Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Ionophore-Based Biphasic Chemical Sensing in Droplet Microfluidics

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Reagents

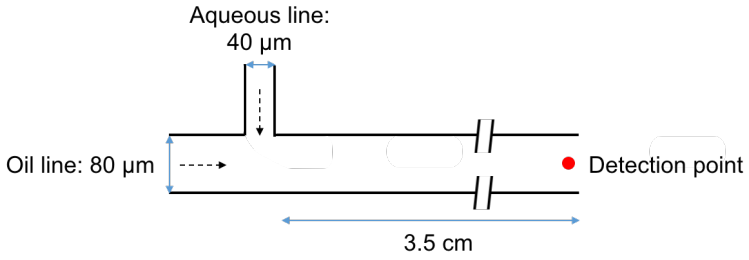
Chromoionophore I, chromoionophore III, potassium ionophore II, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (KTFPB), tetradodecylammonium nitrate ($TDDANO_3$), tridodecylmethylammonium chloride (TDMACl), dioctyl sebacate, 1,2-dichloroethane, protamine sulfate salt from salmon, heparin sodium salt from porcine intestinal mucosa, hydrogen peroxide (30 % (w/w) in H_2O), Trizma® base, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), sodium phosphate dibasic and sodium phosphate monobasic were purchased from MilliporeSigma. Sodium ionophore IV and [9]mercuracarborand-3 were obtained from Euvive, LLC. Dinonylnaphthalene sulfonic acid (DNNSH) is a gift from King Industries. The boronic acid-functionalized boron-azadipyrromethene (azaBDPBA) was obtained from Nanjing Agile Biotec, Inc. Venous sheep blood was obtained from Department of Surgery at the University of Michigan.

Instruments

Fluorescence intensity over time was monitored using an ISS Alba time-resolved confocal microscope equipped with a 20× air objective lens. A Fianium WhiteLaser coupled with an Acousto-Optic Tunable Filter (AOTF) was used as the light source. An avalanche photodiode (APD) detector was used to collect photon signals. Bright-field images of the segmented flow were collected on the confocal microscope by using a high-speed camera (Phantom Miro eX2, Vision Research). A PTI Quanta Master fluorimeter was used to acquire fluorescence spectra of the bulk oil solutions. Visible absorption spectra were obtained on a PerkinElmer LAMBDA 35 UV-Vis spectrophotometer.

Chip fabrication

The PDMS chip bonded onto a PDMS-coated glass side was fabricated according to a conventional soft-lithography protocol.[1] The channel depth is 40 μm. Other channel parameters are shown below:



Sensing oil solutions

The K^+-sensing oil for fluorescent analysis is dioctyl sebacate (Figure 1B) containing 57 μg/mL (100 μM) chromoionophore III, 177 μg/mL (200 μM) NaTFPB, and 216 μg/mL (300 μM) potassium ionophore II. To follow the color change of the oil droplets in the microchannel (Figure 1A), the sensing chemicals are 5-fold more concentrated in the oil. The Na^+-sensing oil is dioctyl sebacate containing 57 μg/mL (100 μM) chromoionophore III, 180 μg/mL (200 μM) KTFPB, and 180 μg/mL (400 μM) sodium ionophore IV. The Cl^--sensing oil is dioctyl sebacate containing 47 μg/mL (80 μM) chromoionophore I, 93 μg/mL (120 μM) $TDDANO_3$, and 370 μg/mL (360 μM) [9]mercuracarborand-3. The protamine-sensing oil is 1,2-dichloroethane containing 2.9 μg/mL (5 μM) of chromoionophore I and 2.3 μg/mL (5 μM) of DNNSH. Notably, higher power of laser was used for protamine sensing and there is a background of fluorescence when the aqueous droplet passed through the detection point. In Figure 4, to facilitate comparison of the fluorescence in the oil segments, such background was not shown because the x-axis does not intersect y-axis at zero. However, they intersect at zero for all other fluorescent traces shown in this paper. The H_2O_2-sensing oil is dioctyl sebacate containing 41 μg/mL (70 μM) azaBDPBA and 40 μg/mL (70 μM) TDMACl. Sonication was employed to dissolve chemicals in dioctyl sebacate. Notably, no additional surfactants were used in any of our sensing systems.

Droplet microfluidics

Both aqueous and oil solutions were loaded into Hamilton glass syringes. Two precision syringe pumps (Pump 11 Pico Plus Elite) were used to infuse the aqueous solution and the oil solution to the microfluidics chip at the same flow rate. The flow rate is 2 μL/min in K^+ and Na^+ measurement. The flow rate is 1.5 μL/min, 3 μL/min, and 1 μL/min for sensing of Cl^-, protamine, and H_2O_2, respectively.

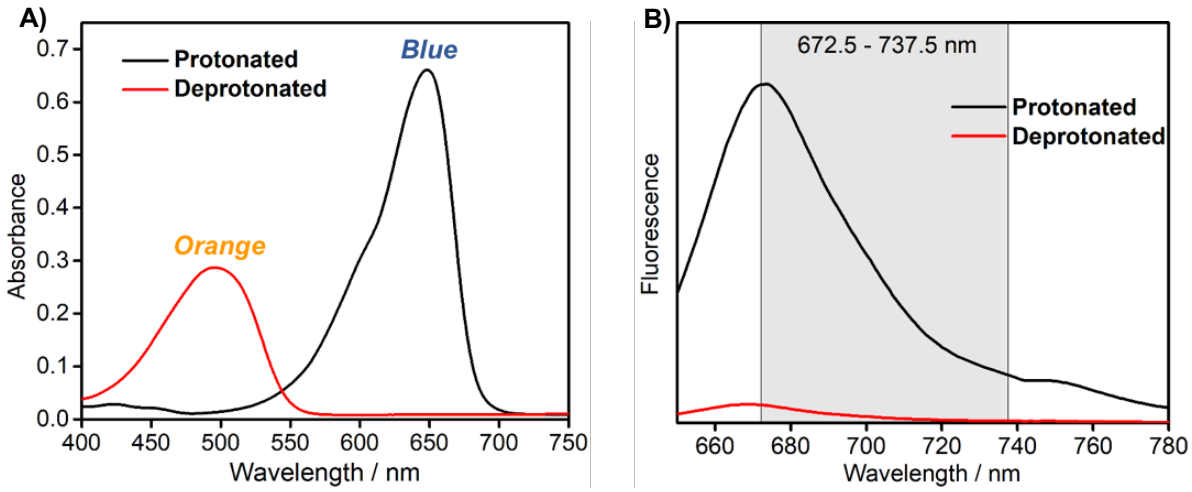


Figure S1. Visible absorbance (A) and fluorescence (B) spectra of the protonated and deprotonated chromoionophore III in dicotyl sebacate.

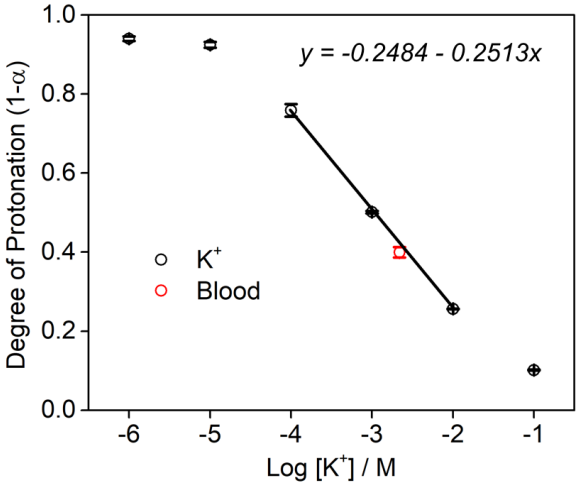


Figure S2. Determination of the K^+ concentration in whole blood based on the mean photon counts in the oil droplets shown in Figure 3 (graph on the left). The sheep blood was diluted with 0.1 M Tris-HCl buffer at pH 7.4 (1:1 dilution). The calculated K^+ concentration in the diluted whole blood is 2.6 ± 0.4 mM (n=3), which reasonably matches the real concentration of 2.2 mM obtained by a Radiometer blood gas analyzer.

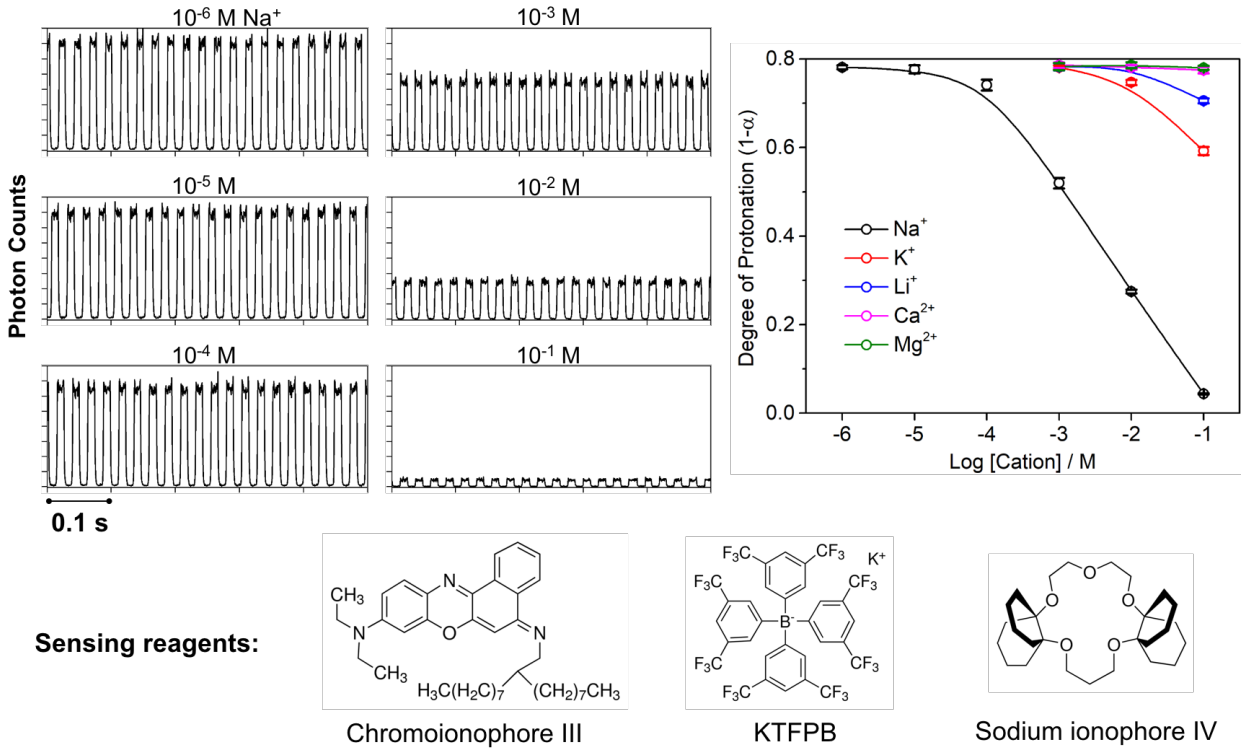


Figure S3. Fluorescence trace of the segmented flow in the presence of different concentrations of NaCl in the aqueous Tris-HCl buffer at pH 7.4 (left) and the corresponding calibration curve (right) based on mean photon counts of the oil droplets in the 0.5-s measurement (data points are average ± SD for n=3 measurements). Excitation wavelength: 630 nm; emission wavelength: 672.5 – 737.5 nm.

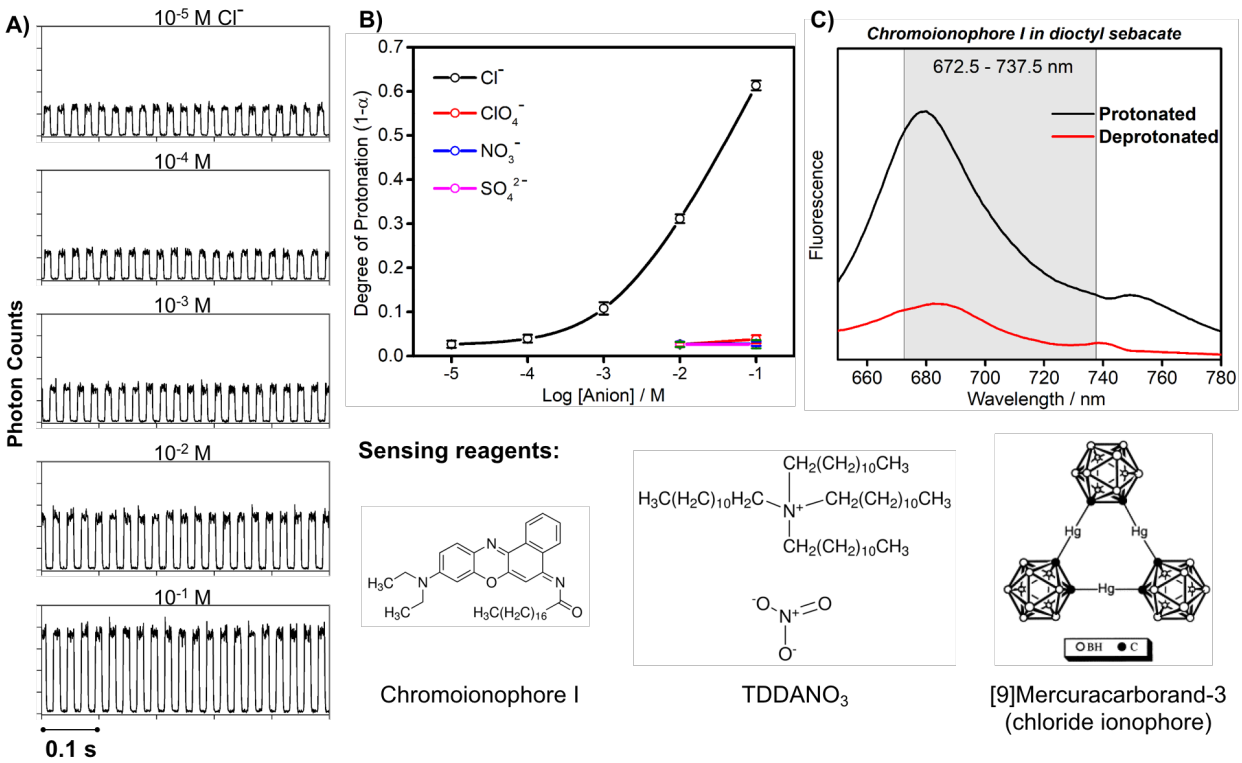


Figure S4. A) Fluorescence trace of the segmented flow at different concentrations of NaCl in 0.1 M HEPES buffer at pH 7.4. An emission wavelength band of 672.5 nm to 737.5 nm and an excitation wavelength of 630 nm were employed; B) The corresponding calibration curve based on the degree of protonation of the chromoionophore, which was calculated from the mean photon counts of the oil droplets in the 0.5-s measurement (data points are average ± SD for n=3 measurements); (C) The fluorescence spectra of the fully protonated and deprotonated chromoionophore dissolved in the bulk dioctyl sebecate. The response principle was previously reported in plasticized polymer membrane-based sensors.[2]

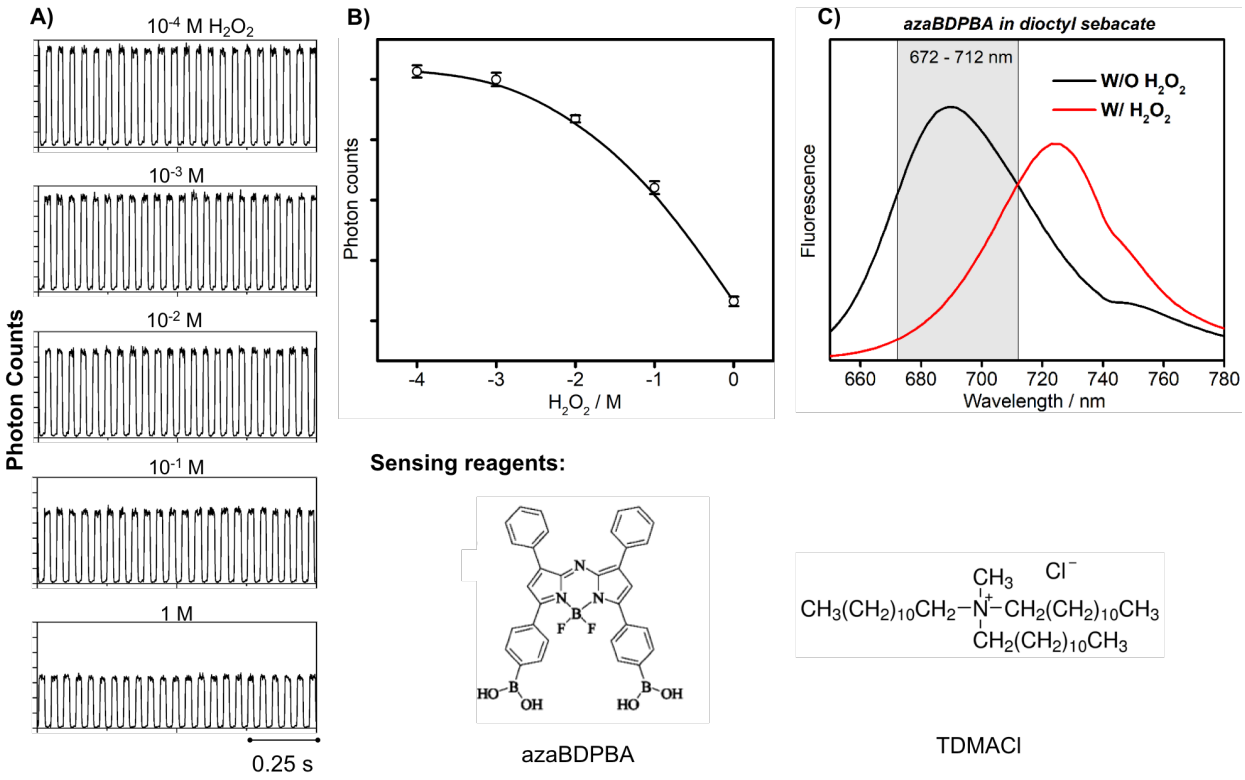


Figure S5. A) Fluorescence trace of the segmented flow at different concentrations of H_2O_2 in 50 mM phosphate buffer at pH 7.4. An emission wavelength band of 672 mm to 712 nm and an excitation wavelength of 640 nm were employed. B) The corresponding calibration curve based on mean photon counts of the oil droplets in the 1-s test (data points are average ± SD for n=3 measurements); C) Fluorescence spectra of the dioctyl sebacate bulk solution containing azaBDPBA and TDMACl before and after reaction with 1 M H_2O_2 in the aqueous solution, which corresponds to the original boronic acids reactants and the phenolic products. Thorough mixing of the oil solution and the aqueous buffer with and without H_2O_2 was performed for 5 min before the phase separation *via* centrifugation. Then the oil phase was transferred to a cuvette for fluorescence measurement. Notably, the lipophilic anion-exchanger, TDMACl was used according to previously reported sensors.[3]

References:

1. S.R. Doonan, R.C. Bailey, *Anal. Chem.* 2017, 89, 4091–4099
2. A. Ceresa, Y. Qin, S. Peper, E. Bakker. *Anal. Chem.* 2003, 75, 133-140
3. Y. Liu, J. Zhu, Y. Xu, Y. Qin, D. Jiang, *ACS Appl. Mater. Interfaces* **2015**, *7*, 11141-11145.