermine stock solution ($[spermine]_{final}$ = 66 μM).

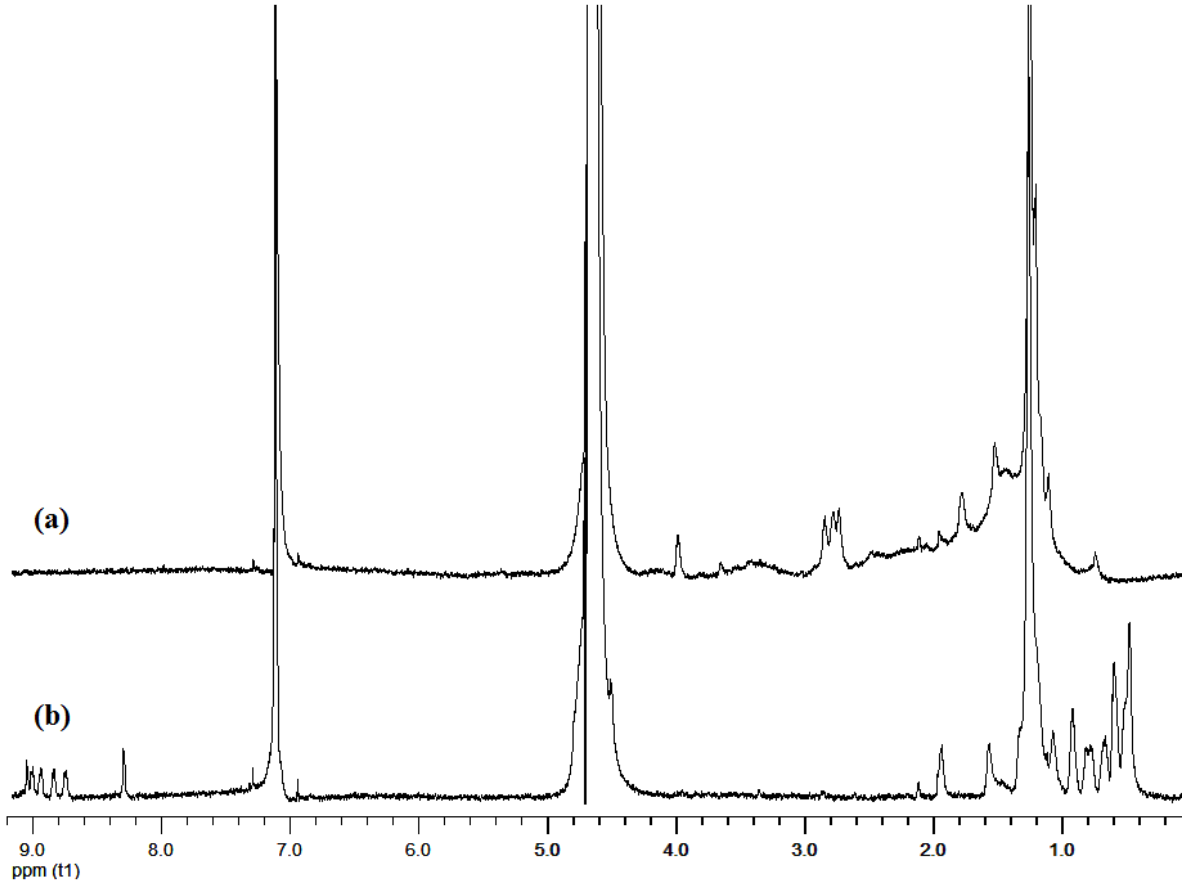


Figure S15. ^{1}H NMR spectra (600 MHz) of solutions (D_2O) containing: (a) dye **1** (66 μM) in the presence of equimolar amounts of spermine, or (b) only dye **1** (66 μM). The intense peaks at 4.7 and 7.2 are due to water and chloroform ($CDCl_3$ was used as reference in a shigemi tube which was inserted). The spectra were acquired using a pulse sequence for suppression of the water peak.

10. References

[1] R. Sasaki and S. Murata, *Langmuir*, 2008, **24**, 2387.
[2] P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305.
[3] M. Ikeda, T. Yoshii, T. Matsui, T. Tanida, H. Komatsu and I. Hamachi, *J. Am. Chem. Soc.*, 2011, **133**, 1670.