CHEMISTRY
A European Journal

A Journal of
ChemPubSoc Europe

Accepted Article

Title: A Ratiometric Fluorescent Probe for K+ in Water based on a Phenylaza-18-Crown-6 Lariat Ether

Authors: Hans-Jürgen Holdt and Thomas Schwarze

To be cited as: *Chem. Eur. J.* 10.1002/chem.201802306

Link to VoR: http://dx.doi.org/10.1002/chem.201802306

Supported by
ACES

WILEY-VCH

10.1002/chem.201802306

WILEY-VCH

FULL PAPER

A Ratiometric Fluorescent Probe for K^+ in Water based on a Phenylaza-18-Crown-6 Lariat Ether

Thomas Schwarze,*[a] Janine Riemer[a] and Hans-Jürgen Holdt*[a]

Abstract: Herein, we present two molecular fluorescent probes **1** and **2** (Scheme 1) for the selective determination of physiologically relevant K^+ levels in water based on a highly K^+/Na^+ building block the *o*-(2-methoxyethoxy)phenylaza-18-crown-6 lariat ether unit. Fluorescent probe **1** showed a high K^+-induced fluorescence enhancement (FE) by a factor of 7.7 of the anthracenic emission and a dissociation constant (K_d) value of 38 mM in water. Further, for **2** + K^+, we observed a dual emission behavior at 405 nm and 505 nm. K^+ increases the fluorescence intensity of **2** at 405 nm by a factor of ~4.6 and K^+ decreases the fluorescence intensity at 505 nm by a factor of ~4.8. Fluorescent probe **2** + K^+ exhibited a K_d value of ~8 mM in Na^+ free solutions and in combined K^+/Na^+ solution a similar K_d value of ~9 mM, reflecting the high K^+/Na^+ selectivity of **2** in water. Therefore, **2** is a promising fluorescent tool to measure ratiometrically and selectively physiologically relevant K^+ levels.

Introduction

Fluorescent probes for biological important cations, such as K^+, are powerful tools to visualize and monitor cations in vitro and in vivo. Therefore, fluorescence spectroscopy is a very popular and sensitive method to track K^+ concentrations in vivo. Thereby, the non-fluorescent K^+ is detected by fluorescent probes, so called fluoroionophores, consisting of a signaling element (fluorophore) and an ion-specific receptor (ionophore). Particularly, the K^+/Na^+ selectivity of the ionophore is of fundamental importance to produce precise and true data. In animal cells, often Na^+ is the antagonist to K^+ and both show contrary concentrations in the extra- ($[K^+]$ = ~4 mM, $[Na^+]$ = ~140 mM) or intracellular ($[K^+]$ = ~120 mM, $[Na^+]$ = ~10 mM) medium. Further, the design of the fluoroionophore architecture affects the fluorescence signal. Fluoroionophores, which are electronically decoupled by a spacer between the electron donor and acceptor unit often use a K^+-induced off switching of a photoinduced electron transfer (PET), which leads normally to intensity enhancements at a single emission wavelength.[1a-d] Most of the developed fluorescent probes for K^+ analysis in vitro and in vivo based on fluorescence intensity changes at a single emission wavelength.[2a-g] However, this K^+ determination method requires a costly calibration and is very sensitive to cellular dye concentration changes. Further, fluoroionophores without a spacer in a donor π-conjugated acceptor arrangement show cation induced spectral shifts of their wavelengths and small intensity changes, caused by the modulation of the intramolecular charge transfer (ICT). A typical representative for an ICT-type fluoroionophore is the ratiometric fluorescent probe fura-2 introduced by Tsien et al..[3] Fura-2 is the most powerful tool to measure physiologically relevant Ca^{2+} levels in vivo. The determination of intracellular Ca^{2+} takes place by the Ca^{2+}-induced change of two wavelengths in the excitation spectrum of fura-2. This method allows a fast and a more precise Ca^{2+} determination in vivo than by the use of single wavelength fluorescent dyes. A benefit of ratiometric metal ion detection in vivo is that this procedure reduces or eliminates distortions caused by photobleaching effects, indicator concentration changes or illumination stabilities.

So far, for the detection of cellular K^+ levels, Minta and Tsien designed in an ICT-type architecture the first fluorescent probe PBFI (potassium-binding benzofuranisophthalate) with a similar signal readout as fura-2. However, K^+ induces only small changes in the excitation spectra of PBFI at two wavelengths.[4] In addition to it, PBFI lacks an approbate K^+/Na^+ selectivity to track exclusively K^+ levels in vivo. Generally, only a few molecular fluorescent probes exist, which show a K^+-dependent dual emission behavior.[5a-c] As a rare example, Boens et al. reported on a highly K^+ selective ratiometric fluorescent indicator, which was only tested in pure acetonitrile.[5a] Teramae et al. showed a ratiometric fluorescent probe based on a K^+-controlled intramolecular exciplex formation in pure acetone.[5b] Further, Clark et al. developed a ratiometric nanosensor for K^+ based on a Förster resonance energy transfer (FRET) between a quantum dot and a chromoionophore in water.[5c]

However, it is a challenge to develop ratiometric ICT-type fluoroionophores for the monovalent K^+, because K^+ induces only small spectral wavelength shifts in such a π-conjugated donor acceptor arrangement.[6a,b] So far, to the best of our knowledge, a ratiometric and K^+-selective fluorescent probe, which shows a K^+-induced change at two emission wavelengths in water has not been reported. Therefore, an improved design concept for fluoroionophores, which are capable for the ratiometric detection of K^+ ions by a dual emission behavior, is required.

Recently, we developed highly K^+ selective PET fluoroionophores to determine exclusively K^+ by fluorescence intensity enhancements[7a-e], when excited by a one photon[7a-c] or two photon process[7d] and by fluorescence lifetime changes[7e]. In a further report, we explored a K^+ responsive fluorescent probe, which consists of a phenylaza-18-crown-6 ionophore and an anthracene unit as a locally excited (LE) fluorophore, which are connected by a 1,2,3-triazole moiety in a fully π-conjugated

[a] Dr. T. Schwarze, J. Riemer, Prof. Dr. H.-J. Holdt
Institut für Chemie, Anorganische Chemie, Universität Potsdam
Karl-Liebknecht-Str. 24–25, 14476 Golm (Germany)
E-mail: holdt@uni-potsdam.de
E-mail: schwarth@uni-potsdam.de

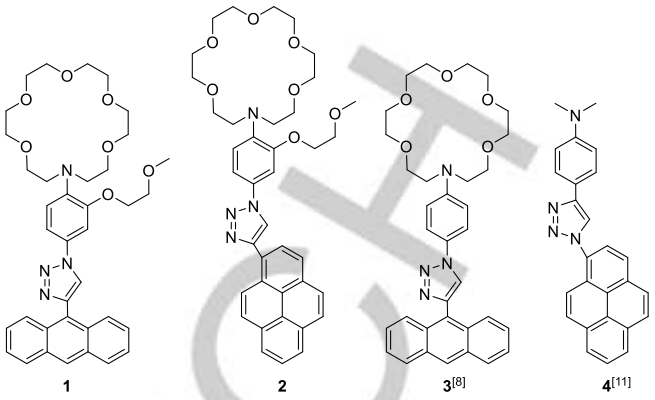
Supporting information for this article is given via a link at the end of the document.

fashion (cf. Scheme 1, fluorescent probe **3**).[8] Further, **3** exhibits a weakly emissive charge transfer (CT) state (S1) at 539 nm and a locally excited (LE) state (S2) at 415 nm in CH_3CN. In the presence of K^+, the CT is decreased and energetically shifted upwards and the LE state becomes the dominating fluorescent state (S1). The fluorescent probe **3** is a light up probe for K^+ with a K^+-induced state reversal in water. Thus, K^+ induces a 12-fold increase of the fluorescence intensity in water, but the K_d value **3** + K^+ (about 300 mM) was too high to measure physiologically relevant K^+ levels. In a further paper, we found that the *o*-(2-methoxyethoxy)phenylaza-18-crown-6 lariat ether moiety is a highly K^+-selective building block for the determination of physiologically relevant K^+ levels.[7b] Nowadays, this K^+ ionophore is part of a set of highly K^+ selective indicators.[9a-c]

Herein, we synthesized fluorescent probes **1** and **2** on the basis of this highly K^+ selective building block the *o*-(2-methoxyethoxy)phenylaza-18-crown-6 lariat ether (ionophore) to measure physiologically relevant K^+ levels. Further, the aniline moieties of fluorescent probes **1** and **2** were connected to position 1 and the fluorophore moiety to position 4 of the triazole unit (cf. Scheme 1), owing to the higher K^+/Na^+ selectivity of this arrangement.[7b] As a fluorophore moiety, we introduced typically LE fluorophores such as anthracene (**1**) or pyrene (**2**) (cf. fluorescent probes **1** and **2** in Scheme 1). As is well known, that these LE fluorophores become a fluorescent CT state in polar solvents, when they were substituted by an electron rich dimethylaniline unit in a fully π-conjugated arrangement.[10] For the pyrene aniline derivative an intense fluorescent CT state with a quantum yield ϕ_f of 0.95 and a fluorescence emission maximum λ_{em} of 532 nm was found in acetonitrile.[10] However, for the corresponding anthracene aniline analogue a lower ϕ_f of 0.47 (λ_{em} = 582 nm) was observed.[10] Further, we found for the 1,2,3-triazol π-linked fluorescent probe **3** a very low fluorescent CT state in acetonitrile and water.[8] However, Bag and Kundu showed for the the 1,2,3-triazol π-linked pyrene derivative **4** (cf. Scheme 1) a dual emission behavior in dioxane/water mixtures.[11a-c] Even a fluorescent CT state in polar solvents is observed, when a dimethylaniline unit is π-linked via a 1,2,3-triazol-1,4-diyl unit with a benzene moiety.[12] Thus, we favored the pyrene substituted fluorescent probe **2** to exhibit a fluorescent CT and LE state for a dual recognition behavior of K^+ in water. Further, for fluorescent probe **1**, we expect a high K^+-induced fluorescence enhancement in water as already observed for fluorescent probe **3**. Overall, the new fluorescent probes **1** and **2** (Scheme 1) should operate in the presence of K^+ in a similar mechanistic way as found for **3** + K^+.

Results and Discussion

The feasible synthesis of the fluorescent probes **1** and **2** were realized by Cu(I)-catalyzed 1,3-dipolar azide alkyne cycloaddition (CuAAC) reactions[13a,b] of the azido functionalized phenylaza-18-crown-6 lariat ether compound[7b] with 9-ethynylanthracene[14] or with the commercial available 1-ethynylpyrene, respectively (cf. Scheme S1).



Scheme 1. Studied fluorescent probes **1**, **2** and reference compounds **3**[8] and **4**[11].

The UV/Vis absorption spectra of **1** and **2** in Tris buffered (10 mM, pH of 7.2) H_2O/DMSO mixtures 99/1 (v/v) show superimpositions of charge transfer (CT) bands and the typical polycyclic aromatic absorption bands (cf. Figures S1a,b). For **1** and **2**, we observed two CT bands about 300 nm and 330 nm and for **2** about 285 nm and 370 nm, respectively. The CT band at 330 nm for **1** and at 370 nm for **2** were mainly superimposed by the typical anthryl or pyrenyl absorption bands. The short-wavelength CT absorptions about 300 nm (**1**) and 285 nm (**2**) correspond to a CT from the nitrogen lone pair of the aromatic ring (donor) to the 1,2,3-triazole acceptor unit, which is also found for π-conjugated anilino-1,2,3-triazol-1,4-diyl – fluoroionophores.[7a,b] Further, the CT absorption band of **1** at 330 nm belongs to a CT from the anilinotriazole donor moiety to the anthryl acceptor unit as also shown for probe **3**.[8] Herein, we observed for **1** compared to **3** (CT at 423 nm) a blue-shifted and less intense CT band about 330 nm (cf. Figure S1a), because **1** possess a weaker electron donor unit as the reference compound **3**, as already discussed for series of crown compounds with a bulky substituent in *ortho* position of the aniline donor moiety.[15] Further, we found for **2** superimposed absorptions about 370 nm, which partially corresponding to a CT from the anilinotriazole donor moiety to the pyrenyl acceptor unit.

The fluorescence spectrum of **1** (λ_{ex} = 365 nm) showed the typical anthracenic emission peaks (cf. Table 1) in Tris buffered (10 mM, pH of 7.2) H_2O/DMSO mixtures of 99/1 (v/v). Further, we observed for **2** in the range from 370 nm to 440 nm the typical pyrene emission from the LE state and a broad emission peaked at 505 nm, which corresponds to a fluorescent CT state, as also observed for **4**[11], but **1** exhibits no intense fluorescent CT state in the range from 450 nm to 650 nm in water. Notably, the fluorescence spectrum of **2** (λ_{ex} = 345 nm) shows compared to **1** a distinctive dual emission in water, which can be underlined by excitation spectra of **2** at a λ_{em} of 390 nm and a λ_{em} of 505 nm, respectively (Figure S3). The excitation spectrum of **2** (λ_{em} = 390 nm) exhibits the typical pyrene fine structure from 300 nm to 370 nm with no superposition of the CT band (cf. Figure S3a) and the excitation spectrum of **2** (λ_{em} of 505 nm) shows only a broad and well-defined CT band in the same region of the spectrum ranging from 300 nm to 400 nm (cf. Figure S3b). Concluding that **2** has a

10.1002/chem.201802306

fluorescent CT state (S1) and a lower fluorescent LE state (S2) in a H_2O/DMSO mixture of 99/1 (v/v). However, the CT emission of **2** at 505 nm becomes more fluorescent, when the volume fraction of H_2O is increased from 1/1 to 99/1 (H_2O/DMSO mixtures, cf. Figure S8). The excitation spectrum of **1** (λ_{em} = 424 nm) showed the typical anthryl bands, respectively (cf. Figure S2), but we found not such a pronounced CT band as observed for **2**, when excitation spectra of **1** were recorded in a λ_{em} range from 450 to 550 nm. Further, we observed for **1** a fluorescence quantum yield ϕ_f of 0.024 and for **2** a higher ϕ_f value of 0.092 (cf. Table 1).

After that, we investigated the influence of K^+ and Na^+ on the fluorescence of **1** and **2** in the physiological relevant concentration range of 1 mM to 160 mM K^+ and Na^+. We observed an extraordinary K^+-induced (*FEF* = 7.7, see Figure 2a and S4a) and a slightly Na^+-induced (*FEF* = 1.7 at 160 mM Na^+, see Figure S5a) fluorescence enhancement of **1**. About 140 mM K^+, we found for **1** + K^+ the maximum signal change with a ϕ_F value of 0.193 (Table 1). The fluorescence signal of **2** + K^+ showed remarkable intensity changes at 405 nm and 505 nm (cf. Figure 1b). K^+ reduces the fluorescene intensity of the CT state at 505 nm by a factor of ~4.8 to a concentration of around 50 mM K^+. However, K^+ enhances the fluorescence intensity (*FEF* = ~4.6, see Figure S7a) of the LE state at 405 nm. Thus, **2** showed a dual fluorescence emission behavior in the presence of K^+. Overall, we observed a reduced ϕ_f of 0.023 for **2** + 160 mM K^+ and a slightly Na^+-influenced ϕ_f with a value of 0.081 (see Table 1).

Table 1. Photophysical data of **1** and **2** in a H_2O/DMSO mixture of 99/1 (v/v) in the absence and presence of 160 mM KCl and 160 mM NaCl.

	λ_{abs} [nm]	λ_{em} [nm]	ϕ_f [a]	K_d^{K+} [mM][b]
1	386, 367, 349, 330, ~300	423, 405	0.024	-
1 + K^+	386, 367, 349, 333, ~300	423 , 405	0.193	38
1 + Na^+	386, 367, 349, 330, ~300	423, 405	0.040	-
2	370, 285	505, 405, 385	0.092	-
2 + K^+	350, 282	495, 405, 385	0.023	8
2 + Na^+	370, 283	505, 405, 385	0.081	-

[a] Fluorescence quantum yield, (±15%). [b] Dissociation constants K_d for K^+ complexes.

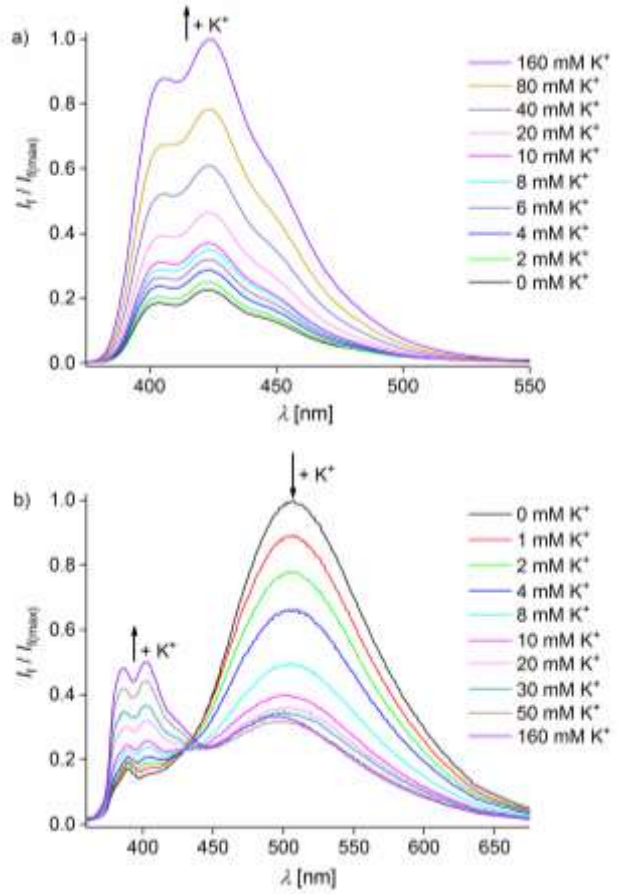


Figure 1. Fluorescence intensity changes of a) **1** (c = 10^{-5} M, λ_{exc} = 365 nm) and b) **2** (c = 10^{-5} M, λ_{exc} = 345 nm) in the presence of various K^+ concentrations under simulated physiological conditions (10 mM Tris-buffer, pH 7.2) with a constant ionic strength of 160 mM by varying Na^+ concentrations equal to [K^+] + [Na^+] = 160 mM.

In the next step, the high K^+/Na^+-selectivity was further verified by using solutions containing both, K^+ and competing Na^+ ions. Figure 1 shows the fluorescence behavior of **1** (cf. Figure 1a) and **2** (cf. Figure 1b) in a buffered H_2O/DMSO mixture (99/1, v/v; 10 mM Tris; pH = 7.2) in combined K^+/Na^+ solutions equal to 160 mM. The FE factor of **1** + 160 mM K^+ (FEF = 4.5) is lower than that in the experiment without Na^+ (FEF = 7.7, cf. Figure 1a and Figure S4b) and for **2** + K^+ in the presence Na^+, we found similar fluorescene intensity changes as in the experiment without Na^+ (cf. Figure 1b and Figure S7b). Further, the run of the titration curves of **1** + K^+ and **2** + K^+ were only slightly influenced by competing Na^+ ions (cf. Figure 2a and 2b), again demonstrating the high K^+/Na^+ selectivity of the fluorescent probes **1** and **2**.
Dissociation constants were calculated from plots of fluorescence intensities versus K^+ concentrations.[16] The K_d of **1** in Na^+ free solutions is ~38 mM and increases to ~41 mM in combined K^+/Na^+ solutions. We also found an slightly increased K_d value for **2** + K^+ from ~8 mM to ~9 mM in the presence of Na^+. Thus, the presence of Na^+ has a negligible effect on the performance of **1** and **2** under simulated physiological conditions.

10.1002/chem.201802306

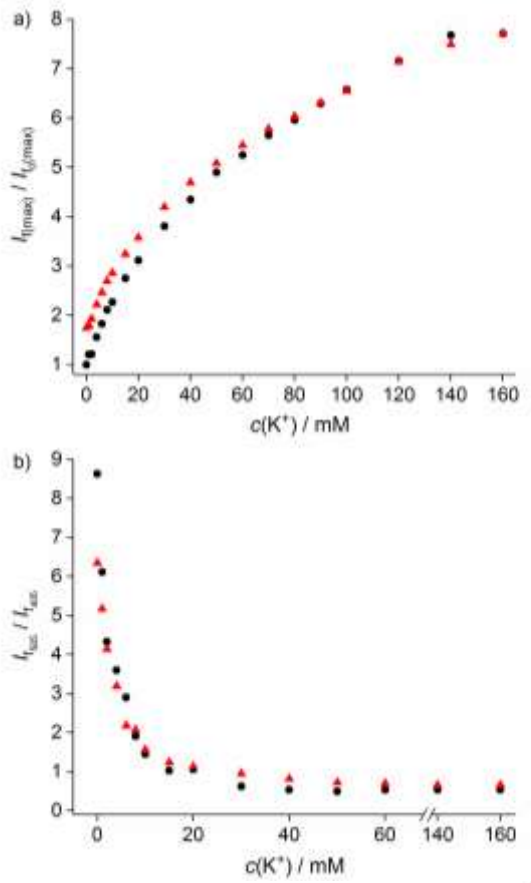


Figure 2. Titration curves of a) **1** (c = 10^{-5} M) and b) **2** (c = 10^{-5} M) in the presence of various K^+ concentrations (black circles) and in combined K^+/Na^+ solutions (red triangles) with a constant ionic strength equal to $[K^+] + [Na^+] = 160$ mM for **1** and **2**, respectively.

Contrary to **1**, **2** exhibits a more pronounced emissive charge transfer (CT) state (S1) at 505 nm and also a locally excited (LE) state (S2) about 400 nm in water (cf. Figure 3). Thus, we observed for **2** in the presence of K^+ a decreased and a slightly energetically upwards shifted CT band and the LE state becomes the dominating fluorescent state (S1) (cf. Figure 3). We assume, that the FE for **1** + K^+ is also caused by a K^+-induced state reversal with the difference that **1** exhibits only a very weak emissive CT state in water

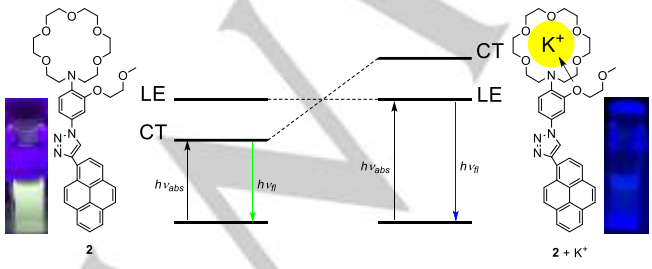


Figure 3. K^+-induced state reversal of fluorescent probe **2** in water.

Conclusions

Herein, we synthesized by CuAAC reactions two fluorescent probes **1** and **2**, which both consisting of a K^+-selective building block the *o*-(2-methoxyethoxy)phenylaza-18-crown-6 lariat ether (ionophore). Fluorescent probes **1** and **2** only differ by the fluorophore unit, whereby **1** possess an anthracene unit and **2** a pyrene moiety. In the presence of K^+, **1** and **2** showed K^+-induced fluorescence intensity changes. For **1** + K^+, we observed a high FE with a factor of 7.7 and for **2** + K^+, we found a dual emission behavior at 405 nm and 505 nm in water. The CT band at 505 nm is decreased and the LE emission at 405 nm is increased by K^+. Thus, we have shown, that the fluorescent probe **2** (K_d = 8 mM) is able to measure extracellular K^+ levels (1 mM - 10 mM K^+) by analyzing the intensity ratio at two emission wavelengths in vitro. This ratiometric behavior of **2** + K^+ is caused by a K^+-induced state reversal in water. Overall, we present an improved design principle for the ratiometric fluorescence analysis of analytes in water. Currently, we functionalizing fluorescent probe **2** with alkyl chains to anchor it into cell membranes to analyze extracellular K^+ levels in vivo. Prospectively, fluorescent probe **2** should embedded in a hydrogel as a part of an optode to measure local K^+ levels.

Experimental Section

General Methods and reagents: All commercially available chemicals were used without further purification. Solvents were distilled prior use. ^{1}H and ^{13}C NMR spectra were recorded on 300 MHz instrument, respectively. Data are reported as follows: chemical shifts in ppm (δ), multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet), integration, coupling constant (Hz). ESI spectra were recorded using a Micromass Q-TOF micro mass spectrometer in a positive electrospray mode. Column chromatography was performed with silica gel (Merck; silica gel 60 (0.04-0.063 mesh)).

General CuACC procedure for 1 and 2: A mixture of *N*-(4-azido-2-methoxyethoxyphenyl)aza-18-crown-6 ether[7b] 189 mg (0.415 mmol) and 0.415 mmol of 9-ethynylanthracene[14] or 1-ethynylpyrene, respectively, $CuSO_4 \cdot 5H_2O$ (5.3 mg) and sodium ascorbate (8.2 mg) in 9 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 then extracted with $CHCl_3$ (30 mL). The organic layer was dried with $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica using $CHCl_3$/CH_3OH (v/v, 9/1) as an eluent mixture to afford **1** and **2** as pale yellow oils.

Fluorescent probe 1: Yield 20% (55 mg); ^{1}H-NMR ($(CD_3)_2SO$, 300 MHz): δ = 9.22 (s, 1H), 8.83 (s, 1H), 8.37 (s, 2H), 8.24 (d, *J* = 8.6 Hz, 2H), 7.91 (d, *J* = 8.6 Hz, 2H), 7.65-7.54 (m, 5H), 4.33-4.26 (m, 2H), 3.82-3.75 (m, 2H), 3.76-3.18 ppm (m, 27H); ^{13}C-NMR ($CDCl_3$), 75 MHz): δ = 144.49, 131.42, 131.30, 128.68, 128.46, 128.41, 127.09, 126.16, 125.96, 125.55, 125.22, 125.02, 112.84, 112.72, 108.73, 106.71, 70.87, 70.73, 70.59, 70.50, 70.31, 69.42, 68.27, 58.97, 53.12 ppm; HRMS (ESI): *m*/*z* calcd for $C_{37}H_{44}N_4O_7+H^+$: 657.3288 [*M*+H^+]; found: 657.3237.

Fluorescent probe 2: Yield 35% (99 mg); ^{1}H NMR (CD_3CN, 300 MHz): δ = 8.85 (d, *J* = 9.3 Hz, 1H), 8.67 (s, 1H), 8.37-8.01 (m, 7H), 7.59 (s, 1H), 7.48-7.37 (m, 2H), 7.19 (d, *J* = 8.6, 1H), 4.22-4.17 (m, 2H), 3.75-3.71 (m, 2H), 3.61-3.40 (m, 24H), 3.37 ppm (s, 3H); ^{13}C NMR (CD_3CN, 75 MHz): δ

= 153.32, 148.26, 132.36, 132.15, 131.84, 129.20, 129.03, 128.81, 128.33, 128.16, 127.40, 126.88, 126.51, 126.29, 126.20, 126.02, 126.00, 125.76, 125.36, 123.02, 113.72, 106.86, 79.15, 71.59, 71.25, 71.12, 71.02, 70.35, 68.88, 59.06, 53.48 ppm; HRMS (ESI): *m/z* calcd for $C_{39}H_{44}N_4O_7+H^+$: 681.3288 [$M+H^+$]; found: 681.3315.

Keywords: fluorescence • potassium • ratiometric • charge transfer • crown compounds

[1] Selected review articles: a) J. F. Callan, A. P. de Silva, D. C. Magri, *Tetrahedron* **2005**, *61*, 8551-8588; b) B. Daly, J. Ling, A. P. de Silva, *Chem. Soc. Rev.* **2015**, *44*, 4203-4211; c) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515-1566; d) J. Li, D. Yim, W.-D. Jang, J. Yoon, *Chem. Soc. Rev.* **2017**, *46*, 2437-2458.

[2] a) H. M. D. Bandara, Z. Hua, Mei Zhang, S. M. Pauff, S. C. Miller, E. A. C. Davie, W. R. Kobertz, *J. Org. Chem.* **2017**, *82*, 8199-8205; b) B. Sui, X. Yue, M. G. Tichy, T. Liu, K. D. Belfield, *Eur. J. Org. Chem.* **2015**, 1189–1192; c) X. Zhou, F. Su, Y. Tian, C. Youngbull, R. H. Johnson, D. R. Meldrum, *J. Am. Chem. Soc.* **2011**, *133*, 18530 – 18533; d) X. Kong, F. Su, L. Zhang, J. Yaron, F. Lee, Z. Shi, Y. Tian, D. R. Meldrum, *Angew. Chem. Int. Ed.* **2015**, *54*, 12053-12057; *Angew. Chem.* **2015**, *127*, 12221-12225; e) B. Sui, X. Yue, B. Kim, K. D. Belfield, *ACS Appl. Mater. Interfaces* **2015**, *7*, 17565-17568; f) T. Hirata, T. Terai, H. Yamamura, M. Shimonishi, T. Komatsu, K. Hanaoka, T. Ueno, Y. Imaizumi, T. Nagano, Y. Urano, *Anal. Chem.* **2016**, *88*, 2693-2700; g) H. He, M. A. Mortellaro, M. J. P. Leiner, R. J. Fraatz, J. K. Tusa, *J. Am. Chem. Soc.* **2003**, *125*, 1468-1469.

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

[4] A. Minta, R. Y. Tsien, *J. Biol. Chem.* **1989**, *264*, 19449-19457.

[5] a) M. Baruah, W. Qin, R. A. L. Vallée, D. Beljonne, T. Rohand, W. Dehaen, N. Boens, *Org. Lett.* **2005**, *7*(20), 4377-4380; b) S. Nishizawa, M. Watanabe, T. Uchida, N. Teramae, *J. Chem. Soc., Perkin Trans.* **1999**, 141-143; c) T. T. Ruckh, C. G. Skipwith, W. Chang, A. W. Senko, V. Bulovic, P. O. Anikeeva, H. A. Clark, *ACS Nano* **2016**, *10*, 4020-4030.

[6] a) B. Valeur, I. Leray, *Coor. Chem. Rev.* **2000**, *205*, 3-40; b) J. R. Lakowicz in *Principles of Fluorescence Spectroscopy*, *Third Edition*, Springer, New York, **2006**, pp. 645-647.

[7] a) S. Ast, H. Müller, R. Flehr, T. Klamroth, B. Walz, H.-J. Holdt, *Chem. Commun.* **2011**, *47*, 4685-4687; b) S. Ast, T. Schwarze, H. Müller, A. Sukhanov, S. Michaelis, J. Wegener, O. S. Wolfbeis, T. Körzdörfer, A. Dürkop, H.-J. Holdt, *Chem. Eur. J.* **2013**, *19*, 14911-14917; c) T. Schwarze, R. Schneider, J. Riemer, H.-J. Holdt, *Chem. Asian J.* **2016**, *11*, 241-247; d) T. Schwarze, J. Riemer, S. Eidner, H.-J. Holdt, *Chem. Eur. J.* **2015**, *21*, 11306-11310; e) T. Schwarze, M. Mertens, P. Müller, J. Riemer, P. Wessig, H.-J. Holdt, *Chem. Eur. J.* **2017**, 23, 17186-17190.

[8] S. Ast, T. Fischer, H. Müller, W. Mickler, M. Schwichtenberg, K. Rurack, H.-J. Holdt, *Chem. Eur. J.* **2013**, *19*, 2990-3005.

[9] a) B. J. Müller, S. M. Borisov, I. Klimant, *Adv. Funct. Mater.* **2016**, *26*, 7697-7707; b) G. Song, R. Sun, J. Du, M. Chen, Y. Tian, *Chem. Comm.* **2017**, *53*, 5602-5605; c) J. Garcia-Calvo, S. Ibeas, E.-C. Antjn-Garcia, T. Torroba, G. Gonzalez-Aguilar, W. Antunes, E. Gonzalez-Lavado, M. L. Fanarraga, *Chemistry Open* **2017**, *6*, 562-570.

[10] A. Wiessner, G. Hüttmann, W. Kühnle, H. Staerk, *J. Phys. Chem.* **1995**, *99*, 14923-14930.

[11] a) S. S. Bag, R. Kundu, *J. Org. Chem.* **2011**, *76*, 3348-3356; b) S. S. Bag, R. Kundu, S, Talukdar, *Tetrahedron Lett.* **2012**, *53*, 5875-5879; c) S. S. Bag, R. Kundu, *J. Fluoresc.* **2013**, *23*, 929-938.

[12] P. Kautny, D. Bader, B. Stöger, G. A. Reider, J. Fröhlich, D. Lumpi, *Chem. Eur. J.* **2016**, *22*, 18887-18898.

[13] a) V. V. Rostovtsev, L. G. Green, V. V. Folkin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599; b) C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057-3062.

[14] Q. Xiao, R. T. Ranasinghe, A. M. P. Tang, T. Brown, *Tetrahedron* **2007**, *63*, 3483-3490.

[15] T. Schwarze, H. Müller, D. Schmidt, J. Riemer, H.-J. Holdt, *Chem. Eur. J.* **2017**, *23*, 7255-7263.

[16] Detailed experimental procedures and data can be found in the Supporting Information.

10.1002/chem.201802306

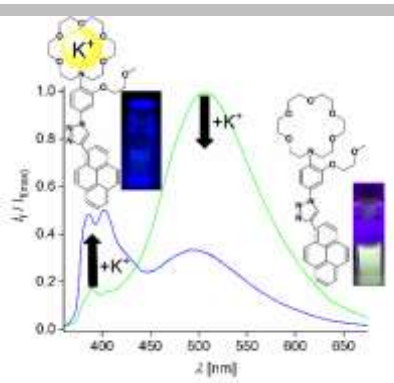
WILEY-VCH

Entry for the Table of Contents (Please choose one layout)

Layout 1:

FULL PAPER

In this paper, we show a ratiometric fluorescent probe to measure physiologically relevant K^+ levels by K^+-induced intensity changes at two emission wavelengths.



Thomas Schwarze, Janine Riemer and Hans-Jürgen Holdt**

Page No. – Page No.

A Ratiometric Fluorescent Probe for K^+ in Water based on a Phenylaza-18-Crown-6 Lariat Ether