

A Highly Selective Mitochondria-Targeting Fluorescent K<sup>+</sup> Sensor

Xiangxing Kong, Fengyu Su, Liqiang Zhang, Jordan Yaron, Fred Lee, Zhengwei Shi, Yanqing Tian,\* and Deirdre R. Meldrum\*

**Abstract:** Regulation of intracellular potassium (K<sup>+</sup>) concentration plays a key role in metabolic processes. So far, only a few intracellular K<sup>+</sup> sensors have been developed. The highly selective fluorescent K<sup>+</sup> sensor **KS6** for monitoring K<sup>+</sup> ion dynamics in mitochondria was produced by coupling triphenylphosphonium, borondipyrromethene (BODIPY), and triazacryptand (TAC). **KS6** shows a good response to K<sup>+</sup> in the range 30–500 mM, a large dynamic range ( $F_{max}/F_0 \approx 130$ ), high brightness ( $\phi_f = 14.4\%$  at 150 mM of K<sup>+</sup>), and insensitivity to both pH in the range 5.5–9.0 and other metal ions under physiological conditions. Colocalization tests of **KS6** with MitoTracker Green confirmed its predominant localization in the mitochondria of HeLa and U87MG cells. K<sup>+</sup> efflux/influx in the mitochondria was observed upon stimulation with ionophores, nigericin, or ionomycin. **KS6** is thus a highly selective semiquantitative K<sup>+</sup> sensor suitable for the study of mitochondrial potassium flux in live cells.

Potassium channels (KCh) are a class of transmembrane proteins, with about 90 human genes coding for the principle subunits.<sup>[1]</sup> They are involved in many physiological functions, such as cell proliferation, growth, and apoptosis.<sup>[2]</sup> By opening or blocking KCh to adjust the K<sup>+</sup> concentration in cellular organelles, the cell can control its membrane potential, contribute to cardiac action potentials and neurotransmitter release, and alter many critical biological functions.<sup>[3]</sup> Recent research found that KCh is a potential pharmacological target in cancer, autoimmune disease, cardioprotection, and diabetes.<sup>[4]</sup> Typical research tools for studying KCh include: 1) patch-clamp techniques,<sup>[5]</sup> 2) fluxOR<sup>TM</sup> assay method with Tl<sup>+</sup> ions and corresponding fluorescent probes,<sup>[6]</sup> and 3) the Rb<sup>+</sup> ion method.<sup>[7]</sup> These methods are well-developed for high-throughput screening of drugs with certain type of KCh, but they have limitations for understanding the relationships in multifactor cellular signaling pathways. Owing to the lack

of fluorescent potassium sensors, most research on KCh makes use of indirect experimental methods, which leave lots of uncertainty in terms of the research conclusions.<sup>[8]</sup> Recent research has demonstrated that K<sup>+</sup> flux through the inner mitochondrial membrane has a significant effect on insulin secretion,<sup>[9]</sup> inflammasome formation,<sup>[10]</sup> and apoptosis.<sup>[11]</sup> The development of a mitochondria-targeting K<sup>+</sup> sensor is critical for the investigation of K<sup>+</sup>-related mitochondrial signaling, analysis of metabolism at the single-cell level, and screening of new drugs.<sup>[12]</sup>

An ideal intracellular K<sup>+</sup> sensor should have a large dynamic range compatible with the intracellular concentrations of K<sup>+</sup> (130–460 mM in different cell compartments<sup>[13]</sup>), as well as insensitivity to pH and competing Na<sup>+</sup> (5–15 mM in intracellular fluid; ca. 145 mM in extracellular fluid) and other metal ions at physiological concentrations.<sup>[14]</sup> The best-known K<sup>+</sup> sensors for molecular biology studies, K<sup>+</sup>-binding benzofuran isophthalate (PBFi) and its cell-permeable form bis(acetyloxymethyl) esterized PBFi (PBFi-AM), suffer from poor selectivity against Na<sup>+</sup>.<sup>[15]</sup> In 2003, He et al. discovered a fluorescent extracellular K<sup>+</sup> sensor based on a highly selective triazacryptand (TAC) ligand, which features an excellent selective response to K<sup>+</sup> over Na<sup>+</sup>.<sup>[16]</sup> More recently, the Verkman group<sup>[17]</sup> and our group<sup>[18]</sup> have developed several fluorescent K<sup>+</sup> sensors based on the TAC ligand. By linking TAC to different positions of BODIPY, two different K<sup>+</sup> ion sensors were reported.<sup>[17a,19]</sup> The fluorescent K<sup>+</sup> ion sensor based on 3-styrylated BODIPY reported by Hirata et al. demonstrated a large apparent disassociation constant ( $K_d$ , 53 mM) from the spectroscopic data obtained in a mixed solution of HEPES (pH 7.0)/MeCN (60:40); no in vivo cell imaging is available. As far as we know, these sensors are unsuitable for mitochondrial K<sup>+</sup> sensing.

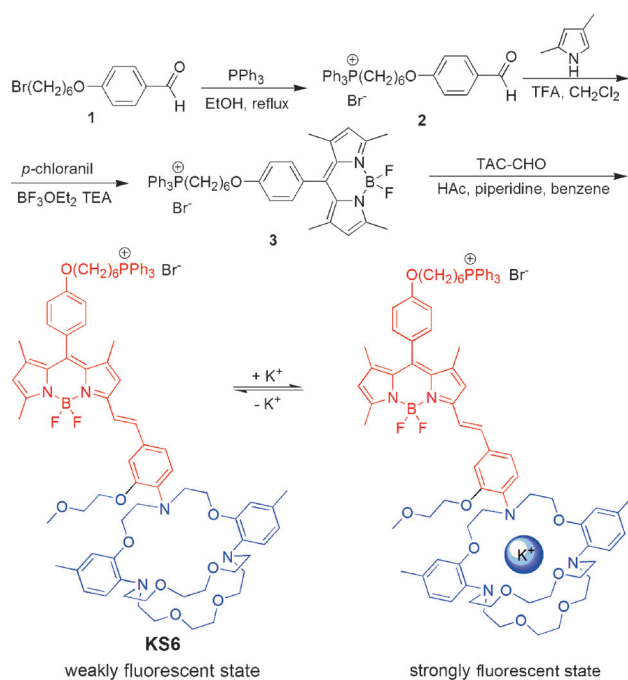
We report herein a mitochondria-targeting K<sup>+</sup> sensor produced by attaching a lipophilic triphenylphosphonium cation (TPP<sup>+</sup>) to TAC-modified 3-styrylated BODIPY. TPP<sup>+</sup> has been used as a mitochondria-targeting moiety because of its accumulation in the mitochondrial matrix.<sup>[20]</sup> Scheme 1 shows the synthetic route to the **KS6** sensor. [6-(4-Formylphenoxy)hexyl]triphenylphosphonium bromide (**2**) was prepared through the reaction of 6-bromohexyloxybenzaldehyde with PPh<sub>3</sub> in ethanol. The TPP<sup>+</sup>-containing BODIPY (**3**) was synthesized by reacting **2** with 2,4-dimethylpyrrole in a reaction catalyzed by trace amounts of trifluoroacetic acid in anhydrous dichloromethane, followed by oxidation with *p*-chloranil and treatment with BF<sub>3</sub>·OEt<sub>2</sub> and triethylamine. **KS6** was obtained through condensation of **3** with TAC-CHO in benzene with piperidinium acetate as the catalyst. The structures of **KS6** and intermediate **3** were characterized by <sup>1</sup>H NMR and high-resolution mass spectroscopy (see the Supporting Information). **KS6** is soluble in organic solvents

[\*] Dr. X. Kong,<sup>[†]</sup> Dr. F. Su,<sup>[†]</sup> Dr. L. Zhang,<sup>[†]</sup> Dr. J. Yaron, F. Lee, Z. Shi, Prof. Dr. Y. Tian, Prof. Dr. D. R. Meldrum  
Center for Biosignatures Discovery Automation  
Biodesign Institute, Arizona State University  
1001 S. McAlister Ave., P.O. Box 876501, Tempe, AZ 85287 (USA)  
E-mail: yanqing.tian@asu.edu  
deirdre.meldrum@asu.edu

Prof. Dr. Y. Tian  
Department of Materials Science and Engineering  
South University of Science and Technology of China  
No. 1088, Xueyuan Rd., Xili, Nanshan District, Shenzhen  
Guangdong, 518055 (China)

[†] These authors contributed equally to this work.

Supporting information for this article (including experimental details) is available on the WWW under <http://dx.doi.org/10.1002/anie.201506038>.

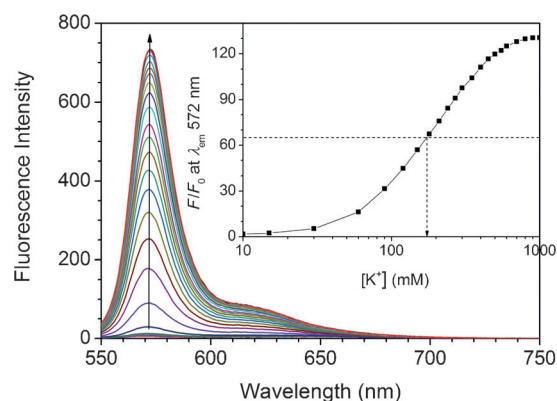


**Scheme 1.** Synthetic route to **KS6** and its response to  $K^+$ .

such as DMSO,  $CH_2Cl_2$ , and chloroform, but is insoluble in water.

To avoid using any organic solvent in the **KS6** titration, we dispersed **KS6** (1.0 mM in DMSO) into three aqueous solutions [Tris HCl buffer (5 mM); **KS6** (5  $\mu$ M), pH 7.4] containing different surfactants: sodium dodecylsulfate (SDS), Pluronic F127, and tetrabutylammonium bromide (CTAB), with the surfactant concentrations below their critical micelle concentrations. Adding KCl stock solution (4.0 M) into **KS6** in SDS solution led to the formation of a white precipitate, thus making it unsuitable for titration. For **KS6** in Pluronic F127 aqueous solution, it takes about 5–6 min for the fluorescence intensity to reach the maximum (or equilibrium) state (Figure S1A in the Supporting Information). The slow response might be caused by competition for  $K^+$  binding between **KS6** and Pluronic F127, which has a similar  $[OCH_2CH_2]$  unit to that of **KS6**. The titration of **KS6** in CTAB solution reaches equilibrium in 10 s after simply shaking the solution (Figure S1B), therefore CTAB was selected for further experiments.

Figure 1 shows the titration result for **KS6** (5.0  $\mu$ M) carried out in Tris/HCl buffer (pH 7.4, 10 mM)/CTAB (0.5 mM) with KCl concentrations from 5 to 800 mM. **KS6** without  $K^+$  binding has a maximum absorbance peak at 582 nm in aqueous solution and an extinction coefficient of  $2.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  (Figure S2A). Upon binding  $K^+$  ions (0.8 M), the maximum absorbance peak is blue-shifted to 567 nm, with an extinction coefficient of  $3.05 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ . **KS6** showed a very weak fluorescence peak at 572 nm in its free form and a quantum yield ( $\phi_f$ ) as low as 0.7% when using rhodamine 101 in ethanol ( $\phi_f = 1.0$ ) as a reference.<sup>[21]</sup> The fluorescence peak at 572 nm increased by 1.3- and 57-fold at  $K^+$  concentrations of 5 mM and 150 mM (typical extracellular and intracellular  $K^+$  concentrations), respectively, which

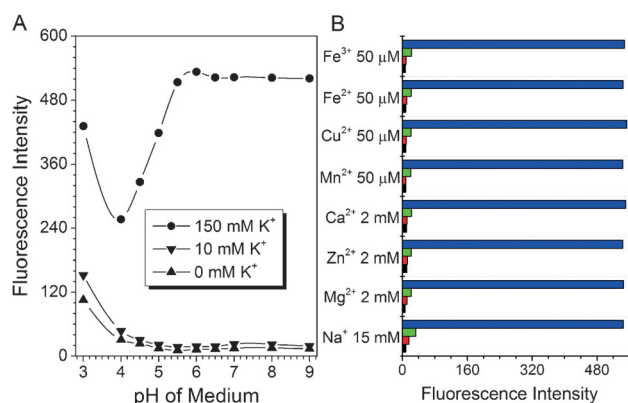


**Figure 1.** Fluorescence spectra for **KS6** (5.0  $\mu$ M) in Tris buffer (pH 7.4, 5 mM)/CTAB (0.50 mM) containing different concentrations of KCl ( $\lambda_{\text{ex}}$ : 540 nm). The inset shows  $F/F_0$  at  $\lambda_{\text{em}}$  572 nm vs.  $[K^+]$ .  $F_0$  is the intensity before adding  $K^+$  ions.  $F$  is the intensity at various concentrations of  $K^+$  ions.

corresponds to  $\phi_f$  values of 1.0% and 14.4%. The excitation spectrum of the **KS6**– $K^+$  complex showed a maximum peak at 565 nm and a shoulder peak at 527 nm (Figure S2B). The emission spectrum of the **KS6**– $K^+$  complex showed a maximum peak at 572 nm and a broad shoulder peak from 600 to 690 nm. Theoretical calculations of  $K_d$  by using either the linear Benesi–Hildebrand equation or Hill plot failed,<sup>[22]</sup> which might be caused by the high ionic strength and the presence of surfactant in the solution. From the  $F/F_0$  vs.  $\log[K^+]$  plot, we found that **KS6** is suitable for monitoring  $K^+$  between 30 mM and 500 mM. We suggest a  $[K^+]_c$  concentration of  $1/2[F_{\text{max}} - F_0]$  (ca. 170 mM) to be used to compare to the  $K_d$  of other  $K^+$  sensors.

For fluorescent sensors with a mechanism involving photoinduced charge transfer, the fluorescence quantum yields often decrease with increasing solvent polarity, and effect that has also been observed in BODIPY fluorophores.<sup>[23]</sup> To clarify the sensing mechanism of the **KS6** sensor, a NaCl aqueous solution (4.0 M) was used to titrate the **KS6** for comparison. Unlike titration with KCl, no UV/Vis spectral change was observed during the titration (Figure S3A). The fluorescence band in the range of 600–700 nm increased with an increase in the concentration of  $Na^+$  up to 0.80 M (Figure S3B). At a  $Na^+$  concentration of approximately 150 mM, which is close to the extracellular concentration of  $Na^+$ , the increase in fluorescence intensity caused by the ionic strength effect can be omitted (Figure S3C), thus indicating that the response of **KS6** is mainly due to the binding of the  $K^+$  ions and not its dispersion in the less polar CTAB phase at a high ionic strength.

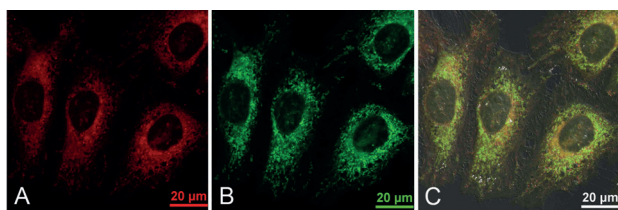
The fluorescence intensity of the **KS6** sensor is independent of pH in the range of 5.5–9.0 (Figure 2A). It started to decrease when the pH value of the buffer solution decreased from 5.5 to 4.0. The pH value in mitochondria (ca. pH 8) would not reach such low levels in live cells; thus it doesn't affect the application of **KS6** in mitochondria. The selectivity of **KS6** was also tested against physiological levels of the following metal ions:  $Na^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Fe^{3+}$ ,  $Fe^{2+}$ ,  $Zn^{2+}$ ,  $Mn^{2+}$ , and  $Cu^{2+}$  (Figure 2B), as well as  $H_2O_2$  (100 mM), and no



**Figure 2.** A) Fluorescence intensity of **KS6** (5 mM) in different Britton–Robinson buffer solutions (0.5 mM CTAB) at different pH values and containing no KCl, 10 mM of KCl, or 150 mM of KCl. B) Fluorescence intensity of **KS6** (5 mM **KS6**, 0.5 mM CTAB) samples containing only sensor (black), metal ions (red), metal ions and 5 mM KCl (green), and metal ions and 150 mM KCl (blue).

significant effects were observed. Therefore, **KS6** demonstrates high selectivity for  $K^+$  in an ambient cell environment, and good chemical stability to  $H_2O_2$ , thus indicating its suitability for monitoring the concentration change of  $K^+$  in intracellular environments.

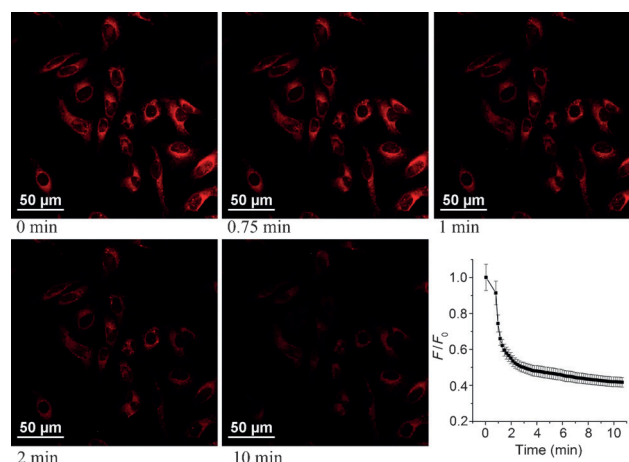
The cytotoxicity of **KS6** towards human HeLa cells was investigated by using the MTT assay. At a concentration of  $3 \mu\text{M}$  of **KS6**, more than 90% of the cells were viable after incubation with the sensor for 3 h (Figure S4). At a lower concentration of  $1 \mu\text{M}$  of **KS6**, more than 95% of the cells were viable after 15 h. In both cases, **KS6** can be used for cell imaging owing to its large absorption coefficient and high fluorescent quantum yield after binding to  $K^+$  ions. A colocalization assay was carried out with the mitochondrial dye MitoTracker<sup>®</sup> Green FM and **KS6** in HeLa cells (Figure 3). The Pearson's correlation coefficient and Man-



**Figure 3.** Confocal fluorescence microscope images of HeLa cells co-stained with **KS6** ( $2 \mu\text{M}$ ) and MitoTracker<sup>®</sup> Green FM. A) Red emission from **KS6**; B) green emission from MitoTracker<sup>®</sup> Green; C) overlay of MitoTracker Green, **KS6**, and bright-field images.

der's overlap coefficient were 0.89 and 0.94, respectively, thus indicating that **KS6** is predominantly localized to the mitochondria of live cells.<sup>[24]</sup> Similar colocalization was observed in U87MG cells.

To monitor the mitochondrial  $K^+$  concentration change under stimulation, HeLa cells, which had been incubated with **KS6** ( $2 \mu\text{M}$ ) for 10 min, were treated with the ionophore ionomycin ( $10 \mu\text{M}$ ) at  $37^\circ\text{C}$ . Fast efflux of mitochondrial  $K^+$



**Figure 4.** Time-dependent confocal fluorescence microscopy imaging of **KS6**-stained HeLa cells stimulated with ionomycin at  $t=0$  (before the addition of ionomycin) and  $t=0.75, 1, 2, 10$  min after adding ionomycin ( $20 \mu\text{M}$  final concentration) into culture medium containing 20 mM of KCl. The average fluorescence intensity ratios as measured by Image J are shown in the graph.  $F_0$  is the average fluorescence intensity at  $t=0$  min;  $F$  is the average fluorescence intensity at a given time point.

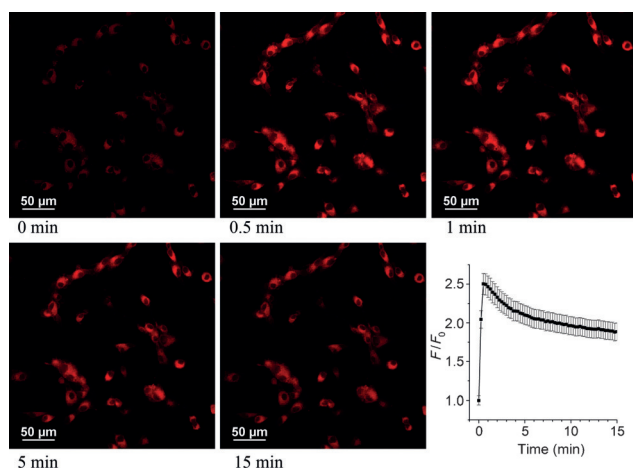
within 2 min was indicated by a decrease in fluorescence intensity (Figure 4). Control experiments without ionomycin stimulation showed no obvious fluorescence intensity change in culture medium containing either 20 or 200 mM KCl, respectively (Figure S5A,B).

Influx and then efflux of  $K^+$  in mitochondria was observed in HeLa cells after stimulation with another ionophore, nigericin ( $10 \mu\text{M}$ ), in a cell-culture medium containing 200 mM of KCl (Figure S6). Within 30 s, the average fluorescence intensity of cells increased by 60%, thus indicating influx of  $K^+$  into the mitochondria. After 2 min, potassium efflux from the mitochondria was indicated by a decrease in fluorescence intensity. The final intensity after stabilization for 10 min was 40% above that before stimulation by nigericin.

U87MG cells can physiologically act as a  $K^+$  buffer to remove excess potassium.<sup>[25]</sup> In contrast to HeLa cells, simple treatment of U87MG cells with a cell-culture medium containing 20 mM of KCl caused a slow decrease in fluorescence intensity to 74% in 12 min. When the concentration of KCl in the medium increased to 200 mM, the fluorescence intensity from the U87MG cells first jumped to 50% above the value measured before the treatment and the slowly decreased to its original state (Figure S7). Stimulating U87MG cells with ionomycin ( $10 \mu\text{M}$ ) in a cell-culture medium caused the fluorescence intensity to decrease to 59% in the mitochondria within 2 min, and it finally reached 30% of its original intensity after 10 min, thus indicating  $K^+$  efflux from the mitochondria (Figure S8).

$K^+$  influx/efflux was also observed when **KS6**-internalized U87MG cells were treated with nigericin ( $20 \mu\text{M}$ ) in the presence of 200 mM of KCl. Within 30 s, the fluorescence intensity in the U87MG cells increased by 250% (Figure 5). After reaching the fluorescence maximum, the fluorescence intensity started to decrease and decayed to 75% of the maximum value, thus indicating efflux of  $K^+$  ions from the





**Figure 5.** Time-dependent confocal fluorescence microscopy imaging of **KS6**-stained U87MG cells stimulated with nigericin at  $t=0$  (before the addition of nigericin) and  $t=0.5, 1, 5, 15$  min after adding nigericin ( $20 \mu\text{M}$  final concentration) into culture medium containing  $200 \text{ mM}$  of KCl.

mitochondria. The rapid influx of  $\text{K}^+$  ions might be caused by nigericin-facilitated diffusion of  $\text{K}^+$  ions into the mitochondria under the influence of the transmembrane potential.

Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), one of the OXPHOS uncouplers that works as a proton transmembrane carrier,<sup>[26]</sup> was used as a typical stimulator to study mitochondrial  $\text{K}^+$  ion flux.<sup>[27]</sup> Both HeLa and U87MG cell lines were used to dynamically detect the potassium flux in mitochondrial matrix in response to changes in membrane potential induced by CCCP. Cells incubated with  $1 \mu\text{M}$  of **KS6** for 30 min were treated with different concentrations of CCCP (0, 10, and  $40 \mu\text{M}$ ). No fluorescence intensity change was observed without CCCP treatment, whereas the fluorescence intensity of **KS6** in mitochondria dropped about 50% in U87MG cells and 20% in HeLa cells depending on CCCP concentration, thus demonstrating the different behaviors of different cell lines (Figure S9 and S10) in response to stimulation.

In summary, we have developed **KS6**, a predominantly mitochondria-targeted  $\text{K}^+$  sensor that selectively responds to  $\text{K}^+$  with a 130-fold fluorescence enhancement, (at a  $\text{K}^+$  concentration of  $0.8 \text{ M}$ ) and a dynamic range with respect to  $\text{K}^+$  ion concentration of 30–500 mM. The presence of the triphenylphosphonium group in **KS6** enables its localization to the mitochondria, thus making it the first mitochondria-specific  $\text{K}^+$  sensor. We have demonstrated that **KS6** is a useful tool for monitoring mitochondrial  $\text{K}^+$  flux (both influx and efflux) under various stimulation conditions, although this is not yet quantitative. We believe that **KS6** has potential applications in the investigation of many potassium related biological processes, especially in single-cell analysis, drug screening, and studies of the inflammatory response

## Acknowledgements

This work was supported by the NIH National Human Genome Research Institute, Centers of Excellence in Genomic Science, grant number 5 P50 HG002360, and the NIH Common Fund LINCS program, grant number 5 U01 CA164250 (Professor Deirdre R. Meldrum, PI). We would like to thank Dr. Honor Glenn and Dr. Kimberly Bussey for useful discussions

**Keywords:** BODIPY · fluorescent probes · ion channels · mitochondria · potassium

**How to cite:** *Angew. Chem. Int. Ed.* **2015**, *54*, 12053–12057  
*Angew. Chem.* **2015**, *127*, 12221–12225

- [1] C. Miller, *Genome Biol.* **2000**, *1*, reviews0004.1.
- [2] a) D. Urrego, A. P. Tomczak, F. Zahed, W. Stuhmer, L. A. Pardo, *Philos. Trans. R. Soc. London Ser. B* **2014**, *369*, 20130094; b) I. Szabó, L. Leanza, E. Gulbins, M. Zoratti, *Pflugers Arch.* **2012**, *463*, 231–246; c) D. Malinska, S. R. Mirandola, W. S. Kunz, *FEBS Lett.* **2010**, *584*, 2043–2048; d) V. Pétrilli, S. Papin, C. Dostert, A. Mayor, F. Martinon, J. Tschopp, *Cell Death Differ.* **2007**, *14*, 1583–1589.
- [3] a) M. Levin, C. G. Stevenson, *Annu. Rev. Biomed. Eng.* **2012**, *14*, 295–323; b) Z. W. Wang, O. Saifee, M. L. Nonet, L. Salkoff, *Neuron* **2001**, *32*, 867–881.
- [4] a) X. Huang, L. Y. Jan, *J. Cell Biol.* **2014**, *206*, 151–162; b) H. Wulff, B. S. Zhorov, *Chem. Rev.* **2008**, *108*, 1744–1773; c) M. P. Mattson, G. Kroemer, *Trends Mol. Med.* **2003**, *9*, 196–205; d) P. Rorsman, M. Braun, *Annu. Rev. Physiol.* **2013**, *75*, 155–179.
- [5] O. B. McManus, *Curr. Opin. Pharmacol.* **2014**, *15*, 91–96.
- [6] a) D. W. Beacham, T. Blackmer, M. O'Grady, G. T. Hanson, *J. Biomol. Screening* **2010**, *15*, 441–446; b) A. P. Wojtovich, D. M. Williams, M. K. Karcz, C. M. B. Lopes, D. A. Gray, K. W. Nehrke, P. S. Brookes, *Circul. Res.* **2010**, *106*, 1190–U1138.
- [7] S. Rezazadeh, J. C. Hesketh, D. Fedida, *J. Biomol. Screening* **2004**, *9*, 588–597.
- [8] T. Muñoz-Planillo, P. Kuffa, G. Martinez-Colón, B. L. Smith, T. M. Rajendiran, G. Núñez, *Immunity* **2013**, *38*, 1142–1153.
- [9] J. C. Xu, P. L. Wang, Y. Y. Li, G. Y. Li, L. K. Kaczmarek, Y. L. Wu, P. A. Koni, R. A. Flavell, G. V. Desir, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 3112–3117.
- [10] D. G. Jackson, J. J. Wang, R. W. Keane, E. Scemes, G. Dahl, *Sci. Rep.* **2014**, *4*, srep04576.
- [11] S. Bonnet, S. L. Archer, J. Allalunis-Turner, A. Haromy, C. Beaulieu, R. Thompson, C. T. Lee, G. D. Lopaschuk, L. Puttagunta, S. Bonnet, G. Harry, K. Hashimoto, C. J. Porter, M. A. Andrade, B. Thebaud, E. D. Michelakis, *Cancer Cell* **2007**, *11*, 37–51.
- [12] M. E. Lidstrom, D. R. Meldrum, *Nat. Rev. Microbiol.* **2003**, *1*, 158–164.
- [13] F. Nolin, J. Michel, L. Wortham, P. Tchelidze, G. Balossier, V. Banchet, H. Bobichon, N. Lalun, C. Terryn, D. Ploton, *Cell. Mol. Life Sci.* **2013**, *70*, 2383–2394.
- [14] B. Alberts, *Essential cell biology*, 3rd ed., Garland Science, New York, **2010**.
- [15] a) A. Minta, R. Y. Tsien, *J. Biol. Chem.* **1989**, *264*, 19449–19457; b) H. Szmajnski, J. R. Lakowicz, *Sens. Actuators B* **1999**, *60*, 8–18.
- [16] H. He, M. A. Mortellaro, M. J. P. Leiner, R. J. Fraatz, J. K. Tusa, *J. Am. Chem. Soc.* **2003**, *125*, 1468–1469.
- [17] a) W. Namkung, P. Padmawar, A. D. Mills, A. S. Verkman, *J. Am. Chem. Soc.* **2008**, *130*, 7794–7795b) R. D. Carpenter, A. S. Verkman, *Eur. J. Org. Chem.* **2011**, 1242–1248; c) P. Padmawar,

- X. M. Yao, O. Bloch, G. T. Manley, A. S. Verkman, *Nat. Methods* **2005**, *2*, 825–827; d) R. D. Carpenter, A. S. Verkman, *Org. Lett.* **2010**, *12*, 1160–1163.
- [18] a) X. F. Zhou, F. Y. Su, W. M. Gao, Y. Q. Tian, C. Youngbull, R. H. Johnson, D. R. Meldrum, *Biomaterials* **2011**, *32*, 8574–8583; b) X. F. Zhou, F. Y. Su, Y. Q. Tian, C. Youngbull, R. H. Johnson, D. R. Meldrum, *J. Am. Chem. Soc.* **2011**, *133*, 18530–18533.
- [19] T. Hirata, T. Terai, T. Komatsu, K. Hanaoka, T. Nagano, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6090–6093.
- [20] a) M. F. Ross, G. F. Kelso, F. H. Blaikie, A. M. James, H. M. Cocheme, A. Filipovska, T. Da Ros, T. R. Hurd, R. A. J. Smith, M. P. Murphy, *Biochemistry* **2005**, *70*, 222–230; b) Q. L. Hu, M. Gao, G. X. Feng, B. Liu, *Angew. Chem. Int. Ed.* **2014**, *53*, 14225–14229; *Angew. Chem.* **2014**, *126*, 14449–14453; c) K. Krumova, L. E. Greene, G. Cosa, *J. Am. Chem. Soc.* **2013**, *135*, 17135–17143; d) C. W. T. Leung, Y. N. Hong, S. J. Chen, E. G. Zhao, J. W. Y. Lam, B. Z. Tang, *J. Am. Chem. Soc.* **2013**, *135*, 62–65.
- [21] T. Karstens, K. Kobs, *J. Phys. Chem.* **1980**, *84*, 1871–1872.
- [22] N. Boens, V. Leen, W. Dehaen, *Chem. Soc. Rev.* **2012**, *41*, 1130–1172.
- [23] a) K. Rurack, M. Kollmannsberger, J. Daub, *Angew. Chem. Int. Ed.* **2001**, *40*, 385–387; *Angew. Chem.* **2001**, *113*, 396–399; b) M. Baruah, W. W. Qin, C. Flors, J. Hofkens, R. A. L. Vallee, D. Beljonne, M. Van der Auweraer, W. M. De Borggraeve, N. Boens, *J. Phys. Chem. A* **2006**, *110*, 5998–6009.
- [24] K. W. Dunn, M. M. Kamocka, J. H. McDonald, *Am. J. Physiol. Cell Physiol.* **2011**, *300*, C723–C742.
- [25] P. Kofuji, E. A. Newman, *Neuroscience* **2004**, *129*, 1045–1056.
- [26] U. Hopfer, Lehninge. Al, T. E. Thompson, *Proc. Natl. Acad. Sci. USA* **1968**, *59*, 484–490.
- [27] a) K. D. Garlid, P. Paucek, *Biochim. Biophys. Acta Bioenerg.* **2003**, *1606*, 23–41; b) S. Chalmers, J. G. McCarron, *J. Cell Sci.* **2008**, *121*, 75–85; c) S. W. Perry, J. P. Norman, J. Barbieri, E. B. Brown, H. A. Gelbard, *BioTechniques* **2011**, *50*, 98–115; d) I. Szabo, M. Zoratti, *Physiol. Rev.* **2014**, *94*, 519–608.

Received: July 1, 2015

Published online: August 21, 2015