Electronic Supplementary Information

# Measuring rapid kinetics by potentiometric method in droplet-based microfluidic devices

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## EXPERIMENTAL SECTION

**Preparation of $Mg^{2+}$ ion-selective microelectrode.** The solid-contact $Mg^{2+}$ ion-selective microelectrode (ISE) was prepared by using 5 mm long Pt wire (diameter ~ 30 μm) as solid substrate. The Pt wire was soldered to Ag wire for electric contact. To prepare the $Mg^{2+}$ ISE, the Pt wire was first dipped into poly(3-octylthiophene-2,5-diyl) (POT) solution for five seconds and then dried in the air for 15 minutes. This process was repeated for three times. Next the Pt wire with POT coating was treated with $Mg^{2+}$ ionophore VI cocktail (Sigma-Aldrich) by dipping the Pt wire into $Mg^{2+}$ ionophore VI cocktail solution for five seconds and then drying the Pt wire in the air for 15 minutes. The process was repeated for three times to produce the $Mg^{2+}$ ISE. The $Mg^{2+}$ ISE was then conditioned in $10^{-6}$ M $MgCl_2$ for 1 day. To prepare the reference electrode, AgCl was deposited electrochemically on a 3 cm-long Ag wire (diameter 50 μm, Sigma-Aldrich) in 0.15 M NaCl aqueous solution for 1 h with 0.5 mA current.

**Fabrication of the microfluidic devices.** The device was fabricated by soft lithography.[1, 2] First, two master molds of fluid layer and control layer respectively were fabricated by photolithography. The fluid layer mold was made with positive photoresist (AZ P4620, AZ electronic materials) on silicon wafer and was treated at 110 $^{o}$C for 1 hour to form channels with cross section of half-moon geometry. The half-moon geometry facilitated full valve closure. The control layer mold was made by SU-8 photoresist (GM 1060, Gersteltec) on silicon wafer also by photolithography.

Two different mass ratios of degassed polydimethylsiloxane (PDMS) base and curing agent (Sylgard 184, Dow Corning) mixtures were prepared. 5:1 ratio mixture was cast as a fluid layer with thickness of 5 mm, and 20:1 ratio mixture was spin-coated at 3000 rpm for 60 s to form a 25 μm-thick control layer. Both fluid and control layers were first cured for 30 min at 80 $^{o}$C and then aligned and sealed to form the microchannels. The combined PDMS pieces were cured for another 60 min at 80 $^{o}$C. Finally, multilayer devices were made by binding fluid and control layers to a flat PDMS slab. The $Mg^{2+}$ ISE and Ag/AgCl microelectrodes were inserted into the microchannels in the fluid layer and sealed with epoxy glue.

**Droplet experiment.** Aqueous streams containing the reactants (RNA, buffer, and $Mg^{2+}$ solutions) were injected into an oil flow to form droplets (Figure 1b). The droplet volume was related to the cross-sectional dimension (~ 200 μm) of the channels and the ratio of the flow rates of aqueous streams to the flow rate of the oil.[3] Typically the droplet volume was about 5 nL. The flow rates of droplets and oil in the microchannels were controlled by syringe pumps (PHD 2000, Harvard Apparatus). The flow rate varied

between 10 and 100 mm/s. All the droplet experiments including measuring the RNA-$Mg^{2+}$ kinetics were performed at room temperature between 21 $^{o}$C and 22 $^{o}$C.

**RNA preparation.** P4-P6 domain of the *Tetrahymena* group I ribozyme constructs was prepared by *in vitro* transcription from PCR-generated DNA templates (TaKaRa, Dalian) using T7 RNA polymerase.[4] Briefly, E. coli competent cell JM109 (TaKaRa, Dalian) was used for transformation. Plasmid was first purified by QIAGEN midi preparation kit and then linearized by Not I restriction enzyme (TaKaRa, Dalian). RNA transcription and purification were done by MEGAshortscript and MEGAclear kit (Applied Biosystems). About 100 μg purified RNA could be obtained from each kit reaction and different concentrations was prepared for RNA-$Mg^{2+}$ binding kinetics experiment.

**Instrumentation.** Pneumatic valves in the control layer were connected to and controlled by a homemade three-way switch through Tygon tubing (Cole-Parmer).

Microphotographs were taken by a stereoscope (MZ 16, Leica) equipped with either a CCD camera (SPOT Insight, Diagnostic Instruments) or a high speed camera (FASTCAM 1024PCI, Photron).

A multifunction data acquisition (DAQ) board (NI USB-6216, National Instruments) connected with a homemade high impedance interface was used for the potentiometric measurements.

REFERENCES

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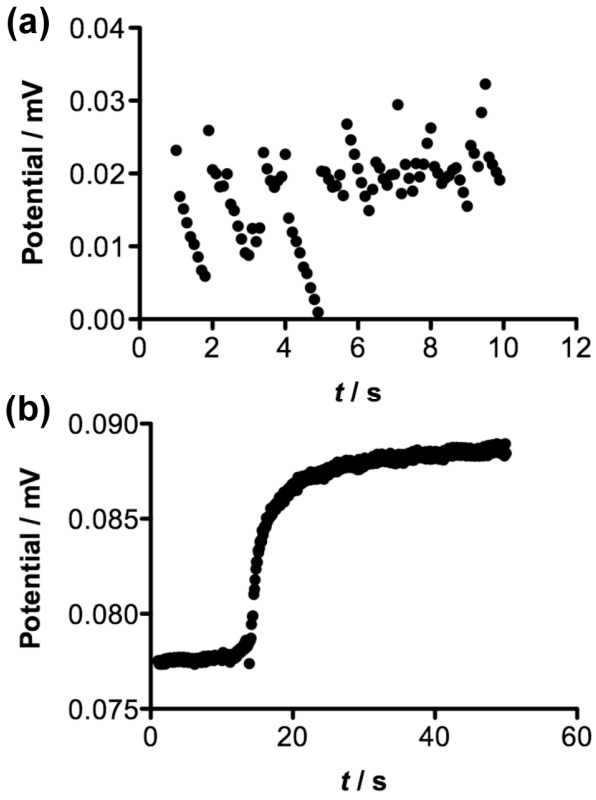


Figure S1. (a) Potential measurement of the droplets in oil by $Mg^{2+}$-selective microelectrode. Potential cannot reach to equilibrium due to the short contacting during when droplets pass through the electrodes. (b) Potential measurement of the droplets by $Mg^{2+}$-selective microelectrode after the oil was separated by the phase separation microchannels.

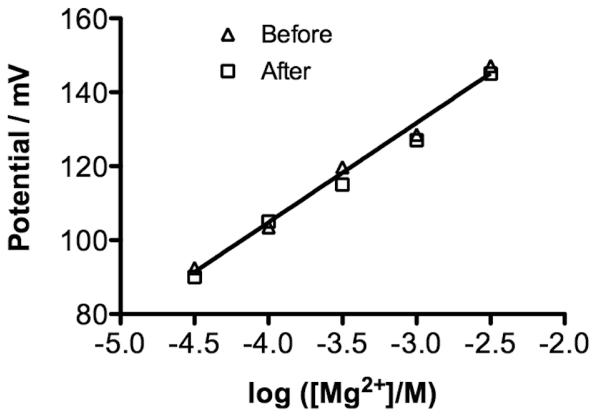


Figure S2. (a) Calibration curve for $Mg^{2+}$ concentration measurement ranging from 0.03 mM to 3 mM with the linear fitting of the experimental data. The on-chip calibration was performed on chip using droplets containing known [$Mg^{2+}$] each time before (triangle) and after (square) the measurement of the RNA-$Mg^{2+}$ binding kinetics. The slope of fitting line is 26.9 mV per ten-fold change of [$Mg^{2+}$], which is a relatively good Nernstian response for the $Mg^{2+}$-selective microelectrode.

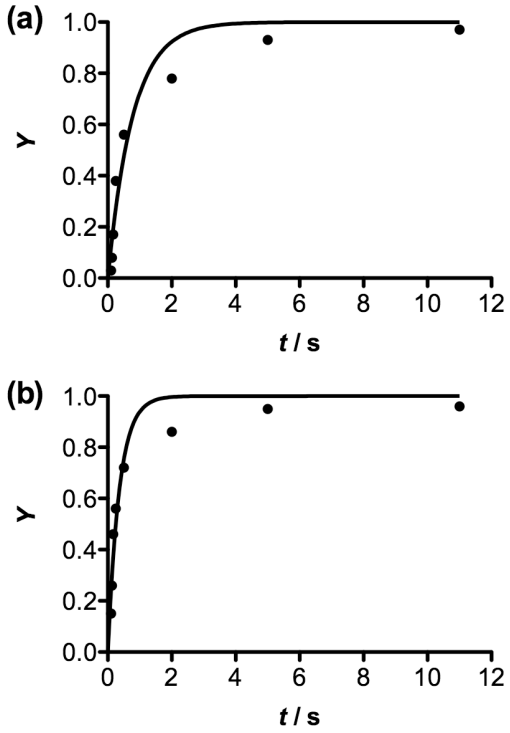


Figure S3. Fitting results of the same data set from Figure 3 with single exponential, $Y = 1 - e^{-kt}$. (a) The initial concentrations are 0.050 mM $Mg^{2+}$ and 0.019 mM RNA. $k$ = 1.3 $s^{-1}$; (b) The initial concentrations are 0.50 mM $Mg^{2+}$ and 0.23 mM RNA. $k$ = 2.8 $s^{-1}$.