Electronic Supplementary Information

Ion-modulated interfacial fluorescence in droplet microfluidics using an ionophore-doped oil

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Experimental Section

Reagents and Materials:

Potassium ionophore I (valinomycin, K-I), sodium ionophore X, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), tetramethylrhodamine ethyl ester (TMRE), methylene blue (MB), bis(2-ethylhexyl) sebacate (DOS), Trizma base, KCl, NaCl, $MgCl_2$, $CaCl_2$ were purchased from MilliporeSigma. SYLGARD™ 184 Silicone Elastomer Kit was obtained from Dow. Human cerebrospinal fluid was purchased from Lee Biosolutions Inc.

Chip Fabrication and Microfluidic Operation:

The microfluidic chip consists of a glass slide spin-coated with a thin layer of PMDS as the substrate and a PDMS cover with 80 μm-deep microchannels. A T-junction design is used for the droplet generation. The channel width is 80 μm except that the inlet channel of the aqueous line is 40 μm wide. The channel after the T-junction has a zig-zag structure. The pressure may cause slight swelling of the channel during use. The PDMS cover is fabricated by a standard soft lithography procedure. Briefly, the designed T-junction pattern is created on a SU8 silicon wafer mold. Then, a mixture of the PDMS elastomer monomer and curing agent at a ratio of 10:1 (w/w) is degassed and poured into a glass petri dish with the SU8 mold on the bottom. After 30 min of vulcanization at 90°C, the PDMS stamp is peeled off carefully and adhered to the glass slide coated with a partially cured PDMS layer. The chip is ready to use after another 30 min of vulcanization at 90°C.

Sensing chemicals are dissolved in DOS via 30 minutes of sonication. The microfluidics operation and sample introduction are the same as those in our previous report.[1] The flow rate for both streams is 2 μL/min controlled by a Pump 11 Pico Plus Elite. A 6-port injection valve is used to introduce ~ 10 μL aqueous sample into a carrier DOS oil. All salt solutions are prepared in 0.1 M Tris-HCl buffer at pH 7.4. Human cerebrospinal fluid is diluted 100-fold with the Tris-HCl buffer before any testing and spiking.

Instruments:

The microfluidic chip is mounted on a Zeiss LSM 710 confocal laser scanning microscope equipped with a 10× dry objective lens for fluorescence detection. Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES) is used as the reference technique for the determination of K^+ in real samples.

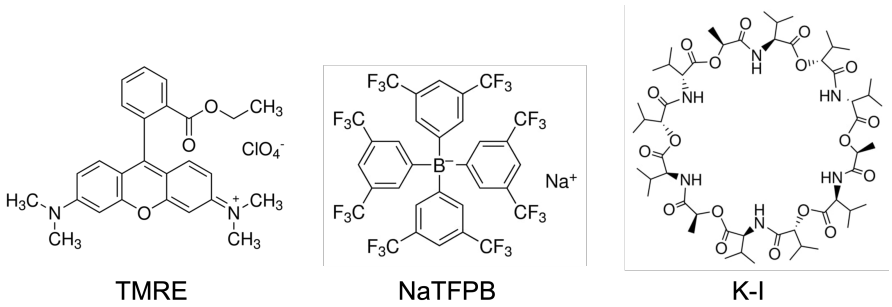

Figure S1. Structures of sensing chemicals used in the K^+-sensing oil.

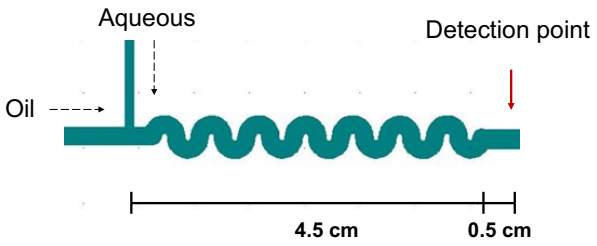

Figure S2. Design of the microfluidic channel for droplet formation.

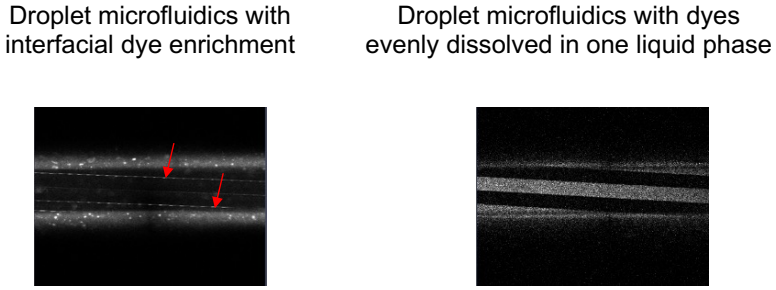

Figure S3. Fluorescence images of the flow at the downstream end of the microfluidic channel captured via the confocal microscope. The distorted pattern is because the oil and water phases are flowing during the scanning process. Left: Droplet microfluidics formed by the K^+-sensing DOS oil and the buffer containing 10^{-2} M KCl. The experimental conditions are the same as those in Figure 1B. Red arrows indicate the oil-to-water interfaces. Right: Droplet microfluidics formed by pure DOS and the buffer containing 0.1 μM resorufin. This system is not related to K^+ sensing but used to demonstrate what the fluorescence image looks like when the dye is dissolved in one phase without interfacial enrichment.

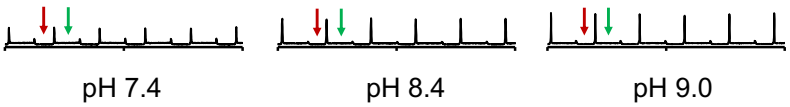

Figure S4. Fluorescence trace of droplet microfluidics formed by the K^+-sensing oil and the Tris-HCl buffer at different pH containing 10^{-4} M KCl. Red and green arrows indicate the aqueous and oil phases, respectively. Fluorescence spikes indicate the oil-to-water interfaces.

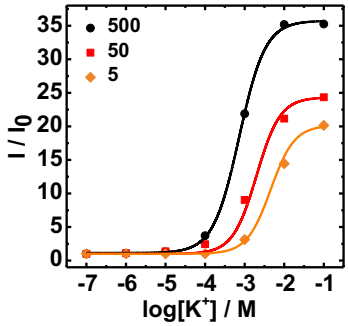

Figure S5. Interfacial fluorescence-based response of sensing oils containing 0.1 μM TMRE, 0.1 μM TFPB, and different ratios of valinomycin as the ionophore. I_0 and I denote the interfacial fluorescence for buffer and KCl concentrations, respectively.

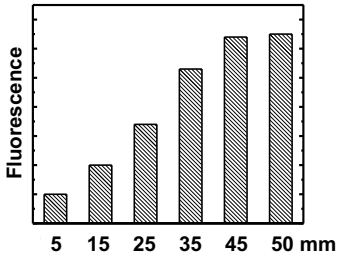

Figure S6. Interfacial fluorescence intensity as a function of the distance from the T-junction along the microchannel. The aqueous phase is 10^{-2} M KCl in Tris-HCl buffer at pH 7.4.

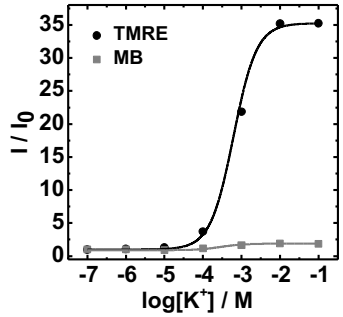

Figure S7. Comparison of TMRE and MB as the dye to generate interfacial fluorescence upon extraction of K^+. The oil contains 0.1 μM dye, 0.1 μM TFPB, and 50 μM K-I. "I" denotes the interfacial fluorescence intensity for each cation concentration. "I_0" denotes the interfacial fluorescence intensity when the sample is the Tris-HCl buffer.

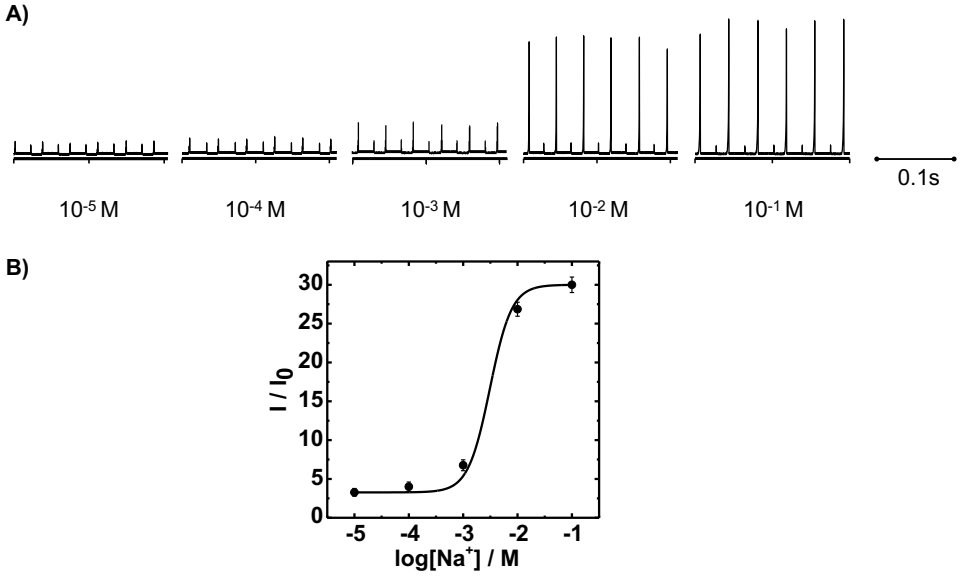

Figure S8. A: Fluorescence trace of droplet microfluidics formed by the sensing oil and 0.1 M Tris-HCl buffer containing 10^{-5} to 10^{-1} M Na^+. The sensing oil is DOS containing 0.1 μM TMRE, 0.1 μM TFPB, and 0.5 mM sodium ionophore X. B: The corresponding calibration curve based on the interfacial fluorescence. "I" denotes the interfacial fluorescence intensity for each cation concentration. "I_0" denotes the interfacial fluorescence intensity when the sample is the Tris-HCl buffer.

Table S1. K^+ concentrations in cerebrospinal fluid determined by the proposed droplet microfluidics and ICP-OES as the standard method.

	Standard method / mM	Proposed method / mM	Percent error	Recovery rate
Sample	8.7	9.2	5.7%	
Sample + 1.0 mM		10.1		90%
Sample + 3.0 mM		11.9		90%

Reference:

1. Anal. Chem. 2021, 93, 13694−13702