**Title:** Light addressable ion sensing for real-time monitoring of extracellular potassium

**Authors:** Ying Yang; Maria Cuartero; Vinicius Romero Gonçales; John Justin Gooding, D. Phil.; Eric Bakker



**To be cited as:** 10.1002/anie.201811268

**Link to VoR:** https://doi.org/10.1002/anie.201811268

# Light addressable ion sensing for real-time monitoring of extracellular potassium

Ying Yang,[a] Maria Cuartero[b], Vinicius Romero Gonçales[a], J. Justin Gooding*[a] and Eric Bakker*[b]

**Abstract:** *Visualization of ion distribution has broad applications. We report here on a light addressable potassium ($K^+$) sensor where light illumination of a semiconducting silicon electrode substrate results in a localized activation of the faradaic electrochemistry at the illuminated spot. This allows one, by electrochemical control, to oxidize surface bound ferrocene moieties that in turn trigger $K^+$ transfer from the overlaid $K^+$-selective film to the solution phase. The resulting voltammetric response is shown to be $K^+$-selective, where peak position is a direct function of $K^+$ activity at the surface of electrode. This concept was used to measure extracellular $K^+$ concentration changes by stimulating living breast cancer cells. The associated decrease of intracellular $K^+$ level was confirmed with a fluorescent $K^+$ indicator. In contrast to light addressable potentiometry, the approach introduced here relies on dynamic electrochemistry and may be performed in tandem with other electrochemical analysis when studying biological events on the electrode.*

The ability to combine electrochemistry with optical microscopy to monitor cells as they respond to stimuli would be very powerful as electrochemistry can monitor, or stimulate, the cells with high temporal resolution over long periods of time without labels whilst microscopy can observe these individual cells as they respond to the stimuli.[1] The missing link has been to obtain electrochemical information from single cells rather than thousands. Semiconducting surfaces offer the possibility to achieve single cell electrochemistry.[2] After absorbing light of appropriate wavelength, a semiconductor generates electron-hole pairs and hence becomes conducting in that region. This phenomenon creates the possibility to perform faradaic electrochemistry anywhere on a monolithic semiconductor surface simply by illuminating this region.[3]

Whilst an old idea,[4] the challenge has been to be able to combine this idea with biological systems. The challenge arises for two reasons. First, the semiconducting electrode must be stable in aqueous media under the potential conditions required. Second the band gap of the semiconductor must be sufficiently small such that the required wavelength of light is within the visible part of the spectrum. This latter requirement is to avoid the use of UV radiation which would be harmful to the living organisms. The band gap requirement makes silicon the distinguished choice for the semiconductor. However, electrochemistry of silicon has proven to be very challenging since it is prone to surface oxidation that results in electrode passivation. To overcome this limitation, efforts have been made to appropriately protect silicon with a monolayer derived from 1,8-nonadiyne and, as a consequence, stable amperometry can be performed.[5] Light-induced conductivity is harnessed by covalently conjugating redox active groups, such as ferrocene, onto the monolayer material by the established Huisgen cycloaddition 'click' reaction.[6] The redox centres promote electron tunnelling between the contacting outside solution and the silicon material, giving rise to well behaved electrochemistry when illuminated with a laser either from the front or back side of the silicon substrate.[3]

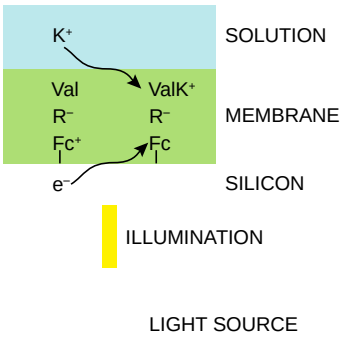
This approach has been shown to be applicable to write electrochemical information to a surface, *via* the electrodeposition of polypyrrole, and to read electrochemical information from a discrete area of a macroscopic surface in terms of DNA hybridization sensing.[3a] A recent demonstration shows the successful application of the concept to a photoelectrochemical platform for the release of rare single cells. The platform showed the compatibility with microscopy imaging and enabled localizing the release of any desired single after comparing cell morphology, surface expression or drug responses.[2]

We demonstrate here for the first time an ion sensing application of this light activation concept by placing a thin ion-selective polymeric film onto the modified silicon substrate. The underlying approach has been introduced recently with spin coated ion-selective films on gold or glassy carbon electrodes.[7] In that work, an intermediate transduction layer based on a conducting polymer film was used to control the amount of functional ion-exchanger sites in the overlaying film. The oxidation of the conducting polymer results in an incorporation of anionic counterions, as the oxidized polymeric form is cationic. To maintain electroneutrality, the original counter ion of the ion-exchanger is expelled from the thin polymeric film. If multiple receptors (ionophores) are present, with ion-exchanger in molar excess, an anodic potential scan results in the consecutive expulsion of cations from the polymeric film, starting from the least retained to the most retained one.[7a] This complete expulsion is evinced by peak-shaped current responses whose peak potential is dependent on each ion activity in solution. As the polymeric film is very thin, the potential can be scanned comparatively rapidly, at over 100 mV $s^{-1}$, thereby giving direct information about the concentration of multiple analytes at a single location.

[a] Dr. Y. Yang, Dr. V.R. Gonçales, Prof. J. J. Gooding
School of Chemistry
University of New South Wales
Sydney, NSW 2052
E-mail: justin.gooding@unsw.edu.au
[b] Dr. M. Cuartero, Prof. E. Bakker
Department of Inorganic and Analytical Chemistry
University of Geneva
Quai E.-Ansermet 30
1211 Geneva 4, Switzerland
Email: eric.bakker@unige.ch

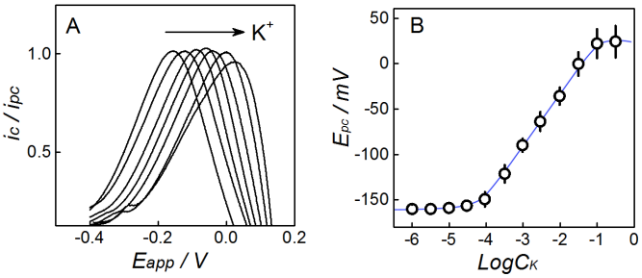
Supporting information for this article is given via a link at the end of the document.((Please delete this text if not appropriate))

The existing approaches that are able to monitor ions distributions mainly rely on fluorescence microscopy,[8] scanning electrochemical microscopy,[9] micro-arrayed electrodes,[10] and light-addressable potentiometric sensor (LAPS).[11] Among them, LAPS has been widely utilized due to its high flexibility for the measured area by light zooming in and out, and no requirement for wire connecting or microfabrication.[12] This work introduces a method with all the advantages as LAPS while using faradaic electrochemistry as a measurement output rather than capacitance current that is used in LAPS. Redox molecules covalently bounded to the oxide-free silicon electrode are expected to offer a direct and amplified signal compared with traditional LAPS and may in principle be extended to the detection of multiple ions.[13] Moreover, this ion sensing system may be compatible with other electrochemical approaches, such as cell-substrate impedance analysis,[1, 14] or integrated with the established single cell release platform,[2] and hence would offer vast opportunities to study complex biological events.




**Scheme 1.** Schematic of the light triggered $K^+$ sensor. A thin layer membrane containing the ionophore valinomycin assists in selective $K^+$ transfer during an electrochemical reduction process, triggered by the redox mediator ferrocene (Fc) that releases the lipophilic cation exchanger $R^-$ upon reduction. Light activation turns on the electrochemical sensing process at the illuminated region only.

Scheme 1 illustrates the strategy for spatially defined $K^+$ detection. With the purpose of achieving light activated electrochemistry at silicon electrode. Monolayers of 1,8-nonadiyne are applied to passivated silicon surfaces using an established procedure.[5a] Briefly, the hydrogen terminated silicon surface, formed by immersing the silicon in a 2.5% HF, is reacted with anhydrous 1,8-nonadiyne under an inert atmosphere. Ferrocene moieties are covalently attached to this monolayer. The ion-selective membrane contains a plasticized polyurethane matrix, a $K^+$ ionophore (valinomycin) and the cation exchanger $TFPB^-$ (shown as $R^-$ in Scheme 1) [7a]. The membrane is spin coated on the ferrocene terminated silicon (Fc-Si). The surface chemistry characterized by X-ray photoelectron spectroscopy was shown in Figure S1. As one of the most reproducible redox-active moieties at an electrode surface, ferrocene-terminated self-assemble monolayers are expected to trigger cation transfer through the ion-selective




membrane as the oxidation state of the ferrocene is changed. When ferrocene (Fc) is oxidized to ferrocenium ($Fc^+$) during cyclic voltammetry, it forms an ion pair with $TFPB^-$ and results in an expulsion of the detected cation (i.e. $K^+$ in this work). The reverse process occurs during a cathodic potential scan, as suggested in Scheme 1. Shifts in the potential of the voltammetric peak to drive this ion transfer are a function of the ion concentration. The shifts obey the Nernst equation and are used as the analytical signals.[13]

**Figure 1.** (A) Normalized cathodic peaks of the cyclic voltammograms for PU/Fc-Si with changing $K^+$ concentration, with $[K^+] = 10^{-6}$–$10^{-0.5}$ M, scan rate: 100 mV $s^{-1}$; (B) plot of cathodic peak potential change *versus* logarithmic $[K^+]$ shows linearity in a range of $[K^+] = 10^{-4}$–$10^{-1}$ M. Error bars represent the standard deviation of five measurements.

Experimental optimization for $K^+$ detection involved varying the thickness of the polyurethane (PU) membrane and the surface coverage of the ferrocene and are given in Figure S2 and S3. Pu/Fc-Si with a ferrocene coverage of $10^{-11}$ mol $cm^{-2}$ exhibited the expected cyclic voltammetry behavior with increasing KCl concentration, suggesting that $K^+$ can be reliably detected. Figure 1 shows the resulting calibration curves for $K^+$ using the optimized Pu/Fc-Si formulation. A Nernstian response was observed for the cathodic peak potential as a function of logarithmic $K^+$ concentration in 10 mM NaCl (slope of 60.1 mV) in the range of $10^{-4}$ M and $10^{-1}$ M, with a detection limit of just below ~0.1 mM in the same background. There are deviations from the linearity at lower and higher target ion activities, and this behaviour has been well-characterized in ion-selective potentiometric sensors. The lower limit originates from other interfering ions while the upper limit depends on the stability of the ionophore-analyte complex and the nature and concentration of co-extracting electrolyte.[15] The lower detection limit observed here satisfies the problem at hand. It is likely caused by the functional groups of polyurethane, which is preferred here over traditional poly(vinyl chloride) because of its superior adhesive properties and resulting sensor lifetime.[7b]

It was found that the electrochemical behavior for a tightly packed monolayer of ferrocene moieties (surface coverage of ~$10^{-10}$ mol $cm^{-2}$ by integration of the cyclic voltammograms) depends on the nature of the counter ion in aqueous solution (see Figure S2). The proposed ion to electron transduction mechanism requires the removal of a bulk $TFPB^-$ from the membrane, to form the surface confined counter ion of the $Fc^+$. It appears that the high packing density of ferrocene does not permit this to occur for steric reasons, resulting instead in a

substantial driving force for the transport and accumulation of smaller solution ions. However, the presence of $Cl^-$ may deteriorate the voltammetric response of PU/Fc-Si by forming irreversible ion adducts between $Fc^+$ and $Cl^-$, as reported earlier in the literature.[16] Although the overlaying sensing film should block extraction of KCl, salt transport is still possible by way of being dissolved in water droplets that exhibit mobility inside ion-selective polymer membranes.[17] This was further evaluated by repeating the experiments using only nitrate salts as solution electrolyte. A linear dependence between peak potential and logarithmic $KNO_3$ concentration was observed in the range of $[K^+]$ = 0.1−10 mM. However, as shown in Figure S3, $NO_3^-$ still gave a dramatic increase in peak current above 1 mM salt concentration, which is again likely relates to the formation of ion adducts between the $Fc^+$ mediator and $NO_3^-$. To minimize the steric hindrance of ion-pairing with hydrophobic anions, ferrocene coverage was reduced by decreasing the concentration of azidomethyl-ferrocene and shortening the time for the *'click'* reaction, giving the desired response characteristics shown in Figure 1. The decreased ferrocene coverage still maintained the silicon in oxide-free state and the redox properties of ferrocene can be fully recovered after removing the $K^+$ selective polymer matrix, as shown in Figure S5.

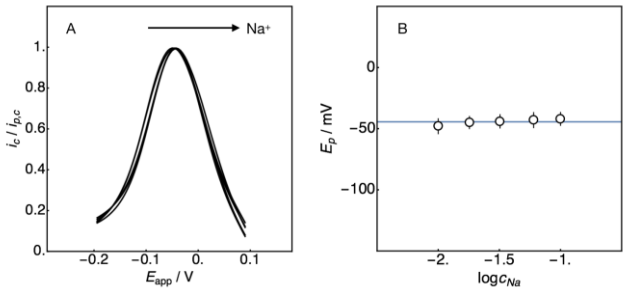



**Figure 2**. (A) Normalized cathodic cyclic sweeps for PU/Fc-Si with increasing [NaCl] in the presence of 10 mM KCl, scan rate: 100 mV $s^{-1}$; (B) plot of cathodic peak potential change *versus* logarithmic $[Na^+]$.

To demonstrate chemical selectivity, the voltammetric behaviour of PU/Fc-Si was evaluated in a 10 mM $K^+$ electrolyte (similar to extracellular levels) with increasing $Na^+$ concentration. Figure 2 shows that the addition of NaCl only gives marginal changes to the reduction peak potential, ca. 6 mV with $[Na^+]$ increasing from 10 mM to 100 mM. This confirms excellent selectivity for $K^+$ over $Na^+$ for the PU/Fc-Si sensing electrode, as expected for a valinomycin-based membrane, and suggests its applicability for physiological measurements.

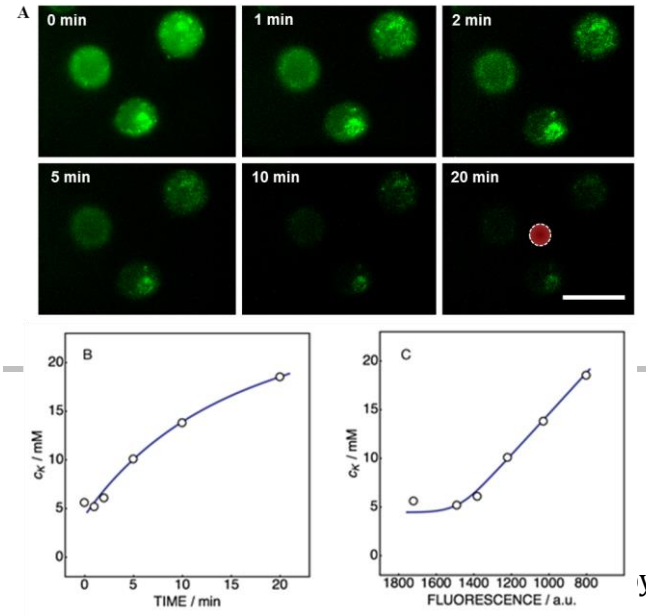



**Figure 3.** (A) Time-dependent fluorescence microscopy imaging (FITC filter) of APG-2-stained MCF-7 cells stimulated with valinomycin at t = 0 (before the addition of valinomycin) and t = 1, 2, 5, 10, 20 min after adding valinomycin (20 μM final concentration) into culture medium containing ~5.4 mM of KCl. Scale bar is 20 μm, the red spot indicates the laser beam size and hence the position during cyclic voltammetry measurement. (B) $[K^+]$ at extracellular region near cells obtained from the cyclic voltammetry in real time; (C) the relationship between extracellular $[K^+]$ and the average fluorescence intensity change of intracellular $K^+$ at a given time point.

The suitability of PU/Fc-Si for the detection of potassium ions in real physiological fluids was tested for real-time monitoring of the extracellular $[K^+]$. A human breast cancer MCF-7 cell line was chosen as model cells. Intracellular $K^+$ was also monitored using Asante Potassium Green-2 (APG-2) dye, which is a fluorescent indicator for $K^+$.[18] Valinomycin, which is a cyclic peptide-like molecule that binds $K^+$, may result in leakage of $K^+$ from the cells, was applied to stimulate the cells.[19] The release of $K^+$ across the cellular membrane would increase extracellular $[K^+]$. Accordingly, cyclic voltammetry was recorded before and after adding valinomycin in the illuminated region close to cells (Figure S5C). Note that a laser beam (FWHM of around 5 μm) was integrated with the microscope used for imaging and applied to localize the electrochemistry to a specific region. According to previous studies, the light intensity change over silicon substrate will alter the space charge layer depth, minor carrier concentration, and the band bending level, as a result, there will be an anodic/cathodic shift in the cyclic voltammetry for the redox species attached on p/n-type silicon with the increase of the illumination intensity.[6, 20] For this reason, a separate calibration curve was obtained by using the laser beam light source in the cell culture medium with increasing $[K^+]$ (Figure S6A). A similar Nernst response was found while the overall redox peaks moved to more negative values due to light intensity increase.

To show this new technique can quantify the amount of ions released by a single or a few cells, the antibiotic valinomycin was used as the stimulant. With the addition of the selective carrier valinomycin to the MCF-7 cells, the fluorescence signals representing the intracellular $K^+$ significantly decreased in 20 min while for those cells without valinomycin stimulus there is no appreciable change of the fluorescence intensity (Figure S9A). This diminution in fluorescence indicates efflux of $K^+$ from the MCF-7 cells after valinomycin stimulation (Figure 3A). Correspondingly, the reduction peaks obtained from the region of these three cells moved to more positive potentials after loading valinomycin (Figure S6C). Together with the calibration curve in Figure S6A, B, the extracellular $[K^+]$ was found to change from ~6 mM to ~17 mM. There were no appreciable current decrease or original redox peak shift except for $[K^+]$ change during continuous extracellular $[K^+]$ monitoring,

indicating the strong stability of PU/Fc-Si surface. The cell free PU/Fc-Si surface with valinomycin treatment also showed no notable change (Figure S6D), ruling out significant contamination of the ion-selective valinomycin doped membrane. This control experiment further confirms that $K^+$ efflux from cells did occur in Figure 3. Importantly, we note that extracellular $K^+$ concentration increased (nearly linearly after loading valinomycin) in tandem with the observed decrease of the fluorescence intensity of $K^+$ inside the cells (Figure 3C). A further attempt to exactly localize the electrochemistry to one single cell by lower cell surface coverage was also conducted, and the results are summarized in Figure S7. Figure S7D shows the change in extracellular $K^+$ concentration obtained from the electrochemical measurements is still positively correlated to the fluorescence decrease, which confirms the high sensitivity the light addressable potentiometric $K^+$ sensor. Trypan blue test assay[21] were performed over the cells after electrochemical measurements, and the results (Figure S9B) showed the cells after electrochemistry on the platform but without valinomycin treatment shows a viability of 88.8% ± 1.4% while the MCF-7 cells in culture have viability of 94.2 ± 1.2%, suggesting that viability was not affected by the electrochemical process. This value slightly dropped to 80.3 ± 3.8% when the cells were treated with 20 μM valinomycin, however, this change is mainly affected by valinomycin which can potentially damage the integrity of the cell membrane[19] and is not related to the electrochemical measurement.

In summary, a light addressable potentiometric $K^+$ sensor based on a polymeric membrane and valinomycin ionophore was successfully developed on a semiconducting silicon electrode. This PU/Fc-Si electrode exhibits Nernstian response to $[K^+]$ from $10^{-4}$ to $10^{-1}$ M in a light-defined spatial region and shows high selectivity for $K^+$ in presence of $Na^+$. Using such a light addressable potentiometric $K^+$ sensor, changes in the $K^+$ concentration assloicated with living cells upon stimulation could be monitored in real-time electrochemiclaly to give extracellular $K^+$ and the intracellualr levels using fluorescence micrscopy simultaneously. These results indicate the potential application of the electrode for analyte imaging, which would also have broader detection range compared with the existed fluoresence probes, such as APG-2 dye. Extending this work to other potentiometric sensing membranes will enable multiple ion sensing or mapping under various conditions. By integrating with other electrochemical sensing platforms, the light addressable potentiometric sensor would have wide applications, such as using microneedle arrays to monitor intracellular ion signals would be an effective approach to study some essential biological events.

## Experimental Section

**Preparation of PU/Fc-Si Electrode.**

The self-assembly of monolayers of 1,8-nonadiyne was performed on silicon following a thermal hydrosilylation reaction as previously reported.[5a] Briefly, the silicon wafer was immersed in hot piranha solution (1:3 v/v 30% $H_2O_2$/98% $H_2SO_4$) and etched with 2.5% hydrofluoric acid for 90s. Silicon was then immediately transferred into thoroughly degassed 1,8-nonadiyne (through more than five freeze−thaw cycles) in a custom-designed Schlenk flask. The reaction vessel was kept at 165 °C for 3 h under an argon atmosphere. After cooling down, the silicon wafer was cleaned thoroughly with dichloromethane. Subsequently azidomethylferrocene was attached to the obtained alkyne-functionalized silicon through the Cu(I)-catalysed alkyne-azide cycloaddition reaction.[5b, 6] In short, the silicon was placed in an isopropanol/water (2:1 ratio) solution containing 45 mM azidomethylferrocene, 0.36 mM copper(II) bromide, and 36 mM sodium ascorbate. The coupling reaction was carried out at room temperature for 5-45 min while excluding air from the reaction environment. The ferrocene terminated silicon (Fc-Si) samples were rinsed consecutively with copious copious amounts of water, ethanol, and dichloromethane. The obtained Fc-Si could be stored under argon atmosphere for subsequent use.

Ion-selective membrane was spin coated on Fc-Si at 2500 rpm for 1 min. An ohmic contact was obtained by first polishing the backside of Si wafer with abrasive paper, and subsequently adding Ga-In eutectic (Alfa-Aesar, 99.99%). Subsequently, the PU/Fc-Si was mounted in electrochemical chamber for further test.

**~~Electrochemical Measurements.~~**

~~All electrochemical experiments were performed in a PTFE three-electrode cell with modified silicon as the working electrode and a platinum mesh as the counter electrode, and all potential values are referenced to as the Ag|AgCl|3 M KCl electrode. An ACE light source (Schott AG, Mainz, Germany) was employed as the light source for whole electrode surface illumination during electrochemical experiments. A 642 nm pigtailed laser diode (LP642-PF20, Thorlabs) mounted with a laser diode and temperature controller (ITC4001, Thorlabs) was connected to a laser fiber and integrated with microscope to perform spatially-defined illumination.~~

**Extracellular $[K^+]$ Detection**

2 μL of APG-2 stock solution (10 mM in DMSO) was mixed with an equal volume of 25 % w/v Pluronic F-127 solution (DMSO) immediately prior to its addition to the 2 mL of MCF-7 cell suspensions ($1\times10^6$ cell $mL^{-1}$). After incubation for 1 h, the well stained cells were centrifuged and resuspended to remove the APG-2 in cell culture medium. The obtained cell suspension was loaded into the electrochemical chamber, and cyclic voltammetry were performed before and after treating with valinomycin (20 μM final concentration). Fluorescence images were taken to indicate the intracellular $K^+$ levels (excitation at 488 nm). The calbiration curves were obtained in cell culture medium but without cells.

## Acknowledgements

E.B. thanks the Swiss National Science Foundation for supporting this research and scientific stay at UNSW. We acknowledge the financial support from the Australian Research Council Centre of Excellence in Convergent Bio-Nano Science and Technology (CE14100036) and the ARC Australian Laureate Fellowship (FL150100060). The authors thank Yanfang Wu for performing film thickness measurements to optimize ion-selective membrane.

**Keywords:** ion-selective electrode · light activated electrochemistry · potassium · physiological condition · potentiometry

[1] M. Parviz, K. Gaus, J. J. Gooding, *Chem. Sci.* **2017**, *8*, 1831-1840.
[2] S. G. Parker, Y. Yang, S. Ciampi, B. Gupta, K. Kimpton, F. M. Mansfeld, M. Kavallaris, K. Gaus, J. J. Gooding, *Nat. Commun.* **2018**, *9*, 2288.
[3] aM. H. Choudhury, S. Ciampi, Y. Yang, R. Tavallaie, Y. Zhu, L. Zarei, V. R. Gonçales, J. J. Gooding, *Chem. Sci.* **2015**, *6*, 6769-

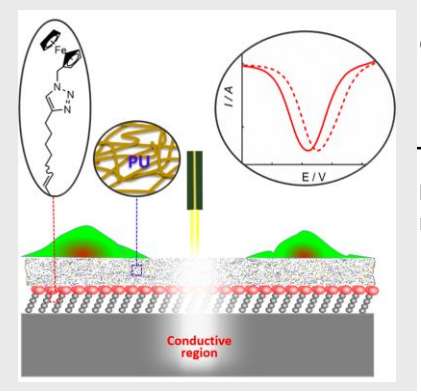
6776; bY. Yang, S. Ciampi, Y. Zhu, J. J. Gooding, *J. Phys. Chem. C* **2016**, *120*, 13032-13038.
[4] B. Fabre, *Chem. Rev.* **2016**, *116*, 4808-4849.
[5] aS. Ciampi, P. K. Eggers, G. Le Saux, M. James, J. B. Harper, J. J. Gooding, *Langmuir* **2009**, *25*, 2530-2539; bS. Ciampi, G. Le Saux, J. B. Harper, J. J. Gooding, *Electroanal.* **2008**, *20*, 1513-1519.
[6] Y. Yang, S. Ciampi, J. J. Gooding, *Langmuir* **2017**, *33*, 2497-2503.
[7] aM. Cuartero, G. A. Crespo, E. Bakker, *Analytical Chemistry* **2016**, *88*, 1654-1660; bM. Cuartero, G. A. Crespo, E. Bakker, *Anal. Chem.* **2016**, *88*, 5649-5654; cX. Shan, U. Patel, S. Wang, R. Iglesias, N. Tao, *Science* **2010**, *327*, 1363-1366.
[8] C. Stosiek, O. Garaschuk, K. Holthoff, A. Konnerth, *Proc. Natl. Acad. Sci.* **2003**, *100*, 7319-7324.
[9] B. P. Nadappuram, K. McKelvey, R. Al Botros, A. W. Colburn, P. R. Unwin, *Anal. Chem.* **2013**, *85*, 8070-8074.
[10] X. J. Huang, A. M. O'Mahony, R. G. Compton, *Small* **2009**, *5*, 776-788.
[11] T. Yoshinobu, K.-i. Miyamoto, C. F. Werner, A. Poghossian, T. Wagner, M. J. Schöning, *Annu. Rev. Anal. Chem.* **2017**, *10*, 225-246.
[12] D. G. Hafeman, J. W. Parce, H. M. McConnell, *Science* **1988**, *240*, 1182-1185.
[13] G. A. Crespo, M. Cuartero, E. Bakker, *Anal. Chem.* **2015**, *87*, 7729-7737.
[14] P. O. Bagnaninchi, N. Drummond, *Proc. Natl. Acad. Sci.* **2011**, 201018260.
[15] E. Bakker, P. Bühlmann, E. Pretsch, *Electroanal.* **1999**, *11*, 915-933.
[16] G. Valincius, G. Niaura, B. Kazakevičienė, Z. Talaikytė, M. Kažemėkaitė, E. Butkus, V. Razumas, *Langmuir* **2004**, *20*, 6631-6638.
[17] aE. Lindner, T. Zwickl, E. Bakker, B. T. T. Lan, K. Tóth, E. Pretsch, *Anal. Chem.* **1998**, *70*, 1176-1181; bR. De Marco, J.-P. Veder, G. Clarke, A. Nelson, K. Prince, E. Pretsch, E. Bakker, *Phys. Chem. Chem. Phys.* **2008**, *10*, 73-76.
[18] X. Chen, W.-t. He, L. Hu, J. Li, Y. Fang, X. Wang, X. Xu, Z. Wang, K. Huang, J. Han, *Cell Res.* **2016**, *26*, 1007.
[19] C. Levinson, *Nature* **1967**, *216*, 74.
[20] Y. Yang, S. Ciampi, M. H. Choudhury, J. J. Gooding, *J. Phys. Chem. C* **2016**, *120*, 2874-2882.
[21] W. Strober, *Curr. Protoc. Immunol.* **1997**, *21*, A. 3B. 1-A. 3B. 2.

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

Layout 1:

## COMMUNICATION

A light addressable electrochemical $K^+$ sensor is introduced that consists of a thin polymer film containing an ionophore deposited on a semiconducting silicon electrode. Ferrocene is covaltently attached on oxide-free silicon and the electrode exhibits surface confined electrochemistry under light illumination. The sensor exhibits a Nernstian response to $[K^+]$ in a light-defined spatial region. The changes in extracellular $[K^+]$ associated with living cells upon stimulation is monitored electrochemically.




*Ying Yang,[a] Maria Cuartero[b], Vinicius Romero Gonçales[a], J. Justin Gooding*[a] and Eric Bakker*[b]*

***– Page No.***

**Light addressable ion sensing for real-time monitoring of extracellular potassium**

Layout 2:

## COMMUNICATION

((Insert TOC Graphic here))

*Author(s), Corresponding Author(s)**

***Page No. – Page No.***

**Title**

Text for Table of Contents

[a] Dr. Y. Yang, Dr. V.R. Gonçales, Prof. J. J. Gooding
School of Chemistry
University of New South Wales
Sydney, NSW 2052
E-mail: justin.gooding@unsw.edu.au
[b] Dr. M. R. Botia, Prof. E. Bakker
Department of Inorganic and Analytical Chemistry
University of Geneva
Quai E.-Ansermet 30
1211 Geneva 4, Switzerland
Email: eric.bakker@unige.ch

Supporting information for this article is given via a link at the end of the document.((Please delete this text if not appropriate))