Supporting information

A Fluorescent Ratiometric Potassium Sensor Based on IPG4-Silica Microparticles for Selective Detection and Fluorescence Imaging of Potassium Cations

Francesco Colella[a,b], Stefania Forciniti[a], Valentina Onesto[a], Giuliana Grasso[a], Helena Iuele[a], Giuseppe Gigli[a, c], Loretta L. del Mercato[a,*]

[a] *Institute of Nanotechnology, National Research Council (CNR-NANOTEC), c/o Campus Ecotekne, via Monteroni, 73100, Lecce, Italy;*

[b] *Department of Mathematics and Physics ''Ennio De Giorgi", University of Salento, c/o Campus Ecotekne, via Monteroni, 73100, Lecce, Italy;*

[c] Department of Experimental Medicine, University of Salento, c/o Campus Ecotekne, via Monteroni, 73100, Lecce, Italy.

Content:

Chemicals

ION Potassium green 4 (IPG4 TMA salt) was purchased from ION Biosciences (ION Biosciences, United States), Rhodamine B isothiocyanate (RBITC, BioReagent mixed isomers), 7-(Diethylamino)coumarin-3-carboxylic acid (7AAC1, BioReagent, suitable for fluorescence, ≥98.0% HPCE), Tetraethyl orthosilicate (TEOS, Reagent grade, 98%), (3-aminopropyl)triethoxysilane (APTES, 99.0%), N,N-Dimethylformamide (DMF, anhydrous, 99.8%), Potassium chloride (KCl, Bioreagent ≥99.0%), 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU, 97.0%) N,N-Diisopropylethylamine (DIPEA, Reagentplus, ≥99.0%), Nigericin sodium salt (TLC ≥98%) Ninhydrin (ACS Reagent), CF™ 660 succimidyl ester (suitable for fluorescence, TLC ≥90%) and 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES, BioReagent, ≥99.0%), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, ≥97.0%), and poly-L-lysine hydrobromide (mol wt 30,000-70,000; CAS Number: 25988-63-0, #P2636)were all purchased from Sigma-Aldrich (Millipore-Sigma, United States), Ethanol 96% vol (EtOH, VWR Chemicals BDH®) was purchased from VWR (United States), Ammonium hydroxide solution (NH_4OH, ACS Reagent 28.0-30.0%) was purchased from Honeywell Fluka™ (United States), Cyanine 3 NHS ester (Cy3, HPLC-MS ≥95%) and Cyanine 5.5 NHS ester (Cy5.5, HPLC-MS ≥95%) were purchased from Lumiprobe (Lumiprobe GmbH, Germany), Alexa Fluor™ 594 NHS ester and Alexa Fluor™ 647 NHS ester were purchased from Thermo Fisher (Thermo Fisher Scientific Inc., United States).

Table S1. Table of the used fluorescent dyes.

Dye	$\lambda_{ex\ max}$ (nm)	$\lambda_{em\ max}$ (nm)	FRET	Ratiometric MPs
7ACC1*	405	450	Yes	No
IPG4	525	545		
RBITC	546	586	Yes	Yes
Cy3	555	570	Yes	Yes
A594	590	617	Yes	No
A647	650	671	Yes	No
CF660	667	685	Yes	No
Cy5.5	683	703	Yes	No

* 7-(Diethylamino)coumarin-3-carboxylic acid

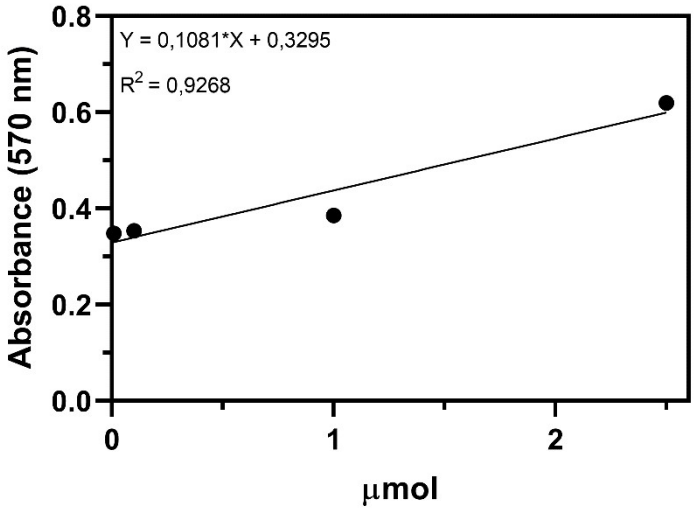

Figure S1. Calibration curve of the ninhydrin test. The calibration curve was performed with Glycine as quantitative standard, the absorption spectra was recorded with ClarioSTAR microplate reader (BMG Labtech, Germany).

Table S2. Results of the ninhydrin assay.

Sample	Amine quantification (µmol/mg)
SiO_2@NH_2	1.265
SiO_2@Cy3	0.605
SiO_2@RBITC	0.423
SiO_2@Cy3-IPG4	0.657
SiO_2@RBITC-IPG4	0.320

The results are shown as µmol over mg of silica microparticles.

Table S3. Kinetic analyses performed on free IPG4 probe and SiO_2@IPG4 MPs.

	λ_{ex} (nm)	K_d K^+ (mM)	B_{max} K^+ (mM)	K_d Na^+ (mM)	B_{max} Na^+ (mM)
IPG4	488	17.55	107.8	2.33	15.64
	525	21.19	110.8	5.86	16.50
SiO_2@IPG4	488	19.17	104.9	3.84	26.90
	525	28.46	106.3	6.18	29.87

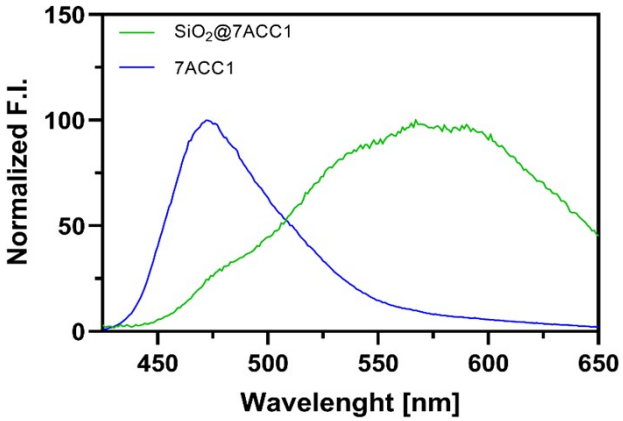

Figure S2. Spectra shift of 7ACC1 from free to bound on silica microparticles. Emission spectra were recorded with ClarioSTAR microplate reader (BMG Labtech, Germany), λ_{ex} 405 nm.

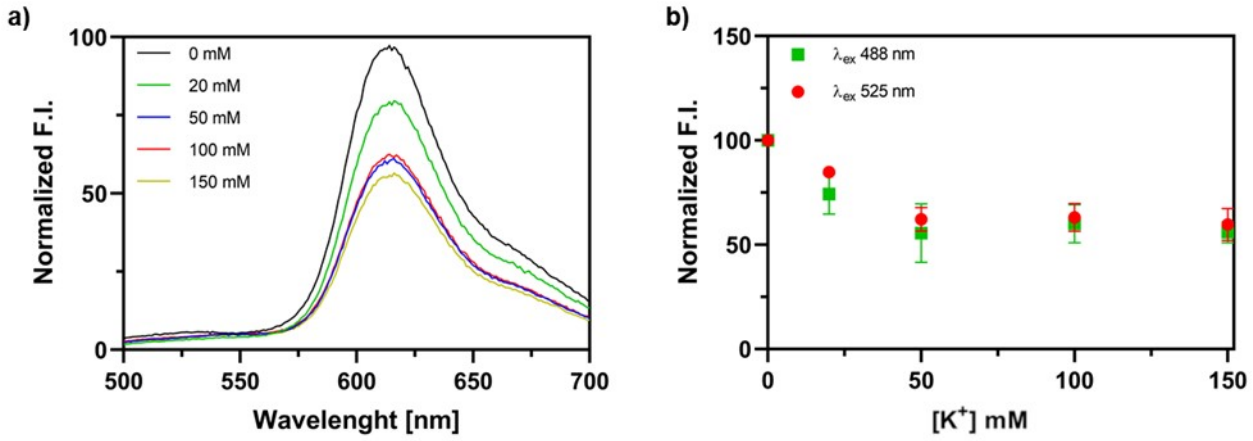

Figure S3. **Calibration of SiO_2@IPG4-A594 silica microparticles. a)** Emission spectra of SiO_2@IPG4-A594 exposed to MES buffer with different K^+ concentrations (λ_{ex} 488nm). **b)** Normalized fluorescence intensity plot of SiO_2@IPG4-A594 exposed to MES buffer with different concentrations of K^+ (λ_{ex} 488 nm and 525 nm). The analysis was performed on a CLARIOstar plate reader (BMG Labtech, Germany) by using spectral scan mode, in a 96 well plate, samples were suspended in MES buffer (50 mM, pH 7.5).

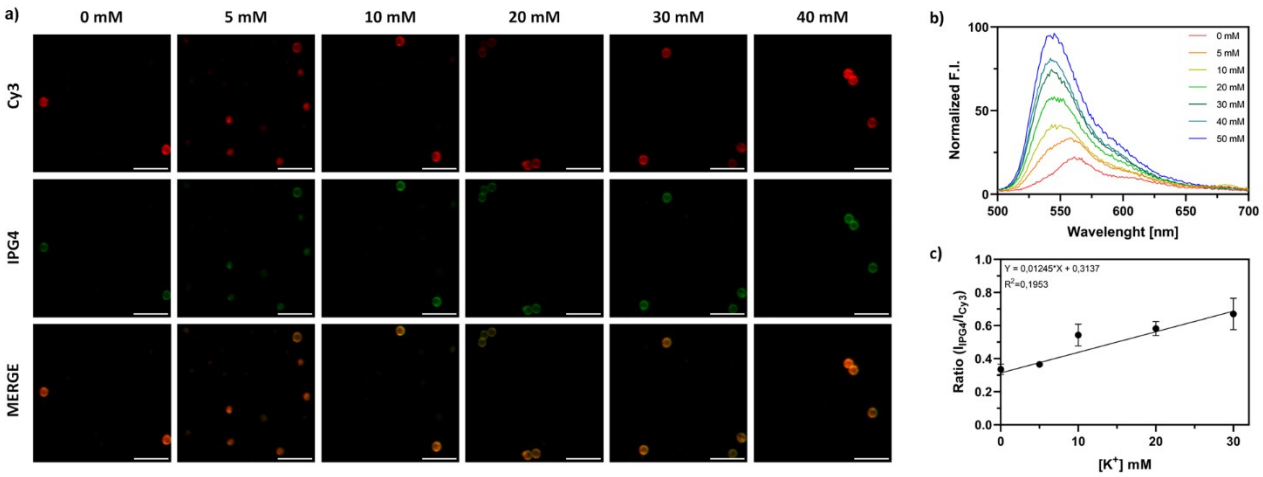

Figure S4. Calibration of K^+-sensors. a) Representative CLSM micrographs of SiO_2@Cy3-IPG4 incubated in MES buffer (50 mM) with adjusted concentration of KCl (0, 5, 10, 20, 30, 40 mM). Cy3 red channel (λ_{ex} 555 nm, λ_{em} 600-700 nm), IPG4 green channel (λ_{ex} 488 nm, λ_{em} 500-600 nm), ZEISS LSM 700 objective 63X, zoom 2, scale bars 5 µm; **b)** emission spectra of SiO2@Cy3-IPG4 microparticles incubated in MES buffer (50 mM) with adjusted concentrations of KCl (0, 5, 10, 20, 30, 40, 50 mM) with λ_{ex} 488 nm; **c)** calibration curve of SiO_2@Cy3-IPG4 based on CLSM image analysis, on the top the plot with K^+ concentration from 0 to 30 mM, on the bottom the plot with K^+ concentrations from 0 to 40 mM, standard error shown as SEM bars.

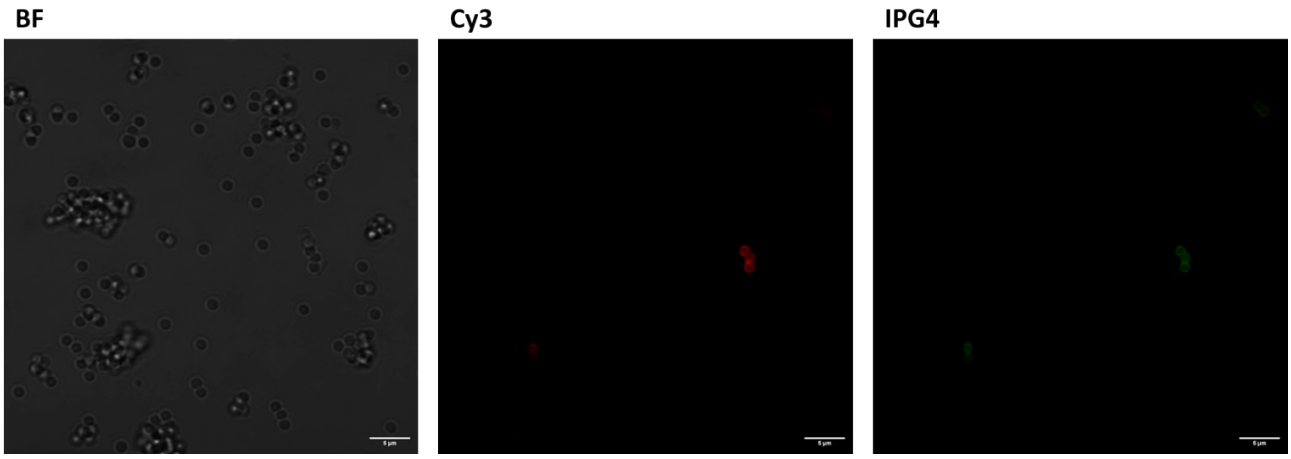

Figure S5. CLSM micrographs of SiO_2@Cy3-IPG4. Micrographs acquired using PlanApochromatic 63X/1.4 oil DIC objective, zoom 2. Bright field acquired with the 555 nm laser line, red channel of Cy3 acquired with 555 nm laser line, green channel of IPG4 acquired with the 488 nm laser line.

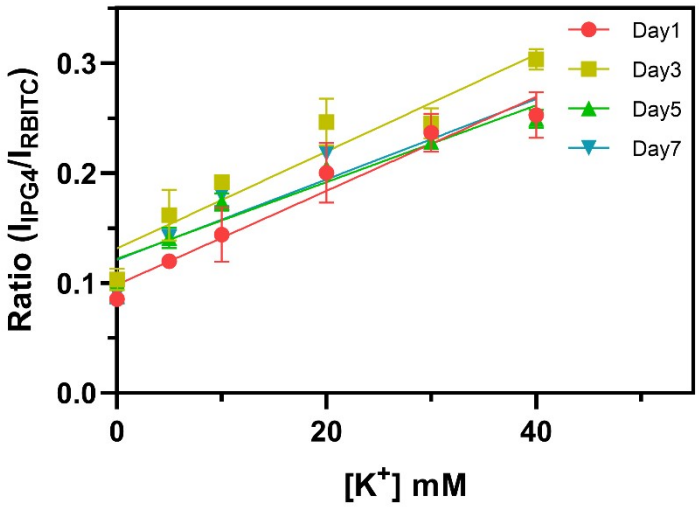

Figure S6. Results of the stability test performed using SiO_2@RBITC-IPG4 MPs over 7 days.

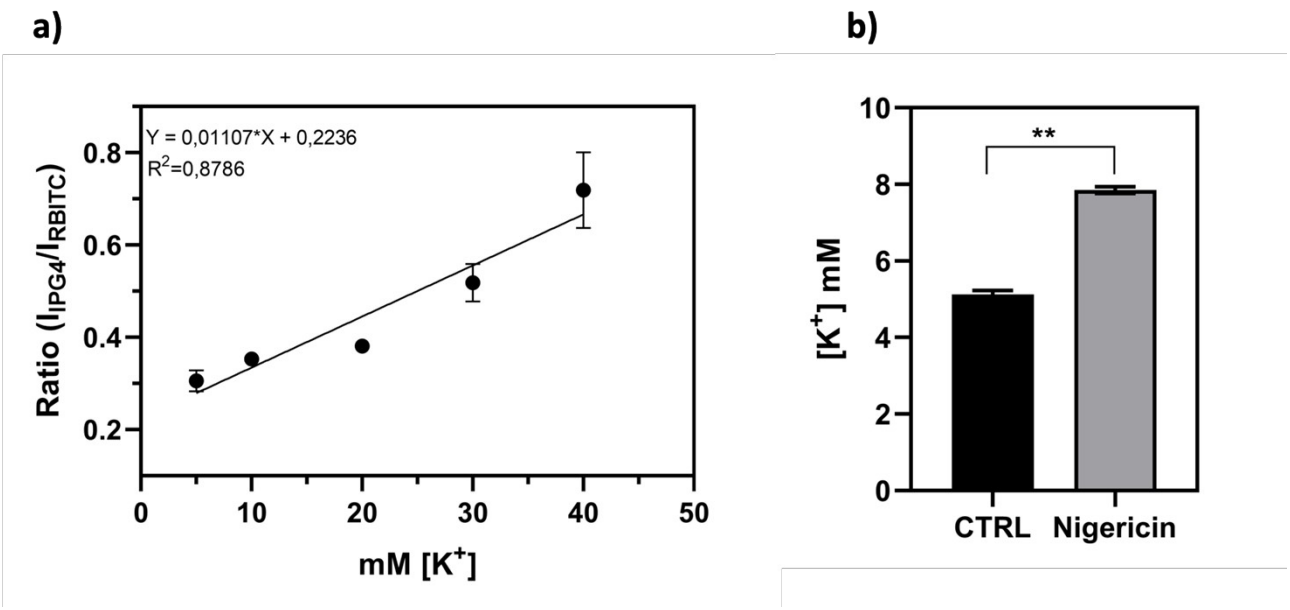

Figure S7: Fluorometric determination of K^+ concentration in cell culture media. a) Ratiometric calibration of SiO_2@RBITC-IPG4 in Leibovitz's L-15 media in the range of [K+] = 5-40 mM (λ_{ex} RBITC 555 nm, λ_{em} 570-650 nm; λ_{ex} IPG4 488 nm, λ_{em} 510-570 nm); **b)** Determination of K^+ concentration variations in cell culture media from SKMEL-2 cells treated with or without 1μM of Nigericin.