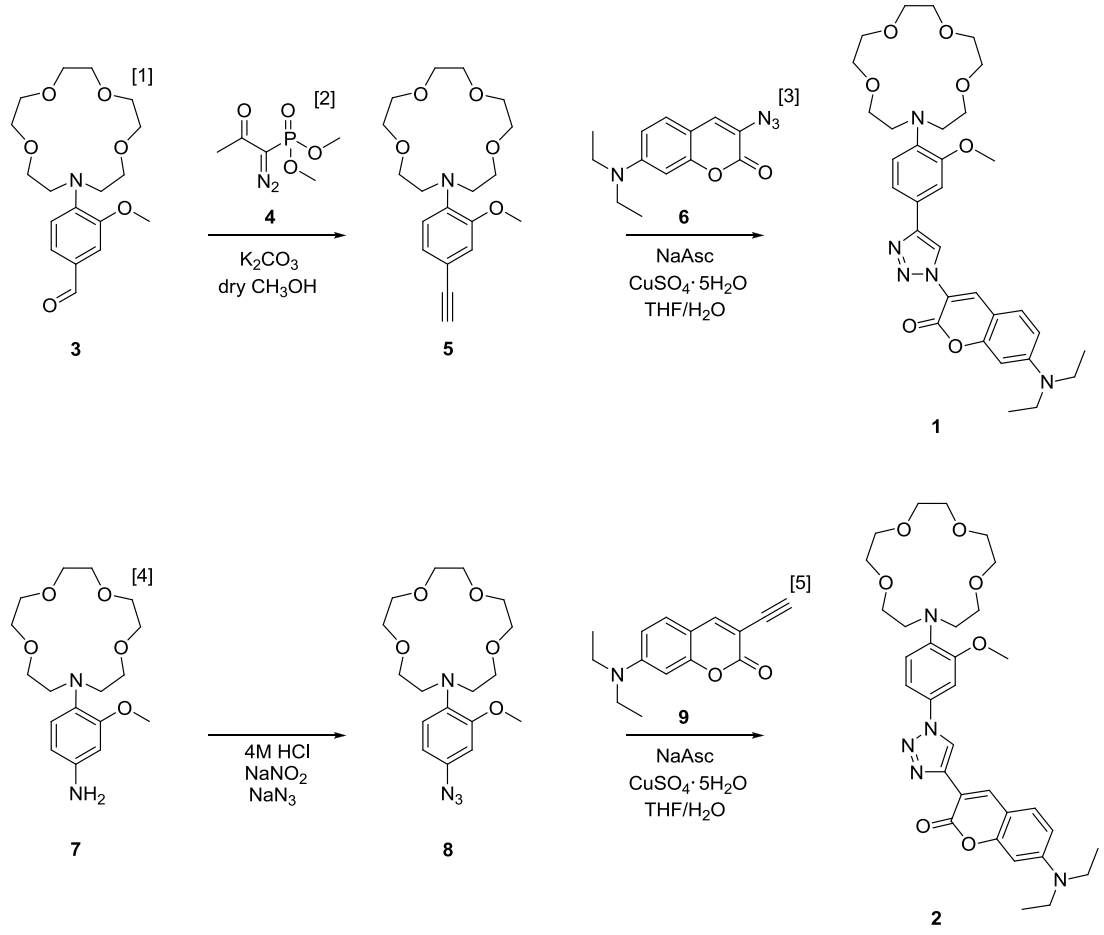
1. General Methods

NMR spectra were recorded with a Bruker Avance 300 or a Bruker Avance 500 spectrometer and all chemical shifts are reported relative to the solvent signal [$CDCl_3$: 7.26 ppm (^1H), 77.00 ppm (^{13}C)]. The assignments of the NMR signals were carried out under using 2D-NMR experiments ^1H-^1H-COSY, HMBC and HMQC. IR spectra were recorded with a Thermo Nicolet NEXUS FTIR instrument. Elemental analyses (C, H, N) were performed with an Elementar Vario EL elemental analyzer. The EI-MS spectra were recorded using Thermo Quest SSQ 710 (70eV). The ESI-MS spectra were recorded using a Micromass Q-TOFmicro mass spectrometer in positive electrospray mode. Electrochemical measurements were performed with a BAS CV-50W system using a platinum working and auxiliary electrode, and a $Ag/AgNO_3$ reference electrode. The experiments were conducted using degassed MeCN solutions using 0.1 M [nBu$_4$N$^+$][PF_6^-] as the supporting electrolyte, and the Ferrocene/Ferrocinium (Fe/Fe^+) couple as a reference $E_{\frac{1}{2}}^{Ox.}$ (Fe/Fe^+) = 0.09 V.

2. Synthesis



Scheme 1. Synthesis of the CuAAC generated 1,2,3-triazol-fluoroionophores **1** and **2**.

4-ethynyl-2-methoxyphenylaza-15-crown-5 (**5**)

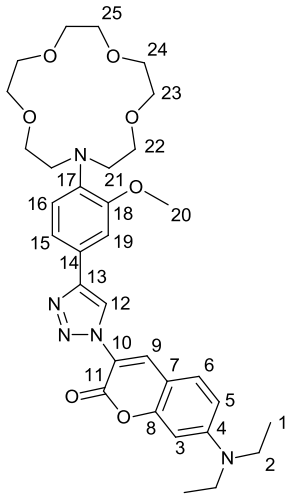
To a mixture of 860 mg (2.43 mmol) 4-formyl-2-methoxyphenylaza-15-crown-5 (**3**)[1] and 673 mg (4.86 mmol) K_2CO_3 in 35 mL dry methanol was added 564 mg (2.93 mmol) dimethyl-1-diazo-2-oxopropylphosphonate (**4**)[2]. This suspension was stirred for 20 hours at room temperature. After addition of 60 mL $CHCl_3$, the organic layer was extracted with water (10 mL), separated, dried with $MgSO_4$ and concentrated in vacuum. The residue was purified by column chromatography on silica with $CHCl_3$/CH_3OH (v/v, 95/5) as an eluent mixture to afford **5** as a yellow oil (435 mg, 51%).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.99 (s, 1H), 3.44-3.48 (m, 4H), 3.58-3.66 (m, 16H), 3.77 (s, 3H), 6.90 (d, 1H, ^4J = 1.70), 6.93 (d, 1H, ^3J = 8.20), 7.01 (dd, 1H, ^3J = 8.20 Hz, ^4J = 1.70 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 52.85, 55.37, 69.94, 70.25, 70.34, 70.85, 75.57, 84.15, 113.94, 115.13, 119.06, 125.21, 140.93, 151.32; MS (EI) m/z (%): 349(100) $[M]^+$; IR (ATR, cm^{-1}): 1110 (s), 1253 (s), 1507 (s), 2102 (m), 2855 (s), 2923 (s); elemental analysis (%) calcd. for $C_{19}H_{27}NO_5$ (349.42): C 65.31, H 7.79, N 4.01; found C 65.12, H 7.38, N 3.98.

7-(diethylamino)-3-(4-(3-methoxy-4-(1,4,7,10-tetraoxa-13-azacyclopentadecan-13-yl)phenyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (**1**)

A mixture of 4-ethynyl-2-methoxyphenylaza-15-crown-5 (**5**) (130 mg, 0.37 mmol), 3-azido-7-diethylaminocoumarin (**6**)[3] (96.1 mg, 0.37 mmol), $CuSO_4 \cdot 5H_2O$ (4.6 mg) and sodium ascorbate (7.3 mg) in 6 ml THF/H_2O (v/v, 2/1) was stirred at 60 °C for 48 hours. After that 5 mL H_2O were added to the mixture and extracted with $CHCl_3$ (30 mL). The organic layer was dried with $MgSO_4$ and concentrated in vacuum. The residue was purified by column chromatography on silica using $CHCl_3$/CH_3OH (v/v, 95/5) as an eluent mixture to afford **1** as a dark yellow oil, which crystallized upon standing in a freezer (90 mg, 40%).

[1] H. He, M. A. Mortellaro, M. J. P. Leiner, S. T. Young, R. J. Fraatz, J. K. Tusa, *Anal. Chem.* **2003**, *75*, 549-555.
[2] J. Pietruszka, A. Witt, *Synthesis* **2006**, *24*, 4266-4268.
[3] K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill, Q. Wang, *Org. Lett.* **2004**, *6*(24), 4603-4606.



^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.24 (t, 6H, ^{3}J = 7.09 Hz, 1-H), 3.45 (q, 4H, ^{3}J = 7.09 Hz, 2-H), 3.51-3.55 (m, 4H, 21-H), 3.64-3.70 (m, 16H, 22-H, 23-H, 24-H, 25-H), 3.93 (s, 3H, 20-H), 6.56 (d, 1H, ^{4}J = 2.21 Hz, 3-H), 6.68 (dd, 1H, ^{3}J = 8.83 Hz, ^{4}J = 2.36 Hz, 5-H), 7.17 (d, 1H, ^{3}J = 7.09 Hz, 16-H), 7.38 (d, 1H, ^{3}J = 7.88 Hz, 15-H), 7.43 (d, 1H, ^{3}J = 8.99 Hz, 6-H), 7.46 (s, 1H, 19-H), 8.44 (s, 1H, 9-H), 8.74 ppm (s, 1H, 12-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.39 (C1), 44.96 (C2), 53.05 (C21), 55.63 (C20), 70.08, 70.37, 70.49, 70.90 (C22, C23, C24, C25), 97.00 (C3), 107.13 (C7), 109.26 (C19), 110.05 (C5), 116.98 (C10), 118.38 (C15), 119.63 (C12), 120.52 (C16), 124.07 (C18), 129.96 (C6), 134.34 (C9), 139.92 (C13), 147.68 (C14), 151.50 (C4), 152.66 (C17), 155.74 (C8), 156.95 ppm (C11); ESI-MS: m/z calcd. for $[M+H]^+$ 608.31; found 608.38; IR (KBr, cm^{-1}): 1130 (s), 1239 (s), 1602 (s),1728 (s), 2855 (s), 2923 (s); UV/Vis (H_2O/DMSO, v/v, 99:1): λ_{max} (ε) = 422 nm (26900); elemental analysis (%) calcd. for $C_{32}H_{41}N_5O_7$ (607.70): C 63.25, H 6.80, N 11.52; found C 62.97, H 6.73, N 11.02.

4-azido-2-methoxyphenylaza-15-crown-5 (**8**)

The 4-amino-2-methoxyphenylaza-15-crown-5 (**7**)[4] (807 mg, 2.37 mmol) was dissolved in 18 ml 4M HCl and cooled to 0°C. After that a solution of 163.6 mg (2.37 mmol) $NaNO_2$ in 10 ml H_2O was slowly added and stirred for 10 minutes at 0°C. Afterwards a solution of 230 mg (3.53 mmol) NaN_3 in 10 ml H_2O was also added. The reaction mixture was stirred for 10 min at 0°C and overnight at room temperature. After that the pH value was adjusted to ~7 with K_2CO_3. The aqueous solution was extracted with $CHCl_3$ (100 mL) and the organic layer was dried with $MgSO_4$ and concentrated in vacuum. The resulting residue was purified by column

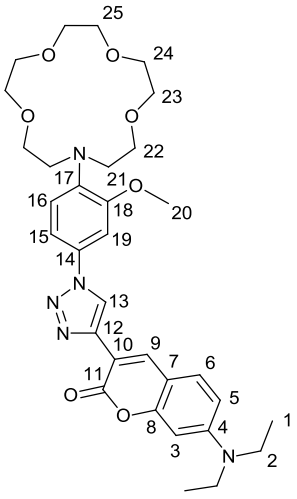
[4] T. Gunnlaugsson, H. Q. N. Gunaratne, M. Nieuwenhuyzen, J. P. Leonard, *J. Chem. Soc., Perkin Trans. 1* **2002**, 1954-1962.

chromatography on silica using $CHCl_3/CH_3OH$ (v/v, 95/5) as an eluent mixture to afford 4-azido-2-methoxyphenylaza-15-crown-5 (**8**) as a dark brown oil (265 mg, 31%).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.39-3.4 (m, 4H), 3.60-3.68 (m, 16H), 3.79 (s, 3H), 6.45 (d, 1H, ^4J = 2.45 Hz), 6.58 (dd, 1H, ^3J = 8.48 Hz, ^4J = 2.45 Hz), 7.10 ppm (d, 1H, ^3J = 8.48 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 53.13, 55.47, 70.08, 70.37, 70.48, 70.93, 103.25, 110.61, 122.16, 133.87, 137.25, 153.89 ppm; MS (EI) m/z (%): 366 (12) $[M]^+$, 338 (100)$[M-N_2]^+$; IR (ATR, cm^{-1}): 1105 (s), 1234 (s), 1504 (s), 2102 (s), 2858 (s).

7-(diethylamino)-3-(1-(3-methoxy-4-(1,4,7,10-tetraoxa-13-azacyclopentadecan-13-yl)phenyl)-1*H*-1,2,3-triazol-4-yl)-2*H*-chromen-2-one (**2**)

A mixture of 4-azido-2-methoxyphenylaza-15-crown-5 (**8**) (136 mg, 0.37 mmol), 3-ethynyl-7-diethylaminocoumarin (**9**)[5] (89.6 mg, 0.37 mmol), $CuSO_4 \cdot 5H_2O$ (8.3 mg) and sodium ascorbate (2.2 mg) in 6 ml THF/H_2O (v/v, 2/1) was stirred at 60 °C for 48 hours. **2** was purified according to the procedure of **1** described above. Thus, **2** was afforded as a dark yellow oil, which crystallized upon standing in a freezer (153 mg, 68%).



^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.22 (t, 6H, ^3J = 7.06 Hz, 1-H), 3.43 (q, 4H, ^3J = 7.06 Hz, 2-H), 3.51-3.55 (m, 4H, 21-H), 3.65-3.69 (m, 16H, 22-H, 23-H, 24-H, 25-H), 3.91 (s, 3H, 20-H), 6.54 (d, 1H, ^4J = 1.98 Hz, 3-H), 6.63 (dd, 1H, ^3J = 8.76 Hz, ^4J = 2.31 Hz, 5-H), 7.13-7.23 (m, 2H, 15-H, 16-H), 7.32 (s, 1H, 19-H), 7.41 (d, 1H, ^3J = 8.85 Hz, 6-H), 8.65 (s, 1H, 9-H), 8.66 ppm (s, 1H, 13-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.42 (C1), 44.82 (C2), 53.03 (C21), 55.78 (C20), 70.03, 70.42, 70.94, (C22, C23, C24, C25), 97.03 (C3), 104.73 (C19), 108.67 (C7), 109.32 (C5), 110.59 (C14), 112.45 (C16), 120.40 (C15), 122.05 (C13), 129.49

[5] D.-N. Lee, G.-J. Kim, H.-J. Kim, *Tetrahedron Lett.* **2009**, *50*, 4766-4768.

(C6), 131.15 (C10), 138.49 (C9), 140.25 (C12), 142.14 (C18), 150.78 (C4), 152.83 (C17), 156.00 (C8), 160.62 ppm (C11); ESI-MS: m/z calcd. for $[M+H]^+$ 608.31; found 608.35; IR (KBr, cm^{-1}): 1130 (s), 1230 (s), 1600 (s), 1694 (s), 1720 (s), 2861 (s), 2969 (s); UV/Vis (H_2O/DMSO, v/v, 99:1): λ_{max} (ε) = 419 nm (24900); elemental analysis (%) calcd. for $C_{32}H_{41}N_5O_7$ (607.70): C 63.25, H 6.80, N 11.52; found C 62.91, H 6.69, N 11.12.

3. UV/Vis absorption measurements in solution

UV/Vis absorption measurements were recorded on a Perkin Elmer Lambda 950 spectrophotometer using 1 cm path length quartz cuvettes. Absorption spectra of **1** and **2** ($c_{1,2}$ = 10^{-5} M) were measured in a buffered H_2O/DMSO mixture (99/1, v/v; 10 mM Tris; pH = 7.2). **1** and **2** were exposed to a saline solution containing 180 mM NaCl. The salt solution (180 mM NaCl) was mixed with a 1 mM DMSO solution of **1** or **2** (v/v, 99/1) to give a final ligand concentration of 10 μM.

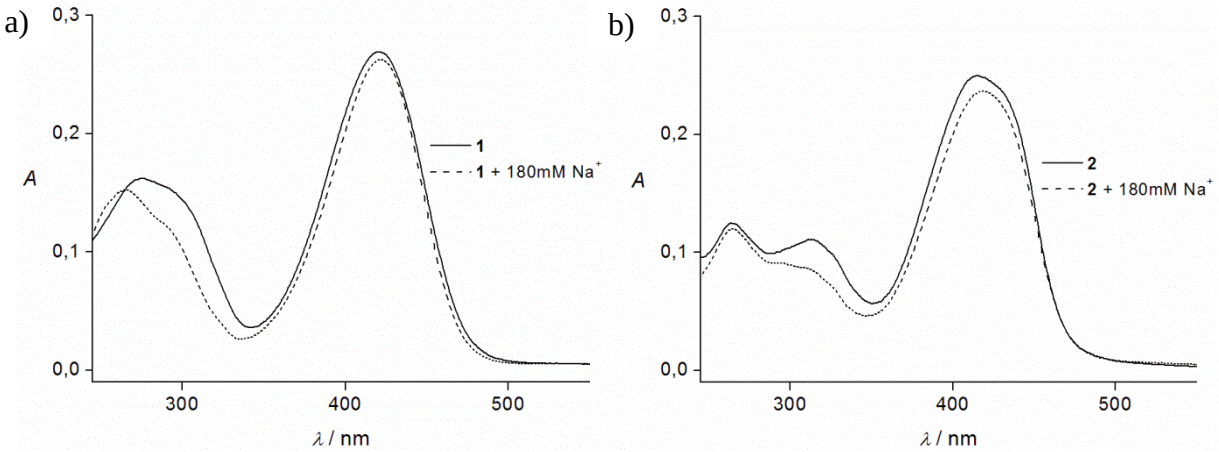


Figure 1 UV/Vis absorption spectra of a) **1** and b) **2** [$c_{1,2}$ = 10^{-5} M, Tris buffer 10 mM, pH = 7.2] in the presence of 0 (—) and 180 mM NaCl (⁻⁻).

4. Fluorescence measurements in solutions

Stationary fluorescence measurements were carried out with a Fluoromax3 spectrometer (Horiba Jobin Yvon) using sealed quartz cuvettes. Fluorescence quantum yields were determined using a PL quantum yield measurement system C9920-2 (Hamamatsu, Japan). The pH value of the Tris buffer solutions was adjusted to 7.2 with 0.1 M HCl. The water used was purified by a Milli-Q Deioniser (Millipore®). Fluorescence spectra of **1** and **2** (c = 10^{-5} M) were measured in a buffered H_2O/DMSO mixture (99/1, v/v; 10 mM Tris; pH = 7.2). **1** and **2** were exposed to different saline solutions containing 10, 20, 30, 40, 60, 80, 100, 120,

140, 160, 180 mM NaCl or KCl, respectively. Each salt solution was mixed with a 1 mM DMSO solution of **1** or **2** (v/v, 99/1) to give a final ligand concentration of 10 μM. Fluorescence response of **1** was recorded exciting at 422 nm and of **2** at 430 nm. Each measurement was repeated with $n = 3$.

The physiological solutions were adjusted to the typical ionic strength of 180 mM by combined NaCl and KCl concentration equal to 180 mM ($[Na^+]+[K^+] = 180$ mM).

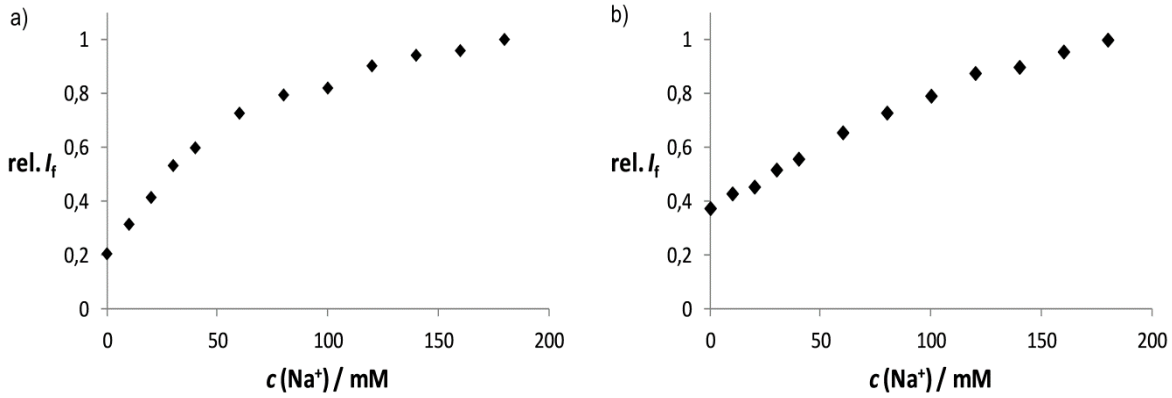


Figure 2 Changes of fluorescence intensities of a) **1** at 500 nm and b) **2** at 493 nm at different $[Na^+]$.

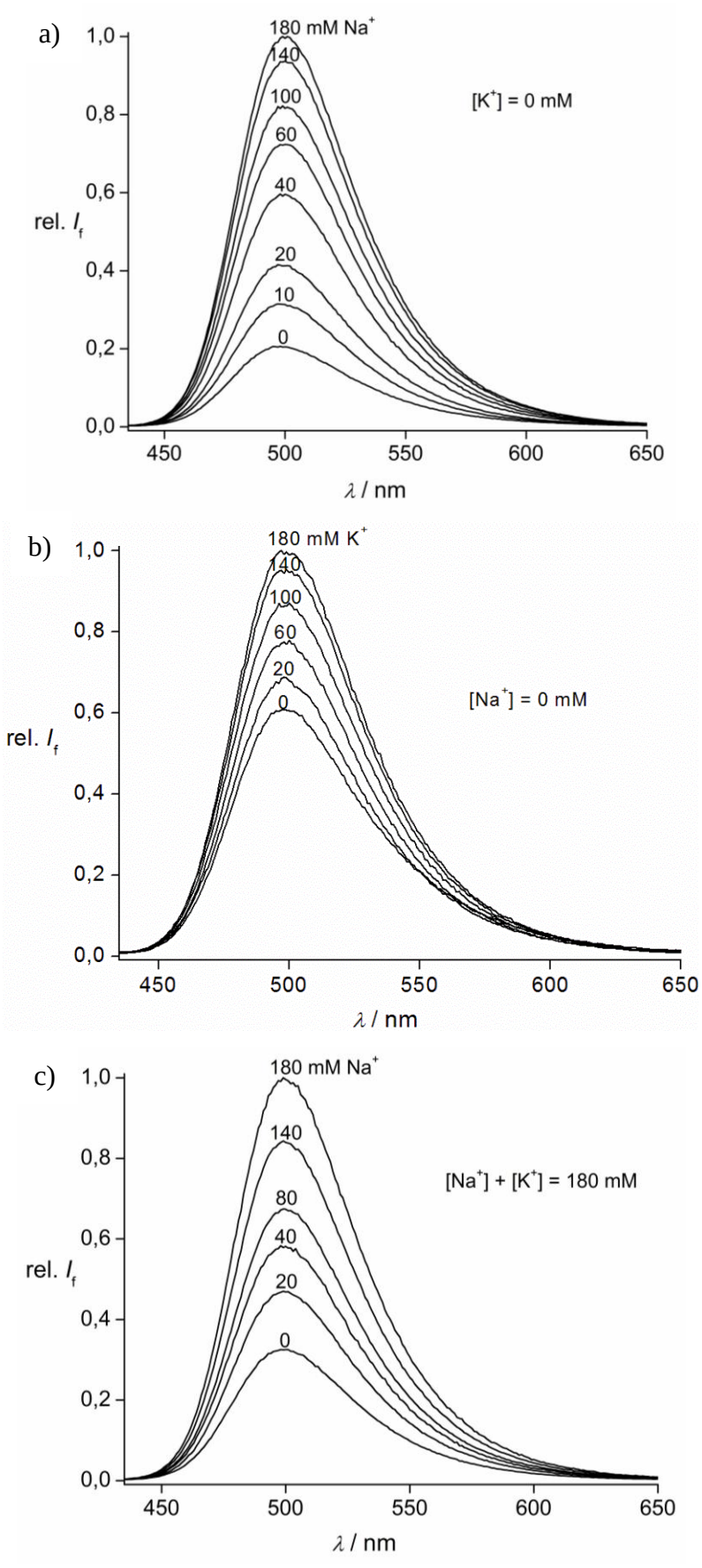


Figure 3 Fluorescence emission spectra of **1** [c(**1**) = 10^{-5} M, λ_{ex} = 422 nm, Tris buffer 10 mM, pH = 7.2] to Na^+ and K^+ a) in K^+-free solution, b) in Na^+-free solutions and c) in solutions containing K^+ with the combined Na^+ and K^+ concentration equal to 180 mM.

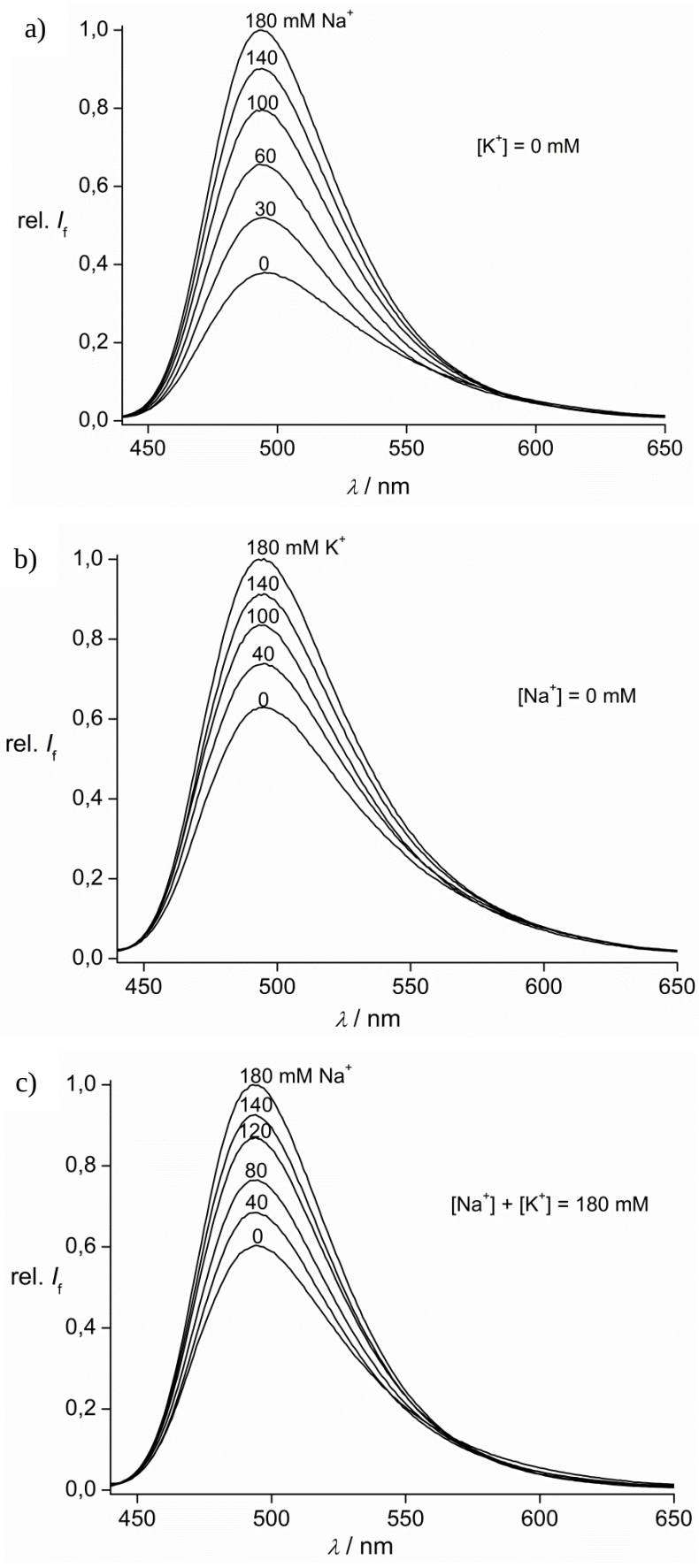


Figure 4 Fluorescence emission spectra of **2** [c(**2**) = 10^{-5} M, λ_{ex} = 430 nm, Tris buffer 10 mM, pH = 7.2] to Na^+ and K^+ a) in K^+-free solution, b) in Na^+-free solutions and c) in solutions containing K^+ with the combined Na^+ and K^+ concentration equal to 180 mM.

Determination of dissociations constants (K_d values)

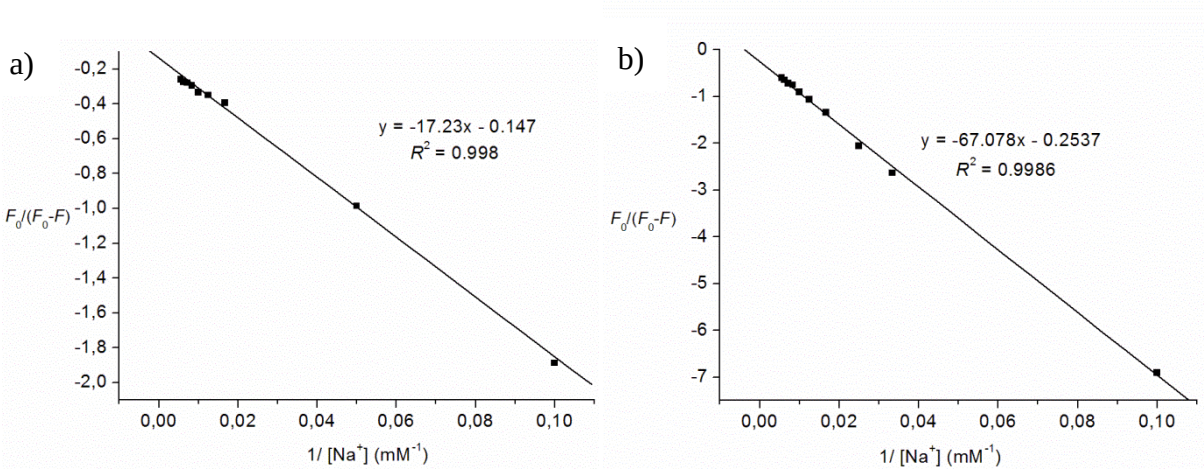


Figure 5 Benesi-Hildebrand plots for a) **1** + Na^+ and b) **2** + Na^+.

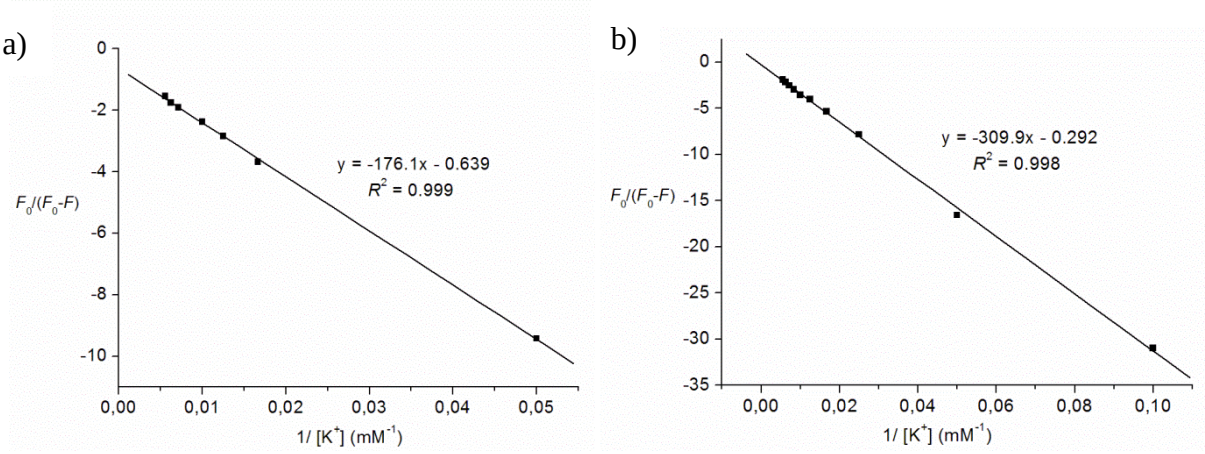


Figure 6 Benesi-Hildebrand plots for a) **1** + K^+ and b) **2** + K^+.

The K_d values were determined by the Benesi-Hildebrand equation (1)[6],

$$F_0/(F_0\text{-}F) = F_0/(F_0\text{-}F_{max}) + F_0/(F_0\text{-}F_{max}) \cdot K_d \cdot 1/[M] \qquad (1)$$

Where F_0 is the integrated fluorescence intensity of the free fluoroionophore, F is the observed integrated fluorescence intensity, F_{max} is the integrated fluorescence intensity at saturating [Na^+] or [K^+] and [M] is the ion concentration. When $F_0/(F_0\text{-}F)$ is plotted against 1/[M], the binding constant is given by the intercept/slope ratio. From curve fitting of the fluorescence intensity of **1** and **2** against the reciprocal of the Na^+ or K^+ concentration 1/[M], this Benesi-Hildebrandt plot yielded a linear fit (Figure 5a and 5b for Na^+, Figure 6a and 6b for K^+), from which the K_d values were estimated to be for **1** + Na^+, K_d = 117.2 mM and for **2** + Na^+, K_d = 264.4 mM; **1** + K^+, K_d = 275.6 mM and for **2** + K^+, K_d = 1061.3 mM. The linear fit suggested that a 1:1 complex is formed.

[6] H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, *71*, 2703-2707.

The dissociation constants K_d for Na^+ (mol/l) were also calculated from the fluorescence spectra using plots of $\log[(I_F-I_{Fmin})/(I_{Fmax}-I_F)]$ against $\log[Na^+]$, following a previously described method[7] (I_F: fluorescence intensity, I_{Fmax}: maximum fluorescence intensity at saturating $[Na^+]$, I_{Fmin}: minimum fluorescence intensity at zero $[Na^+]$).

To obtained the maximum fluorescence intensity I_{Fmax} at saturating $[Na^+]$ we prepared a saline solution containing 1000 mM NaCl.

This double log plot gives an x-intercept that is the logarithm of the K_d (in mol/l). For **1** the x-intercept is -0.924 (Figure 7a) and for **2** the x-intercept is -0.592 (Figure 7b). The inverse logarithm of -0.924 for **1** is 0.1192 mol/l (119.2 mM) and of -0.592 for **2** is 0.2561 mol/l (256.1 mM). The slopes of the plots are ~ 1, reflecting the 1:1 binding of **1** or **2** with Na^+.

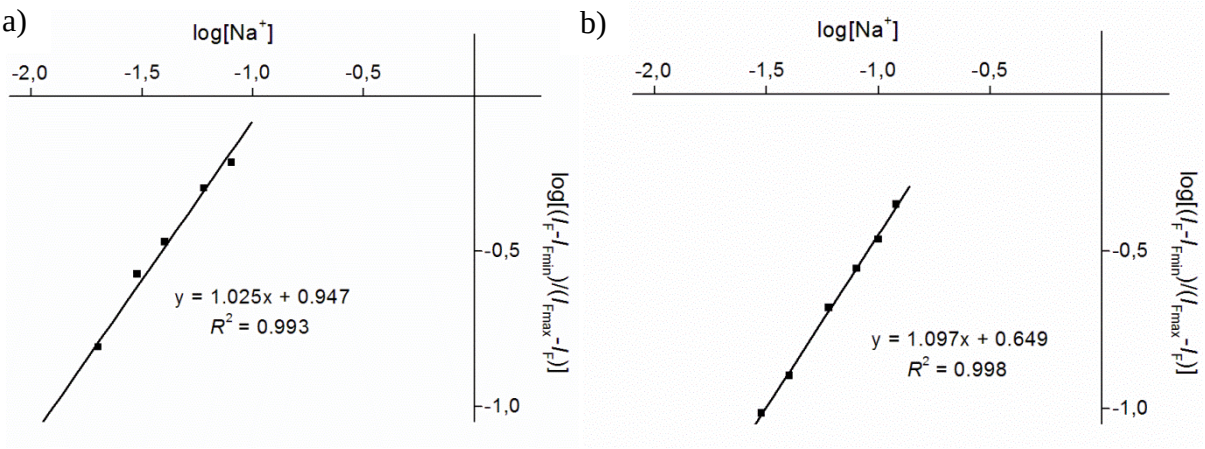


Figure 7 Plot of $\log[(I_F-I_{Fmin})/(I_{Fmax}-I_F)]$ of a) **1** and b) **2** against $\log[Na^+]$ for determination of K_d.

pH sensitivity of **1** and **2**

Aqueous solutions ranging from pH 1 to 6 were prepared by diluting 1 M HCl with deionized water (from pH 8 to 11 with ~1.5 M $[n\text{Bu}_4\text{N}^+][OH^-]$, pH 7.2 with Tris buffer). Solutions of fluoroionophores **1** and **2** at pH 1–11 were prepared by adding 20 µL ($c = 10^{-3}$ M) DMSO solutions to aqueous solutions (1980 µL) of either HCl (at pH 1–6), $[n\text{Bu}_4\text{N}^+][OH^-]$ (at pH 8–11) or Tris buffer (pH 7).

[7] G. Grynkiewicz, M. Poenie, R. Y. Tsien, *J. Biol. Chem.* **1985**, *260*, 3440-3450.

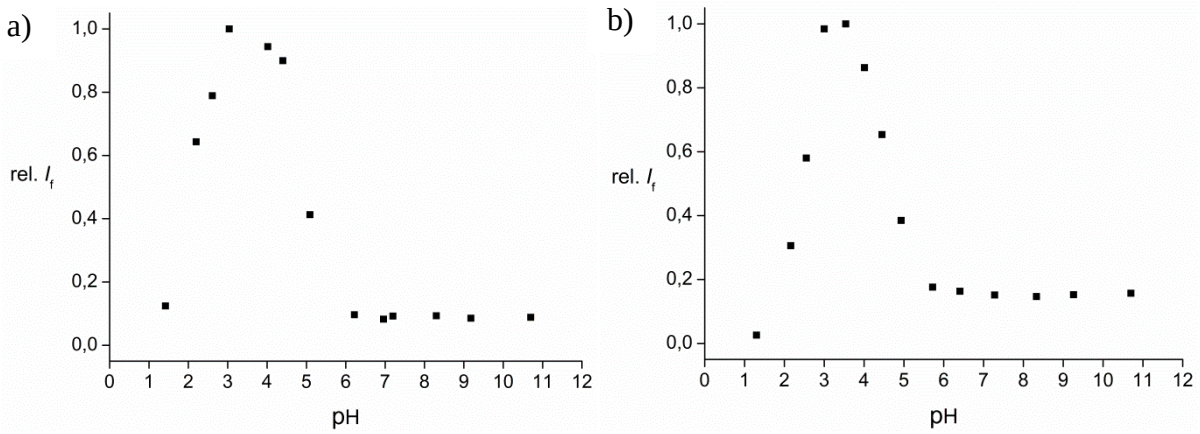


Figure 8 Fluorescence intensity of of a) **1** (c = 10^{-5} M) at 500 nm (λ_{ex} = 422 nm) and b) **2** (c = 10^{-5} M) at 493 nm (λ_{ex} = 430 nm) as function of pH.

The pK_a values of **1** and **2** were determined by the equation (2)[8]

$$\log[(I_{Fmax}-I_F)/(I_F-I_{Fmin})] = \text{pH-p}K_a \qquad (2),$$

where I_F is the fluorescence intensity, I_{Fmax} is the maximum fluorescence intensity (protonated form), I_{Fmin}: minimum fluorescence intensity (unprotonated form). When $\log[(I_{Fmax}-I_F)/(I_F-I_{Fmin})]$ is plotted against the pH value the p$K_\mathbf{a}$ value is given by the intercept with the x-axis. From curve fitting of the fluorescence intensities of **1** and **2** against the pH values, the plot using equation (2) yielded a linear fit (for **1**, y = 1.457x-7.167, R^2 = 0.993; and for **2**, y = 1.264x-5.795, R^2=0.999), from which the p$K_\mathbf{a}$ values were estimated. The resulting pK_a of **1** is 4.9 (Figure S8a) and that of **2** is 4.6, respectively (Figure S8b) meaning that **2** is less pH sensitive than **1**. We assume that the electron-withdrawing character of the 1,2,3-triazole unit in **1** and **2** makes it more difficult to protonate the anilino nitrogen. Moreover, protonation of the anilino nitrogen inhibits the PET quenching process in **1** and **2** which results in an increased fluorescence intensity (Figure S8). The fluorescence signal of **1** and **2** are unaffected in the pH range of 6–8 (Figure S8a and S8b).

Quantum yields and fluorescence quenching of **1** and **2**

The fluorescence quantum yield of **1** in Tris buffer (ϕ_f(**1**) = 0.009, Table 1) is lower than for **2** (ϕ_f(**2**) = 0.051, Table 1). Probably, a reductive PET occurs from the anilino-triazole unit to the

[8] R. A. Bissell, E. Calle, A. P. de Silva, S. A. de Silva, H. Q. N. Gunaratne, J.-L. Habib-Jiwan, S. L. A. Peiris, R. A. D. D. Rupasinghe, T. K. Shantha, D. Samarasinghe, K. R. A. Samankumara Sandanayake, J.-P. Soumillion, *J. Chem. Soc. Perkin Trans. 2* **1992**, 1559-1564.

coumarin moiety [9], but we found an increased oxidation potential of the anilino-triazole unit in **2** ($E_{ox}(2)$ = 0.79 V, $E_{ox}(1)$= 0.68 V).[9] This might be caused by the fact that the anilino unit in **2** is more twisted against the triazole ring as in **1**. Thus, the more planar anilino-triazole unit in **1** is a better PET donor and an efficient fluorescence quenching is realized in **1**. As found in 1,2,3-triazol-1,4-diyl-coumarin fluoroionophores for K^+ the twisting at the coumarin-triazole link caused by the steric hindrance of the carbonyl group results in a virtual spacer. We assume that **1** and **2** are also PET-fluoroionophores with a virtual spacer between the anilino-triazole electron donor and the coumarin electron acceptor moiety. Hence, as a result the fluorescence is switched off.

Fluorescence decay time measurements of **1** and **2** in solution

The fluorescence decay times of **1** and **2** in buffered solution were determined with time-correlating single photon counting (TCSPC) method. The analysis of the resulting decay curves was based on a biexponential rate law [10]: $\boldsymbol{I(t) = I_0 + \sum_i \alpha_i e^{-t/\tau_i}}$. The calculated fraction f_i represents the percentage of the respective lifetime τ.[10] An averaged fluorescence decay time can be calculated by the fraction-weighted addition of both fluorescence decay times according the following equation: $\boldsymbol{\tau_{av} = \sum_i f_i \tau_i}$.

Table 1. Photophysical properties of **1** and **2** in Tris-buffer.[a]

Compound	λ_{abs} [nm]	λ_{fl} [nm]	Φ_f[b]	τ_f[c] (f [%]) [ns]	FEF[d]
1	422	500	0.009	0.084 (90), 0.677 (10)	-
1 + Na^+	422	500	0.048	0.08 (1), 0.385 (99)	5.0
1 + K^+	422	500	0.017	0.140 (87), 0.494 (13)	1.5
2	419	493	0.051	0.57 (89), 3.32 (11)	-
2 + Na^+	419	493	0.110	1.02 (98), 3.12 (2)	2.5
2 + K^+	419	493	0.076	0.80 (91), 2.50 (9)	1.5

[a] All data were measured in a buffered H_2O/DMSO mixture (99/1, v/v; 10 mM Tris; pH = 7.2) in the absence and presence of Na^+- or K^+ ions (180 mM). [b] Fluorescence quantum yield, (± 15)%. [c] Fluorescence lifetime, (± 15)%. [d] Fluorescence enhancement factor, [$FEF = (F-F_{min})/F_{min}$], (± 0.1).

[9] For **1** and **2** a reductive PET process from the anilino-triazole unit to the excited coumarin has different ΔG_{PET} values according to the Rehm-Weller equation. The oxidation potential E_{ox} of the anilino-triazol unit in **1** is 0.68 V and of **2** 0.79 V. The reduction potential E_{red} of **1** is –1.61 V and of **2** –1.82 V (Fc/Fc^+ = 0.09 V in CH_3CN). In CH_3CN the $E_{00}^{h\cdot\nu}$ of **1** is observed at 448 nm (≙2.77 eV) and of **2** at 440 nm (≙2.82 eV). Finally: ΔG_{PET} (**1**) = -0.48 eV and ΔG_{PET} (**2**) = -0.21 eV.

[10] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 2006.

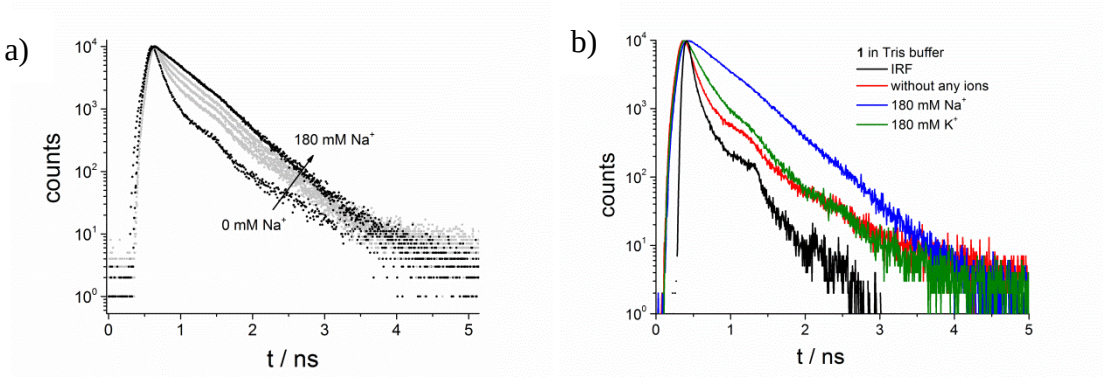


Figure 9 Fluorescence decay curves of **1** in Tris buffer a) in the presence of increasing Na^+ concentrations and b) in the presence of 180 mM Na^+ and 180 mM K^+.

5 Sensing

The steady-state fluorescence measurements were performed on a Fluoromax3-P spectrometer (Horiba Jobin Yvon). A FLS920 Spectrofluorometer (Edingburgh Instruments) was used in the time-resolved fluorescence measurements and operated in the time-correlated single photon counting (TCSPC) mode. For excitation (λ_{ex} = 420 nm; 20 MHz,) a pulsed light emitting SuperContinuum Source SC400 pp-2 (Fianium) was used.

The fiber-optical measurements were performed with a frequency domain phase modulation spectroscopy technique. A laser diode (*NICHIA*, λ_{ex} = 440 nm) was modulated with a frequency of 30 MHz by a function generator *Tektronix* AFG 3102 coupled to a laser driver system from *ELOVIS* Dynalase-C System (in Figure 9 Point C). The emissions of the dyes were detected with a photomultiplier (*Hamamatsu* H6780-20, Point D). The signal was transferred to a Lock-In Amplifier for the determination of the phase shift (*Stanford Research System*, Model SR844). The steady-state measurements were done with a fiber-optical spectrometer from *Stellar Net Inc* BLACK-Comet UV-VIS Spectrometer (Point E).

By using a one to three fiber (Point F in the picture below) it was possible to perform simultaneously the frequency domain phase modulation spectroscopy technique and the measurement of the whole spectra. In one of the three arms of the triple-fiber the excitation light was coupled into (Point C), the other two were used to focus the emission signal to the two different detectors (D and E). To the end with only one fiber the optical fiber with the optode at the surface was coupled by simple SMA to SMA-connector (above Point A).

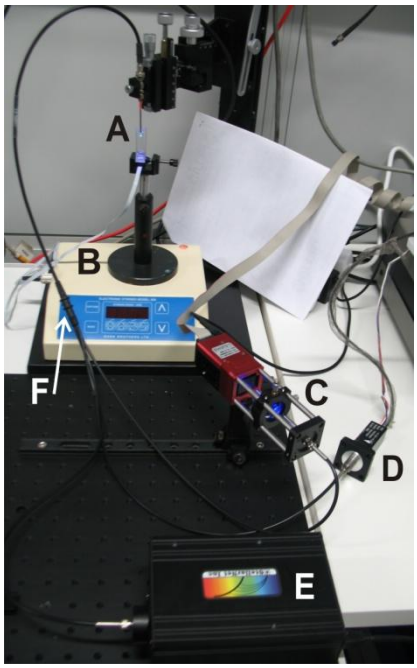


Figure 10 Experimental set-up for fiber-optical measurements.

During the calibration and all measurements the sample solution (10 mM TRIS puffer with different Na^+ concentrations) were stirred (Point B).

Optode preparation

500 mg of the polymer Polyhydroxypropylmethacrylat (PHPMA, purchased from Sigma-Aldrich) were added to 2.5 mL ethanol and the mixture was shaked for 3 hours, within this time a highly viscous solution is generated. Into 0.5 mL of the polymer/EtOH mixture 25 µL of a $7.5 \cdot 10^{-4}$ mol $\cdot L^{-1}$ dye solution in ethanol was added and shaked for 3 hours.

One end of an optical fiber (1 mm in diameter) were polished with fiber-optical polishing sheets (purchased from Thorlabs) and cleaned finally with iso-propanol. This tip was dipped into the prepared polymer/ethanol/dye-solution and then dried over night in an oven at 75°C. Figure 10 illustrates the resulting fluorescence microscopic picture.

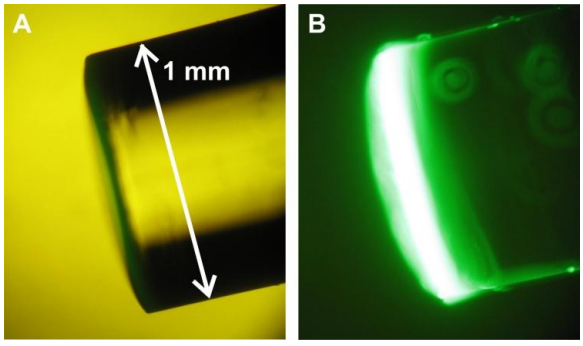


Figure 11 A: Transmission microscopy picture of the dipped optical fiber and B: Fluorescence microscopic picture with an excitation-filter transmitted light from 445 nm to 450 nm and an emission filter transmitted light between 505 nm to 550 nm.

Characterizations of 1 embedded in PHPMA

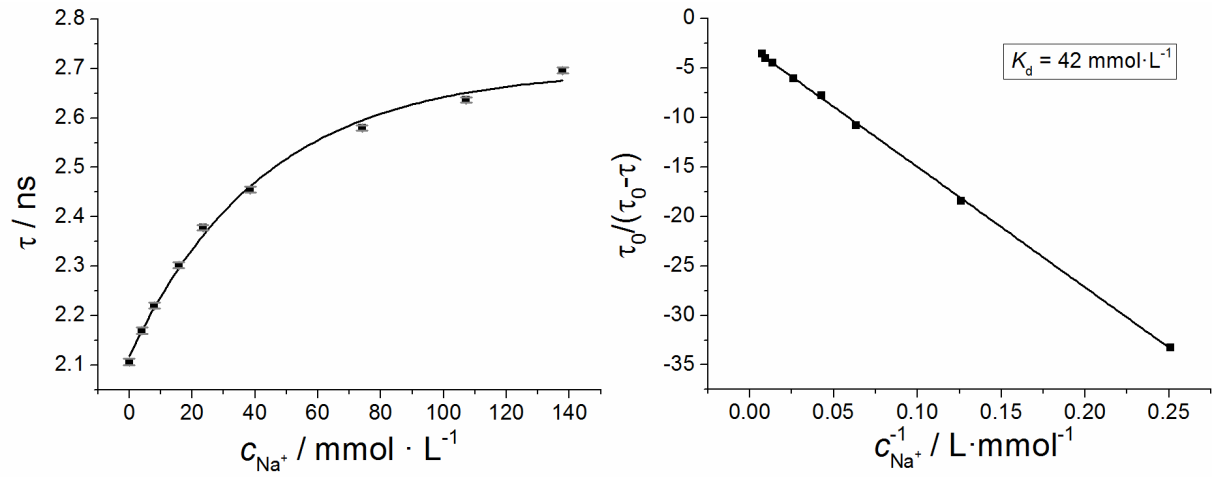


Figure 12 TCSPC measurement of **1** immobilized in PHPMA, thin films on slides, prepared for use in standard cuvette.

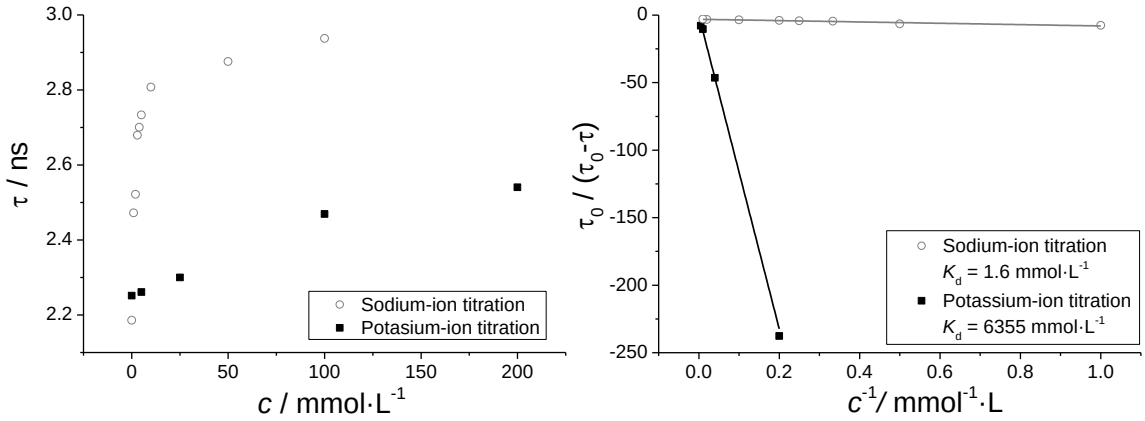


Figure 13 Same measurements like in Fig. 11, but with a higher concentration of dye **1.** We had to double the dye concentration for the fiber-optical determinations to get an observable and stable fluorescence signal.

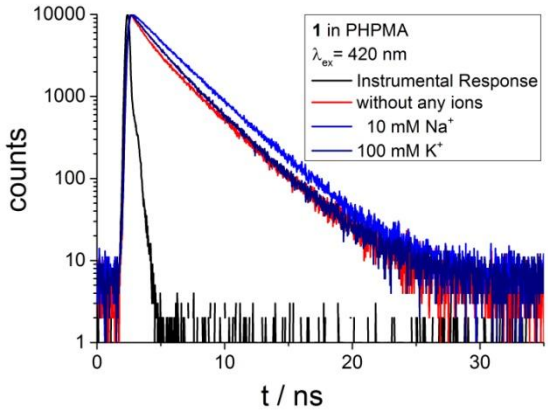


Figure 14 Fluorescence decay curves of **1** in PHPMA in the presence of Na^+ or K^+.

Characterisation of Optodes with fiber-optical measurements

a) Steady-state measurements

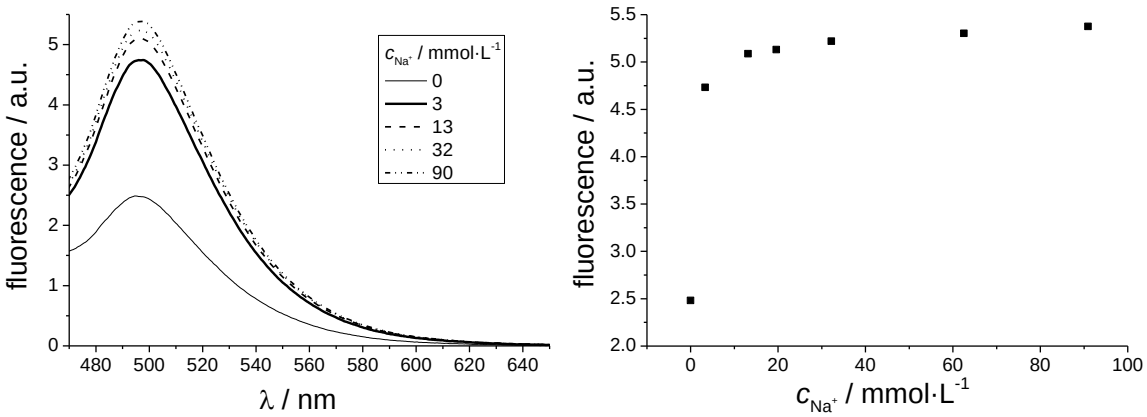


Figure 15 Emission spectra (λ_{ex} = 450 nm) of a fiber-optical sensor at different sodium ions concentrations.

b) Frequency-Domain Spectroscopy (FD-S)

In figure 14 the determined decay times of an optode prepared like describe above are shown. The decay times ranges from 2.6 ns in sodium free buffered solution to 3.1 ns at a Na^+ concentration of 30 mM and higher.

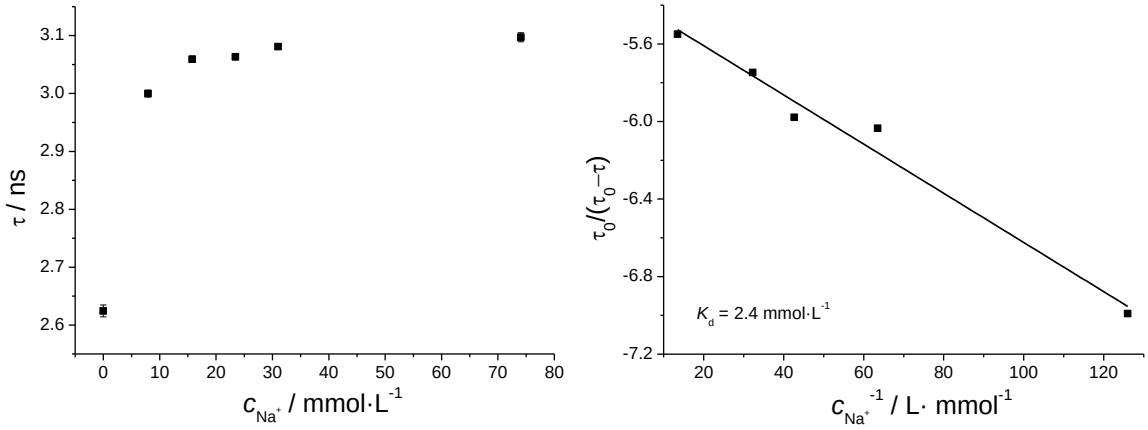


Figure 16 Calibration of an optode with fiber-optical frequency-domain spectroscopy (FD-S). The used solution contains 10 mM Tris buffer.

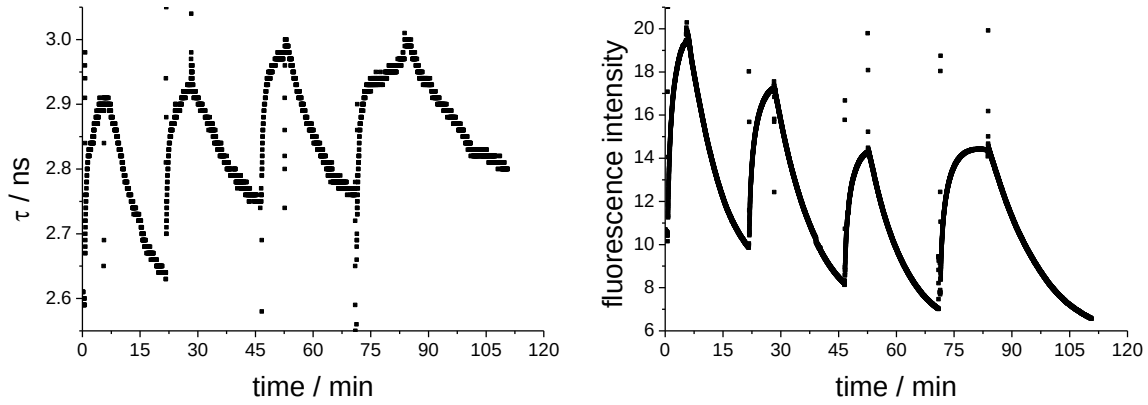


Figure 17 Response time and signal stability of an optode by periodically switching the concentration of sodium from 0 to 10 mM.