Supporting Information

DNAzyme-powered nucleic acid release from solid supports

Ting Cao,[a, b, c] Yongcheng Wang,[a, b, d] Ye Tao,[a] Lexiang Zhang,[a] Ying-Lin Zhou,[*c] Xin-Xiang Zhang,[*c] John A. Heyman,[*a] David A. Weitz[*a, b]

[a.] John A. Paulson School of Engineering and Applied Sciences and Department of Physics, Harvard University, Cambridge, MA 02138, USA. * *E-mail: jheyman@g.harvard.edu, weitz@seas.harvard.edu.*

[b.] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA.

[c.] Beijing National Laboratory for Molecular Sciences (BNLMS), MOE Key Laboratory of Bioorganic Chemistry and Molecular Engineering, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China. * *E-mail: zhouyl@pku.edu.cn, zxx@pku.edu.cn.*

[d.] Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA.

Experimental section

Reagents and Apparatus

NaCl, KCl, *N,N,N′,N′*-Tetramethylethylenediamine (TEMED), mineral oil, Acrylamide solution (AA, 40%), AA/bis-acrylamide solution (40%, molar ratio 19:1), Span-80, Hexane, Ammonium persulfate (APS), Triton X-100, Tris base and boric acid were all purchased from Sigma-Aldrich (USA). 0.5 M EDTA (pH 8.0), UltraPure 20xSCC buffer and UltraPure distilled water were purchased from Life Technologies (USA). 1H,1H,2H,2H-Perfluorooctanol (PFO) was purchased from Alfa Aesar (USA). 3M Novec 7500 Engineered Fluid (HFE-7500 oil) was bought from 3M Science (USA). 1 M $MgCl_2$, 1 M Tris–HCl (pH 8.0), Fetal Bovine Serum (FBS), penicillin-streptomycin (P/S), 5x First Strand Buffer, SuperScript II Reverse Transcriptase, DPBS, RNaseOUT Recombinant Ribonuclease Inhibitor and Dithiothreitol (DTT) were bought from Thermo Fisher Scientific (USA). Deoxynucleotide (dNTP) Solution Mix was ordered from NEB (USA). Kapa 2x HiFi HotStart PCR mix was purchased from Kapa Biosystems (USA). SeaKem LE Agarose was obtained from Lonza (ME, USA). 20X Evagreen Dye was obtained from Biotium (USA). DNA oligos were all ordered from Integrated Device Technology, Inc. (USA) and listed in Table S1.

10 mL 4x Acrylamide/bis-acrylamide solution (AB) was prepared with 3.6 mL of acrylamide/bis-acrylamide (AA/BIS), 2.58 mL of AA, and 3.82 mL of nuclease-free water and stored at 4°C for use. 10% (wt/vol) ammonium persulfate (APS) was prepared and stored at −20 °C as aliquots. Tris-buffered saline–EDTA–Triton buffer (TBSET) was prepared by combining 822 mL of nuclease-free water, 10 mL of 1 M Tris–HCl (pH 8.0), 137 mL of 1 M NaCl, 1.35 mL of 2 M KCl, 20 mL of 0.5 M EDTA, and 10 mL of 10% (vol/vol) Triton X-100 in nuclease-free water and stored at room temperature. 5x TBE buffer was prepared with 54 g Tris, 27.5 g boric acid 20 mL 0.5 M pH 8.0 EDTA in 1L solution and stored at room temperature. All these solutions were filtered through 0.2 μm membrane (Corning, USA). 20% (vol/vol) 1H,1H,2H,2H-Perfluorooctanol in HFE-7500 oil (PFO) was prepared and used in a well-ventilated area. 1% (vol/vol) Span-80 in hexane was prepared by combining 99 mL of hexane and 1 mL of Span-80 in a fume hood and stored in a glass bottle.

3-inch silicon wafer was obtained from University Wafer, Inc. (South Boston, MA, USA). SYLGARD 184 Silicone Elastomer Kit was obtained from Sigma Aldrich (USA). Base and curing agents were mixed in a 10 (base) :1 (curing agent) ratio by weight and mixed by Thinky Mixer (Thinky Corporation, USA). 1 mL and 3 mL syringe were bought from Becton, Dickinson and Company (BD, USA). Syringe pumps were Harvard apparatuses. PE2 tubing was bought from Scientific Commodities, Inc. (USA). Solution was vortexed by Analog Vortex Mixer and centrifuged by Eppendorf centrifuge (VWR International Company, USA). Beads generation was monitored by Axiovert 10 microscope and beads fluorescence signal and intensity were evaluated by Axio Observer Z1 Inverted Microscope (Carl Zeiss, Germany). Samples run a PCR program

in Veriti 96 well Thermal Cycler (Applied Biosystem Inc., USA) and a Q-PCR program in C1000 Thermal Cycler (Bio-Rad Laboratories, Inc., USA). Electrophoresis system for DNA agarose gel was also bought from Bio-Rad (USA).

Fabrication of microfluidic equipment

A computer assisted design (CAD) microfluidic design drawing for hydrogel beads generation was printed on a transparent film, which was a mask for transferring microfluidic pattern into an embossed master on a silicon wafer. The master as a reusable mold could be used to set and produce poly (dimethyl siloxane) (PDMS) polymerization slabs. After punching inlet and outlet ports and dealing with oxygen plasma, the slab was bonded to a glass slide and treated with hydrophobic coating on channel surface. The microfluidic fabrication process was done in Center for Nanoscale System (CNS) in Harvard University. A detailed fabrication procedure can refer to previous description.[1]

DNAzyme linker hydrogel beads generation

200 μL solution mix with finial concentration of 0.1x TBSET buffer, 1x AB solution, 10 μM acrydite-modified DNA oligo and 0.3% APS was prepared and transferred to a 1 mL syringe. The syringe was prefilled with 300 μL HFE-7500 oil that was used for carrying forward all aqueous solution to microfluidic channel. 1 mL carrier oil containing 4 μL TEMED was transferred to 3 mL syringe. Both syringes with needles were connected to corresponding inlets of the microfluidic device through PE2 tubing. The 1 mL syringe was hold upright to avoid aqueous and oil phase mixing. Aqueous solution was pumped into microfluidic channel with 300 μL/h flow rate, while oil solution was pumped with 1000 μL/h. The intersection of the two phases would generate water-in-oil droplets (Figure S10). Collected stable droplets into a 2 mL tube from device outlet through PE2 tubing. Covered the solution with 200 μL mineral oil and incubated overnight at 60 ^{o}C. Removed the bottom and top oil phases, and used 20% PFO, 1% Span-80 in hexane and 1X TBSET buffer to wash the hydrogel beads twice respectively. The washed beads were thought clean and used in the next steps without other treatment (Figure S11). Previous published protocol shows a more detailed hydrogel beads generation and wash steps.[2]

DNAzyme cleavage process

2 μL Mg-DNAzyme-linking hydrogel beads and 2 μL 10 uM Mg^{2+} DNAzyme Enzyme strand solution were added into working buffer (10 mM Tris-HCl, pH 8.0, 500 mM NaCl). Then added $MgCl_2$ and incubated the 50 μL reagent mix for 2h at 20 ^{o}C. After that, centrifuged and removed the clear supernatant. Used water to wash the beads three times. 0.5 μM fluorescence oligo was incubated with hydrogel beads in 6XSCC buffer for 30 min at room temperature. Then used 6XSCC buffer to wash the beads three time. Added 50 μL 6XSCC buffer and vortexed to make the beads disperse in

buffer. Transfered 10 µL dispersed beads into a cell counting slide (NanoEntek, USA) and imaged the beads by Zeiss Axio Observer Z1 Inverted Microscope with 1600 ms Expose time. Average fluorescence value was calculated by subtracting background fluorescence signal from fluorescence value of six fluorescence beads.

biological comparability test

Jurkat cells (ATCC number: TIB-152) were cultured in RPMI-1640 Medium (ATCC number: 30-2001) containing 10% FBS, 100 units/ml penicillin and 100 µg/ml streptomycin in 5% CO_2 at 37 ^oC. Treated with Trypan Blue (NanoEntek, USA) and counted cell number with Countess automated cell counter (Invitrogen, USA). Cell lysis buffer was made by adding 1 µL RNaseOUT into 19 µL 0.2% Triton. 1 µL 2E6 cells/mL Jurkat cells were added into 9 µL reverse transcription solution mix, which contained 2 µL 5x First strand buffer, 2 µL cell lysis buffer, 1 µL 10 mM dNTP, 0.5 µL 0.1 M DTT, 0.25 µL 40 U/µL RNaseOUT, 0.5 µL SuperScript II Reverse Transcriptase, 0.5 µL TDMgDSS-GAPDH (Table S1) hydrogel beads, 0.5 µL 10 µM DNAzyme catalysis strand and 1.75 µL H_2O.[3] As a control, we used TDMgDSS-GAPDH without hydrogel beads and Adaptor-GAPDH (Table S1) to capture GAPDH gene mRNA at the same time. Samples were kept at 20 ^oC for 5 min and then 42 ^oC for 90 min. Then run 11 cycles of 50 ^oC for 2 min and 42 ^oC for 2 min. After 70 ^oC for 15 min to inactive the reverse transcriptase, kept the sample 4 ^oC for hold. Centrifuged the samples and collected the clear supernatant. 1 µL this supernatant, together with 5 µL KAPA HIFI mix, 0.5 µL 1 µM Acry-DNA/GAPDH, 0.5 µL 1 µM GAPDH primer, 0.5 µL 20x Evergreen Dye and 2.5 µL H_2O were put into Q-PCR machine and run a PCR program: 98 ^oC for 3 min; 31 cycles of 98 ^oC for 20 s, 64 ^oC for 15 s, 72 ^oC for 1 min; kept sample at 4 ^oC. Melt Curve was recorded from 65.0°C to 95.0°C with an increment of 0.5°C for 0:05. After that, 2% Agarose gel electrophoresis with SYBR Safe DNA stain was conducted in 1xTBE buffer under 120 V for 40 min. The gel was image with FluorChem M system (ProteinSimple, California, USA).

Table S1. DNA oligoes used in the experiment

ID	DNA sequence (5'-3')
MgDSS-IP	Acryd[1]-CGATGA*TTCTCTCTrAGGACAAAA*[2]CGATACTACACGACGCTCTTCCGATCT[3]
Mg-Enz	TTTTGTCAGCGATCCGGAACGGCACCCATGTGAGAGAA
FP	FAM[4]-AGATCGGAAGAGCGTCGTGT
TDMgDSS-IP	Acryd-CGATGA*TTCTCTCTrAGGACAAAA*CGA*TTCTCTCTrAGGACAAAA*ACACGACGCTCTTCCGATC
TDMgDSS-GAPDH	Acryd-CGATGAT*TCTCTCTrAGGACAAAA*CGA*TTCTCTCTrAGGACAAAA*CCATGTAGTTGAGGTCA[5]
Adaptor-GAPDH	**AAGCAGTGGTATCAACGCAGAGTAC**[6]CCATGTAGTTGAGGTCA
GAPDH primer	ATGGGGAAGGTGAAGGTCGGAGTCAACG
UV-triggered DNA oligo	Acryd-*photocleavable spacer*-TTTTTTTTTTTTTT ($264.05 for 100nmol with 4.1 nmol guaranteed yield)
Mg^{2+}-triggered DNA oligo	Acryd-*TTCTCTCTrAGGACAAAA*TTTTTTTTTTTTTT ($102.07 for 100nmol with 15.6 nmol guaranteed yield) TTTTGTCAGCGATCCGGAACGGCACCCATGTGAGAGAA ($8.1 for 25nmol with 8.1 nmol guaranteed yield)

Notes:

1. Acryd means acridite-modification in 5' end of the DNA sequence.
2. The sequence in italic means Mg^{2+}-dependent DNAzyme substrate strand. The adenosine ribonucleotide base is the cleavage site.
3. The sequence in underline means a random DNA sequence, which is complimentary with FP and functions as an indicator sequence.
4. FAM means FAM-fluorophore modification in 5' end of the DNA sequence.
5. The sequence in wave underline means a reverse transcript primer for GAPDH gene mRNA.
6. The sequence in bold means a random DNA sequence.

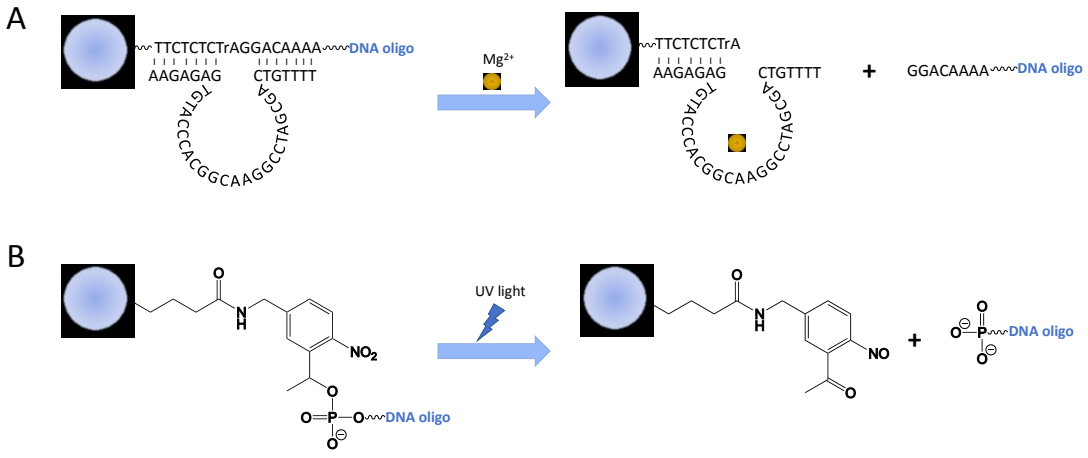

Figure S1. (A) Mg^{2+}-triggered cleavage process depending on the DNAzyme system; (B) The conventional UV-triggered cleavage process.

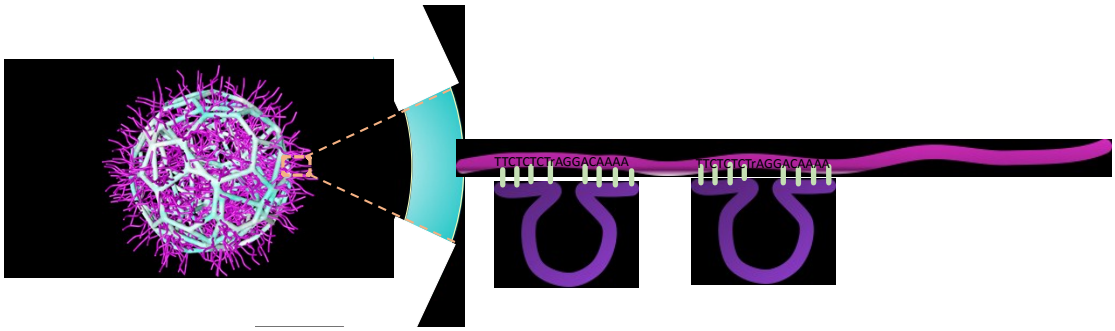

Figure S2. Schematic diagram of tandem DNAzyme linking hydrogel beads.

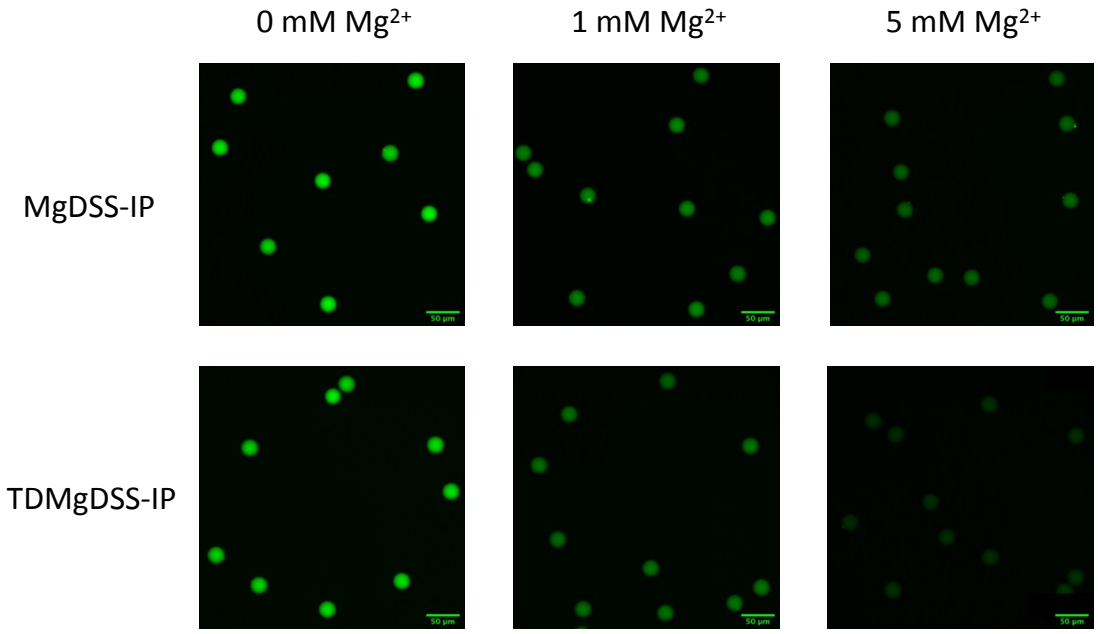

Figure S3. Fluorescent images for MgDSS-IP system and TDMgDSS-IP system with different Mg^{2+} concentration.

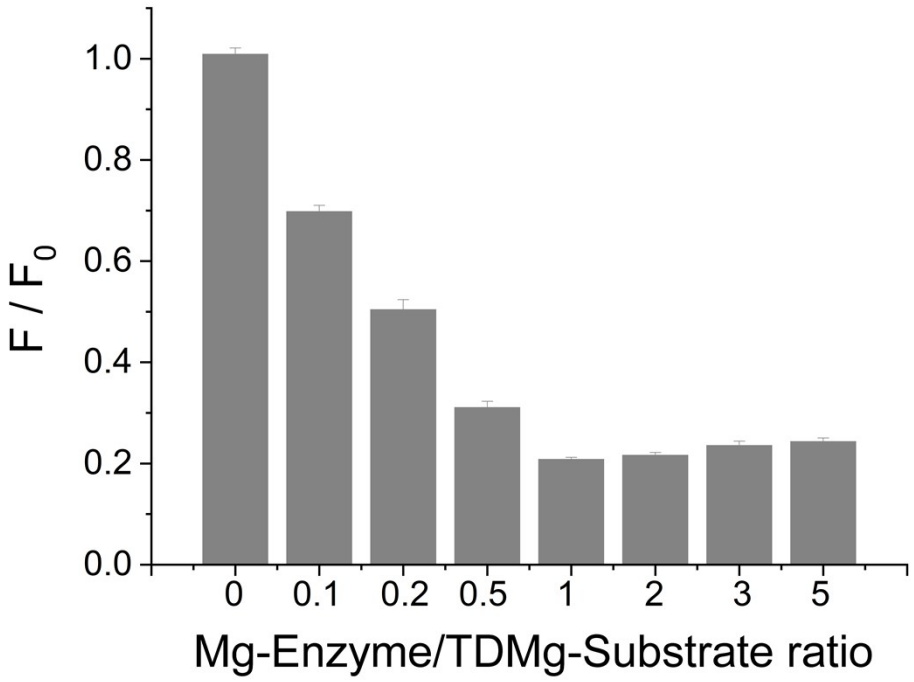

Figure S4. The influence of Mg-Enzyme/TDMg-Substrate strand concentration ratio on cleavage efficiency of the TDMgDSS-IP design.

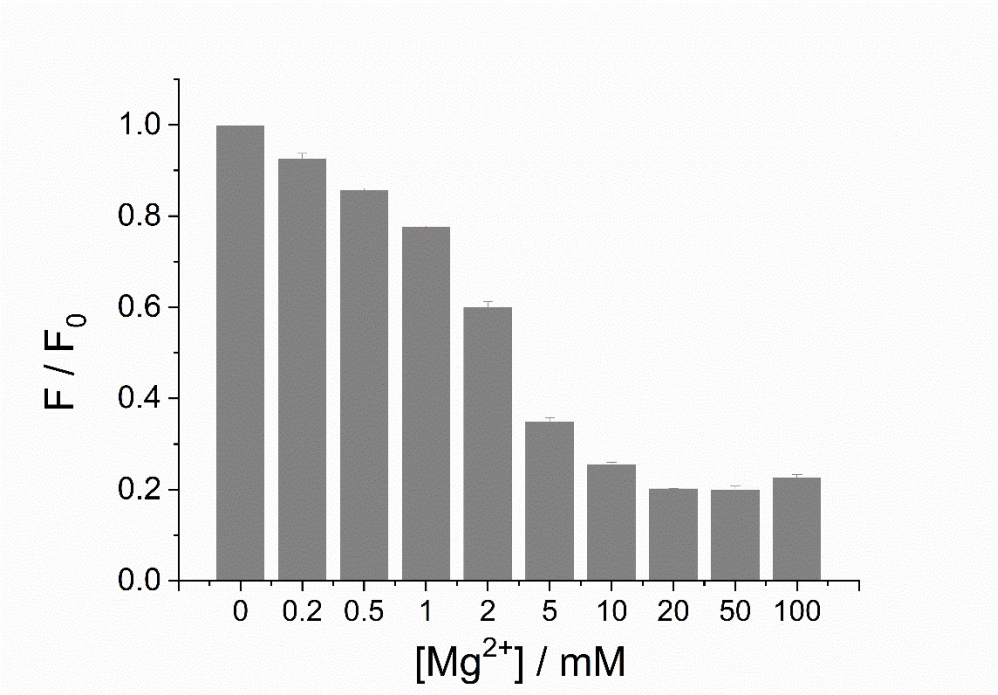

Figure S5. The influence of Mg^{2+} concentration on cleavage efficiency of the TDMgDSS-IP design.

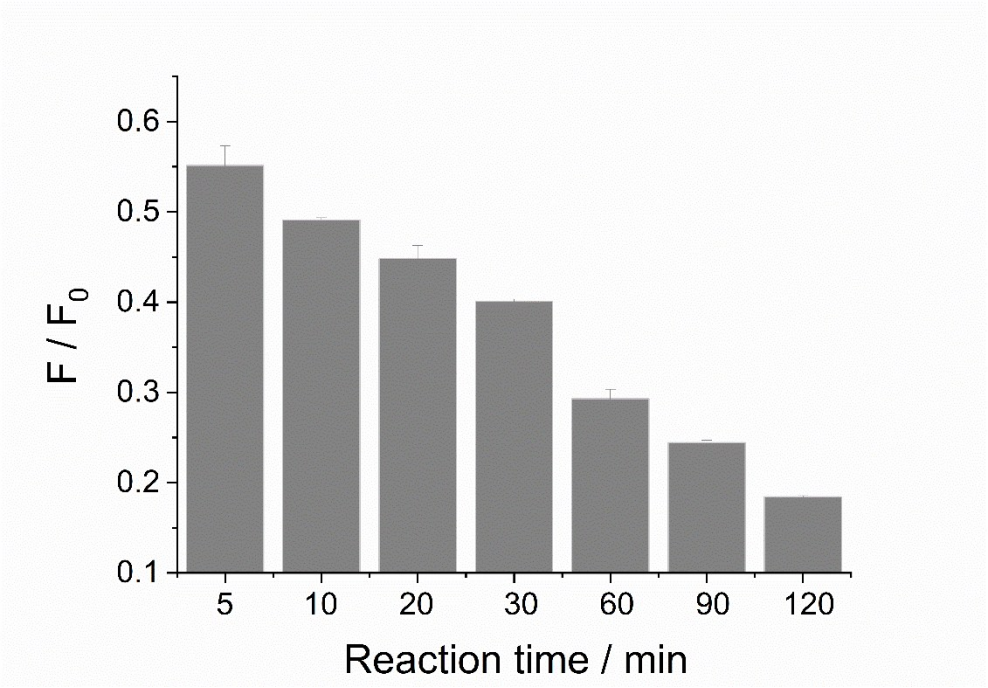

Figure S6. The influence of reaction time on cleavage efficiency of the TDMgDSS-IP design.

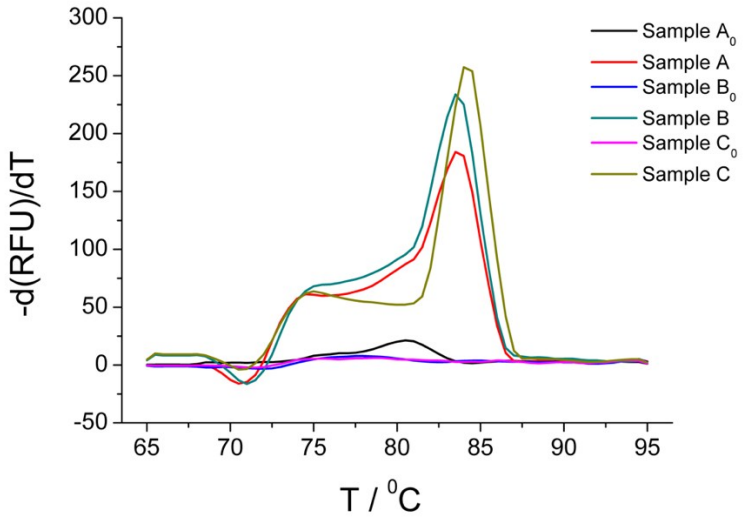

Figure S7. T_m curve for samples A-C (A_0, B_0 and C_0 have no cells as control experiments).

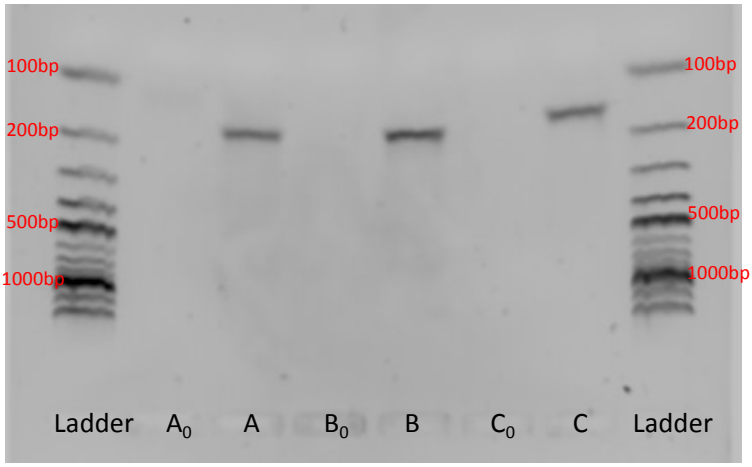

Figure S8. Agarose gel electrophoresis image for samples A-C (theoretically samples A and B should produce a new 173bp band and sample C should produce a new 155bp band).

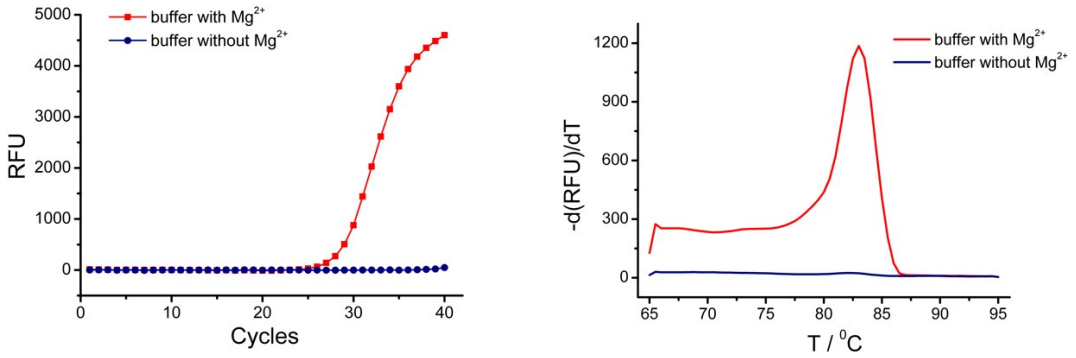

Figure S9. Q-PCR data and T_m curve for samples with magnesium ions (red line) and without magnesium ions (blue line). Both samples contain cells and use TDMgDSS-IP beads to capture mRNAs in cells.

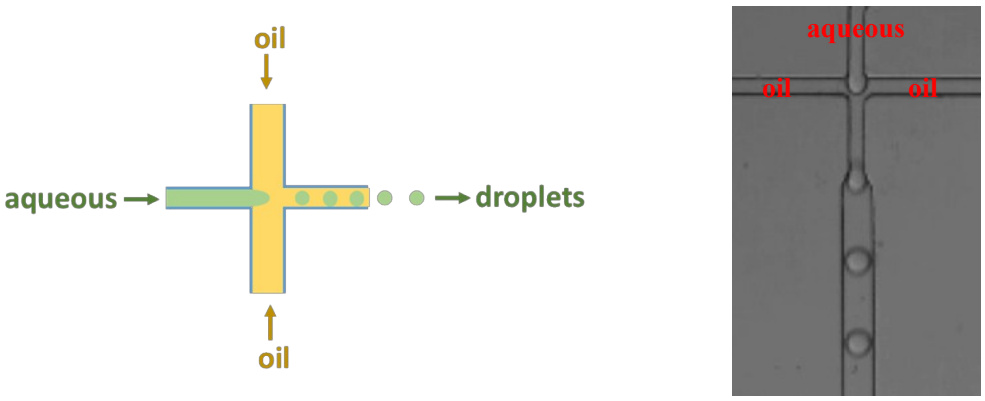

Figure S10. Diagrammatic drawing for droplet generation at the intersection of the aqueous phase and the oil phase (left) and the droplet generation image under a microscope (right).

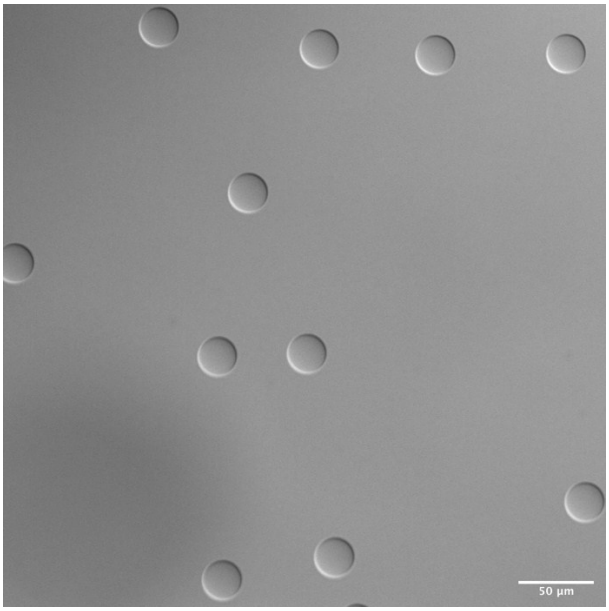

Figure S11. Microscopic image for polyacrylamide hydrogel beads.

References:

1 L. Mazutis, J. Gilbert, W. L. Ung, D. A. Weitz, A. D. Griffiths and J. A. Heyman, *Nat. Protoc.*, 2013, **8**, 870–91.

2 R. Zilionis, J. Nainys, A. Veres, V. Savova, D. Zemmour, A. M. Klein and L. Mazutis, *Nat. Protoc.*, 2017, **12**, 44–73.

3 S. Picelli, O. R. Faridani, Å. K. Björklund, G. Winberg, S. Sagasser and R. Sandberg, *Nat. Protoc.*, 2014, **9**, 171–181.