Supporting Information

Artificial signal feedback network mimicking cellular adaptivity

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Material and Methods

Cell culture

HeLa cells were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA) and cultured in DMEM medium supplemented with 10 % fetal bovine serum (FBS, heat inactivated) and penicillin (100 U mL^{-1})-streptomycin (100 μg mL^{-1}) in a cell culture incubator at 37 °C with 5 % CO_2.

Preparation of GMVs

Micron-scale giant membrane vesicles (GMVs) were derived from HeLa cells according to our previous report. Briefly, HeLa cells were incubated with carboxyfullerenes for 4 hours at 37 °C and then washed by DPBS three times and supplemented with Tris-HCl buffer (pH 8.0, containing 10mM Tris-HCl and 100mM NaCl (Beyotime Shanghai, China)). After that, cells were exposed under white light for 4 hours. After 8 hours incubation, GMVs dispersed in supernatant were used as prepared.

Construction of DNA-based artificial adaptive system

200 μL of GMVs solution were incubated with 0.4 μM DNA hybrid (containing FAM-labeled cholesterol-trigger and Cy5-labeled DNAzyme) for 30 minutes on a glass bottom cell culture dish (NEST, China). Afterwards, DMs (containing FAM-labeled DM2 and DM1) (1.5 μM), dNTP (1 mM, Takara (Dalian, China)), ATP (1 mM, Energy-Chemical (Shanghai, China)), 1×reaction buffer A for T4 PNK (containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 5 mM DTT, and 0.1 mM spermidine) were added. To observe DNA polymer disassembly, T4 PNK (0.1 U μL^{-1}) and Bsm DNA polymerase (0.08 U μL^{-1}), both purchased from Thermo Fisher Scientific Company (MA, USA), were added to the above solution. Fluorescence intensity on the membrane surface was recorded by FV1000 confocal laser scanning microscope (Olympus, Japan), and the fluorescent images were analyzed by ImageJ software.

Agarose gel analysis

Annealed DM1 (1 μM) and DM2 (1 μM), Trigger (0.2 μM), DNAzyme (0.4 μM), stimulus (2 μM), T4 PNK (0.5 U μL^{-1}), Bsm DNA polymerase (0.16 U μL^{-1}), dNTP (1 mM), ATP (1 mM), 1×reaction buffer A for T4 PNK (containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 5 mM DTT, and 0.1 mM spermidine) were used to construct DASsys. To perform adaptivity in solution, trigger and DNAzyme were mixed for 1 hour to produce DNA hybrids. DNAzyme-induced cleavage of stimulus and DNA polymer formation and disassembly were carried out in Tris-HCl buffer solution at room temperature for 8 hours. DNA ladder (20-bp/50-bp) was purchased from TaKaRa (Dalian, China). Agarose gel (2 %) was run 100 min under 100V, stained with ethidium bromide (EB). The Bio-Rad ChemiDoc XRS System was used to image and analyze the gels, and the intensity of DNA band was analyzed with FlowJo 7.6 software.

Native polyacrylamide gel electrophoresis analysis

DM1 (1 μM), trigger (0.2 μM), DNAzyme (0.4 μM), stimulus (2 μM), T4 PNK (0.5 U μL^{-1}), Bsm DNA polymerase (0.16 U μL^{-1}), dNTP (1 mM), ATP (1 mM), 1×reaction buffer A for T4 PNK (containing 50 mM Tris-HCl (pH 7.5),10 mM $MgCl_2$, 5 mM DTT, and 0.1 mM spermidine) were used to amplify cleavage-based stimulus. 10 % native polyacrylamide (ultrapure water, 10×TAE/Mg^{2+}, 30 % Acryl-Bis, 10 % APS and TEMED) was run 70 min under 110V at 1×TAE/Mg^{2+} (containing 40 mM Tris-acetic acid, 1 mM EDTA, and 12.5 mM magnesium acetate, pH 7.4). The cleavage of stimulus in GMVs solution was also analyzed by PAGE, consisting of 10 μL GMVs solution, stimulus (5 μM) and DNAzyme (1 μM). 2 μL 6×loading buffer were mixed with samples, respectively, for electrophoresis experiments. Gels were stained by Stains-All (Thermo Fisher), washed by water 10 minutes later, and then imaged with a Bio-6000 (Microtek, Shanghai, China) with ScanWizard Bio software.

Fluorescence Measurements

All fluorescence measurements were carried out on a Fluorescence Spectrometer (F-7000, Hitachi, Japan). Briefly, λex=488 nm with 5 nm bandpass, λem=500-600 nm

filter was selected for FAM dye and λex=550 nm laser with 2 nm bandpass, λem=560-700 nm filter was selected for FRET experiment in a 200 μL quartz cuvette. All experiments were repeated at least three times.

Flow cytometry analysis

FAM-labeled cholesterol-trigger and Cy5-labeled DNAzyme were used during the experiments. 1 mL GMVs solution was incubated with trigger-DNAzyme hybrids, FAM-labeled cholesterol-trigger (10 nM), and Cy5-labeled DNAzyme (20 nM) for 30 minutes, respectively. Then, GMVs solution was analyzed by flow cytometry using a BD FACSVerse™ system operated at a mid-pressure. The fluorescence intensity of GMVs solution was detected by following λex and λem: λex=488 nm blue laser, λem=527 ± 32 nm filter for FAM and λex=640 nm laser, λem=660 ± 10 nm filter for Cy5. All FACS data were determined for a total of 10,000 events. Data were analyzed with FlowJo 7.6 software.

Confocal microscopy analysis

A 200 μL solution containing GMVs equipped with DASsys constructed as described above, with membrane surface anchored by cholesterol-labeled trigger and DNAzyme and the solution supplemented with DMs (DM1 and FAM-labeled DM2) and enzymes, was added to a glass bottom cell culture dish 15 mm in diameter (NEST, China). The GMVs solution was observed under the LSM 880 with Airyscan confocal laser scanning microscope (Carl Zeiss GmbH, Jena, Germany). Upon the addition of stimulus, the fluorescence intensity was recorded during the whole process. At least 20 GMVs were recorded at each time point. Image analysis was performed with ImageJ software. To record the recyclable property of the adaptive system, the average intensity of FAM-labeled DM2 on the membrane surface was measured by ImageJ software. We set a threshold value to measure average intensity of each GMV and then obtained the average intensity of at least 20 GMVs.

∆ G calculation

The ΔG values of the different structures of DNA were calculated with oligonucleotides analyze tool from Integrated DNA Technologies, Inc. (https://www.idtdna.com/calc/analyzer) under the condition of $[Mg^{2+}]$ = 10 mM, $[Na^{+}]$ = 100 mM, and at the temperature of 37 °C.

Statistical analysis

All the experiments were done in triplicate. Results are expressed as means ± standard deviation of the mean value (SD). The statistical significance of the observed differences was analyzed by t tests. Statistical significance was set at $p < 0.05$ (*P < 0.05, **P < 0.01, ***P < 0.001).

Table S1: DNA sequences used for construction of DASsys. DNA was purchased from Sangon (Shanghai, China).

Name	DNA Sequences (5'-3')
FAM-Trigger-Chol	FAM-CCTCATCCCACTCCTACCTAAACCTTTTTT-Cholesterol
Trigger-FAM	CCTCATCCCACTCC/i6FAMdT/ACCTAAACCTTTTTT
DNAzyme-Cy5	TAGGAGTGGGATGAGGAGGCTAGCTACAACGAATGACCTCAGG-Cy5
DNAzyme-BHQ1	BHQ1-TAGGAGTGGGATGAGGAGGCTAGCTACAACGAATGACCTCAGG
Stimulus	GGGCTGAGGTCATrArUCCTCATCCCACTC
Stimulus-mismatch	GGGCTGAT<u>T</u>GTCATrArUCCT<u>A</u>ATCCCACTC
Stimulus-insertion	GGGCTG<u>T</u>AGGTCATrArUCCTCATCCCACTC
Stimulus-deletion	GGGCTGAGTCATrArUCCTCATCCCACTC
DM1	GGTTTAGGTAGGAGTGGGATGAGGCCAAATCCTCATCCCACTCCTACCTATGACCTCAGCCC
DM1-Cy3	GGTTTAGGTAGGAGTGGGATGAGGCCAAA/iCy3dT/CCTCATCCCACTCCTACCTATGACCTCAGCCC
DM2-FAM	CCTCATCCCACTCCTACCTAAACCGGTAGGAGTGGGATGAGGATTTGGAA-FAM
DM2-Cy5	Cy5-CCTCATCCCACTCCTACC*TAAACC*GGTAGGAGTGGGATGAGGATTTGGAA

Supporting Figures

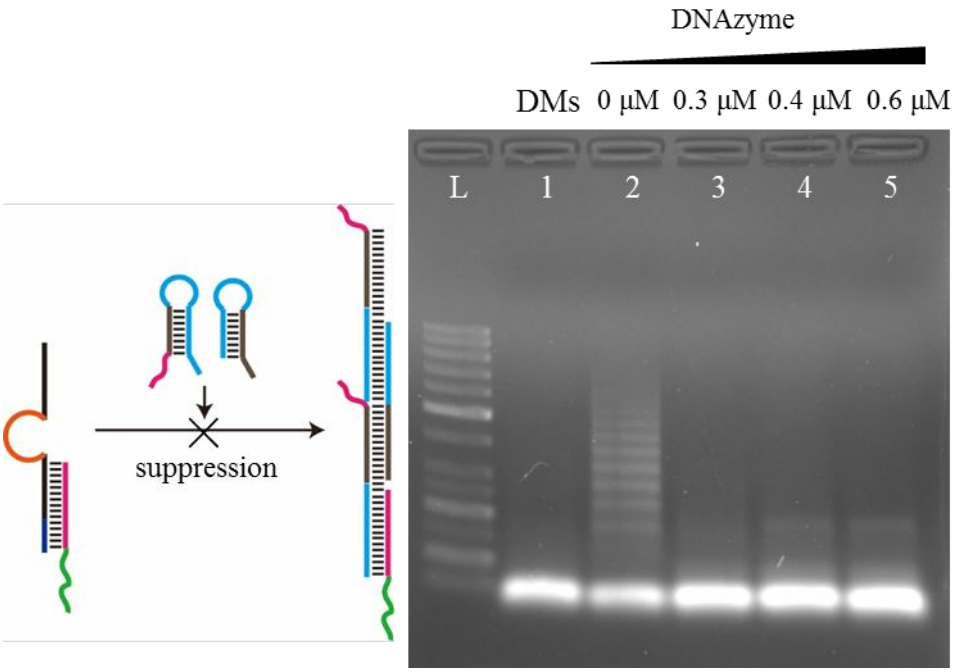

Figure S1. Agarose gel electrophoresis analysis of DNAzyme-trigger hybrid unable to induce the formation of DNA polymer in solution. L: 50-bp DNA ladder. Line 1: DMs. Line 2: DNA polymer formation induced by trigger. Line 3-5: formation of DNA polymer under different ratios (1.5:1, 2:1, 3:1) of DNAzyme and trigger.

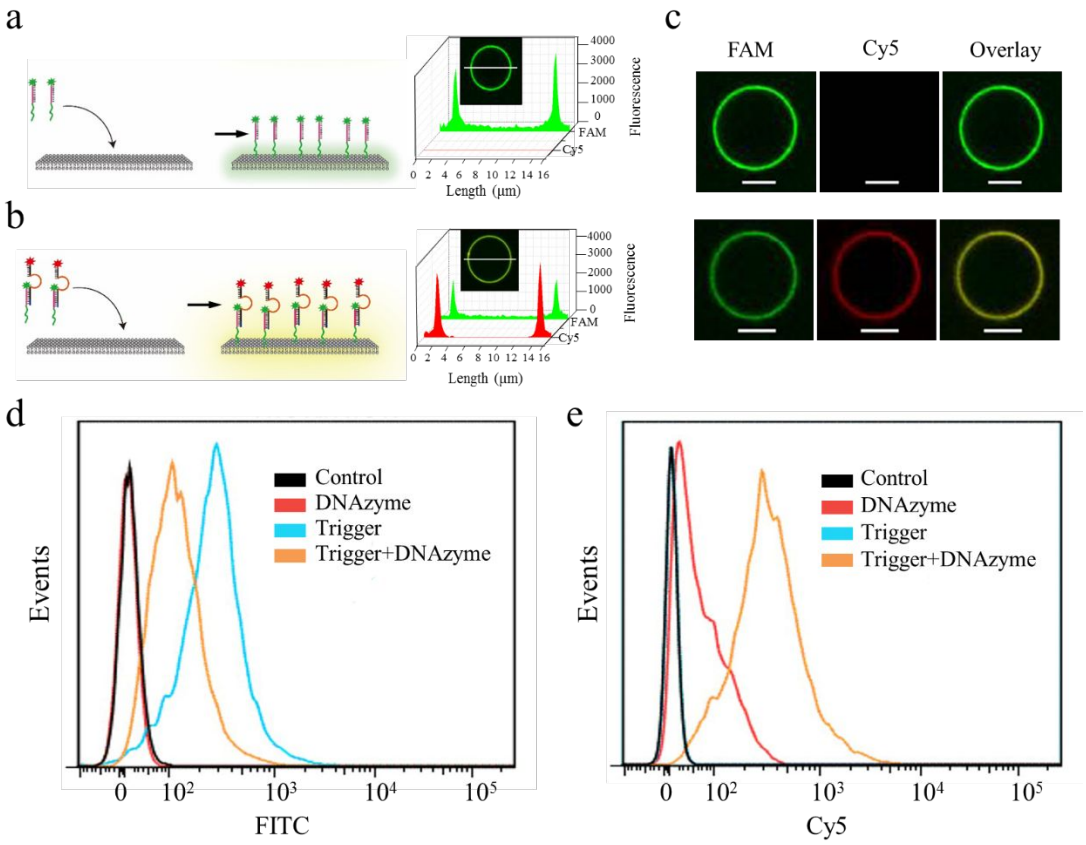

Figure S2. Construction of DNA-based adaptive sensor on membrane surface. Confocal image of GMVs incubated with FAM-labeled DNA trigger (a) and Cy5-labeled DNAzyme (b) at 37 °C for 30 min. Colocalization image of FAM-labeled DNA trigger and Cy5-labeled DNAzyme on membrane surface (c). Flow cytometry analysis for GMVs incubated with FAM-labeled DNA trigger (d) and Cy5-modified DNAzyme (e). About 10000 events were counted for all samples. Scale bar is 5 μm.

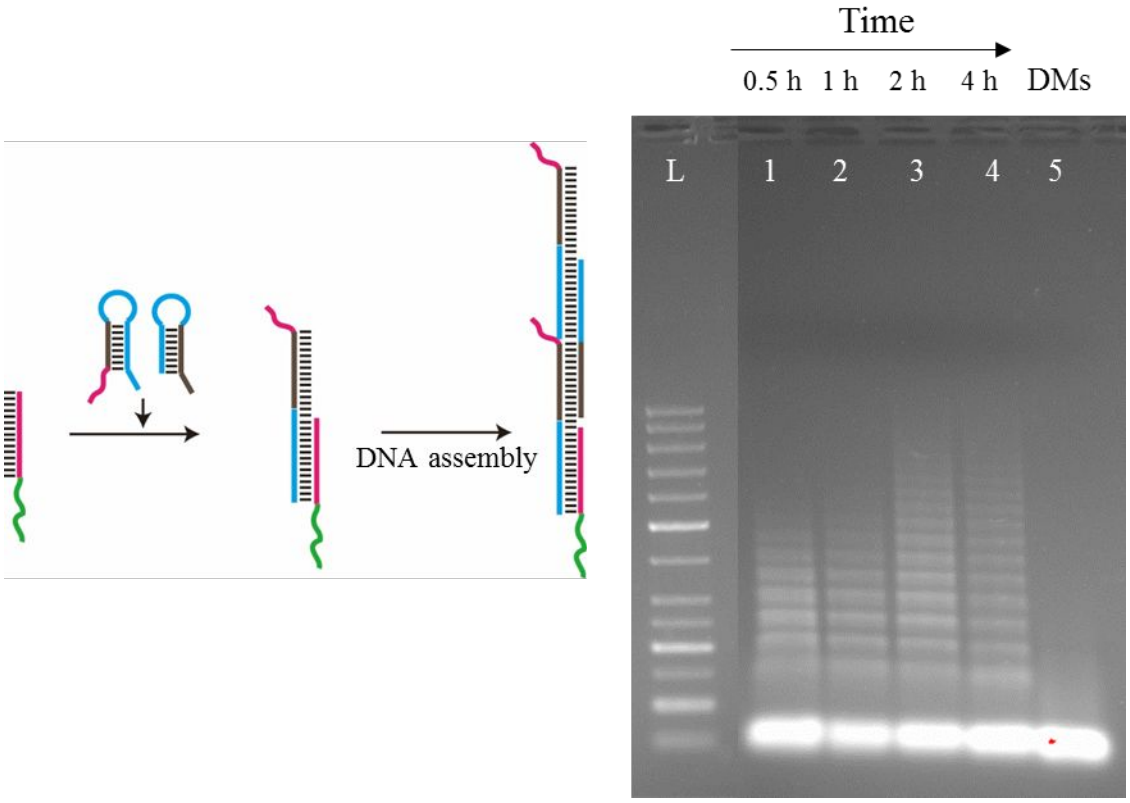

Figure S3. Agarose gel electrophoresis analysis of DNA polymer formation in Tris-HCl buffer solution. L: 50-bp DNA ladder. Line 1: DMs. Line 2-5: different reaction (4 h, 2 h, 1 h, 0.5 h) time of DNA polymer.

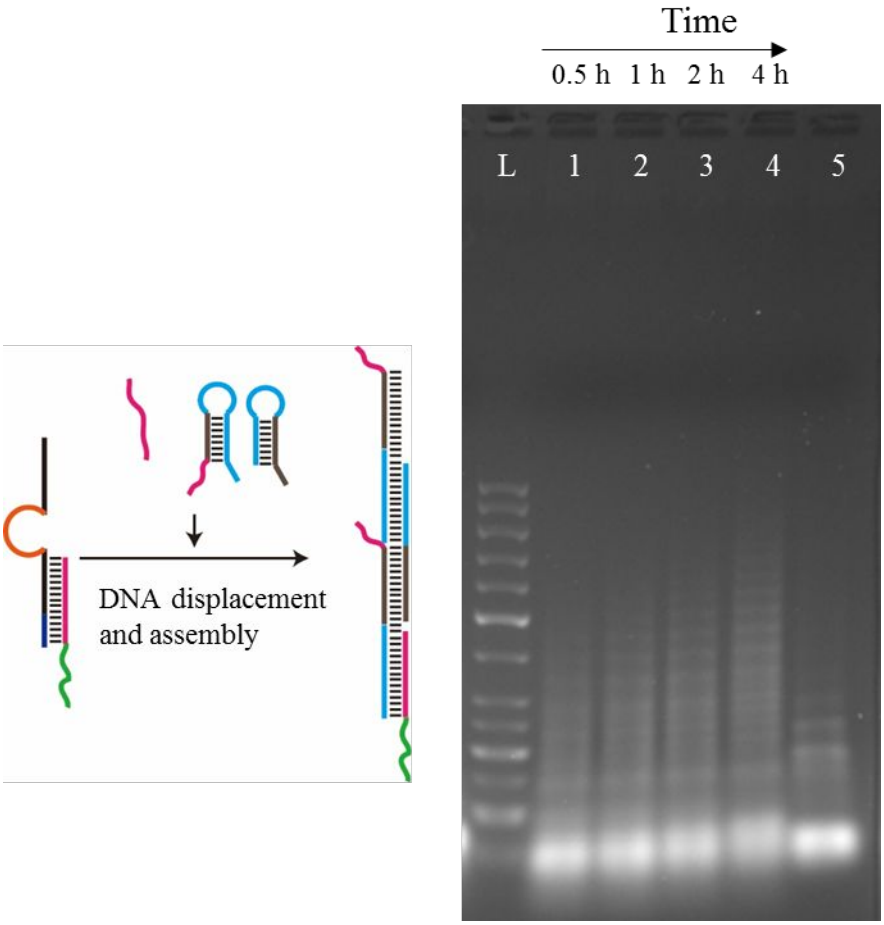

Figure S4. Agarose gel electrophoresis verified the stimulus-mediated dissociation of DNAzyme from trigger, which initiated the formation of DNA polymer. L: 50-bp DNA ladder, Line 1-4: different reaction time (0.5 h, 1 h, 2 h, and 4 h), indicating the formation of DNA polymer.

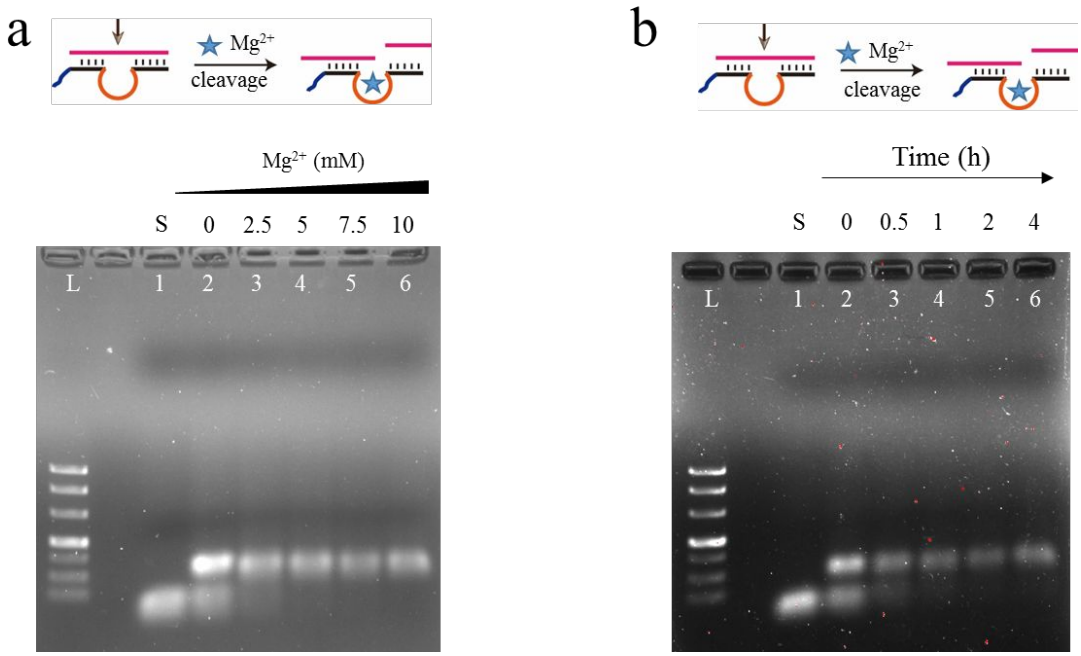

Figure S5. Agarose gel electrophoresis analysis of stimulus cleaved by DNAzyme. (a) Lane L: 20-bp DNA ladder. Line 1: stimulus. Line 2: cleavage of stimulus by DNAzyme (without Mg^{2+}). Line 3-6: cleavage of stimulus by DNAzyme in the presence of different Mg ion concentration (2.5 mM, 5 mM, 7.5 mM, and 10 mM). (b) L: 20-bp DNA ladder, Line 1: stimulus, Line 2: cleavage of stimulus by DNAzyme (without Mg^{2+}), Line 3-6: cleavage of stimulus by DNAzyme (in the presence of 10 mM Mg^{2+}) for different reaction time (0.5 h, 1 h, 2 h, and 4 h).

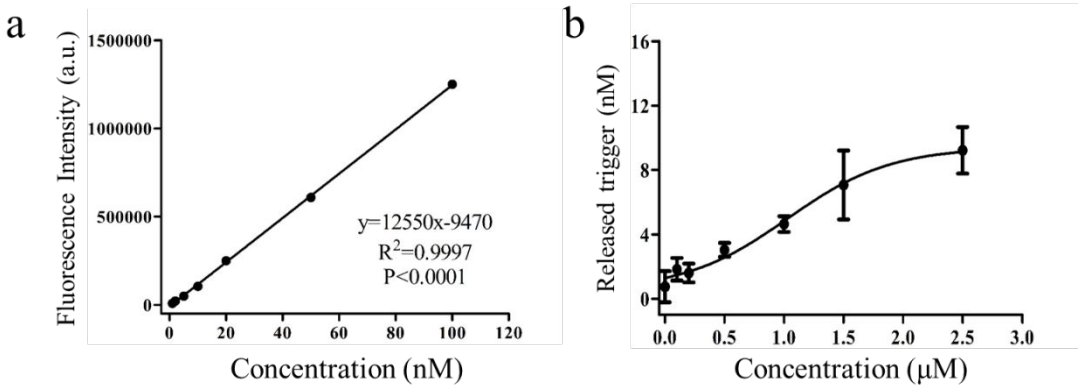

Figure S6. Fluorescence analysis of trigger-FAM/DNAzyme-BHQ-1 upon addition of different concentrations of Stimulus. The linear relationship between the fluorescence intensity and the concentration of Trigger-FAM was established using free trigger-FAM strand (a). Based on this relationship, the amount of trigger-FAM released from the hybrid of trigger-FAM/DNAzyme-BHQ-1 was evaluated upon the addition of stimulus (b). The DNAzyme concentrations was 0.8 μM and the concentration of trigger-FAM is 0.4 μM.

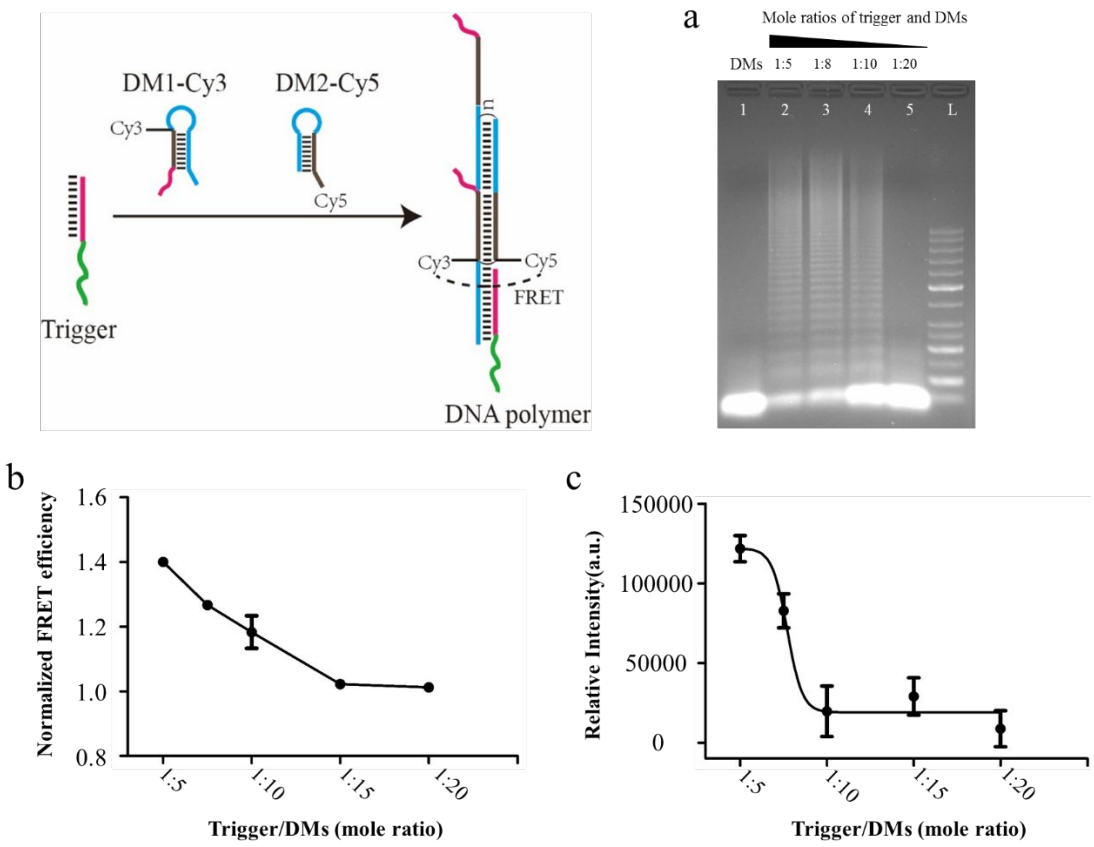

Figure S7. FRET efficiency between DM1-Cy3 and DM2-Cy5 upon the addition of Trigger with different concentration. Agarose gel electrophoresis analysis of DNA polymer formation by different mole ratio of Trigger/DMs (a). FRET efficiency was calculated according to the value of F_{Cy3}/F_{Cy5} (b). The increased fluorescence intensity of Cy5 also confirmed the increased amount of Trigger facilitated the DNA polymer formation (c). The concentration of DM1-Cy3 and DM2-Cy5 is 1.5 μM, and the concentration of Trigger ranges from 0.075 μM to 0.3 μM.

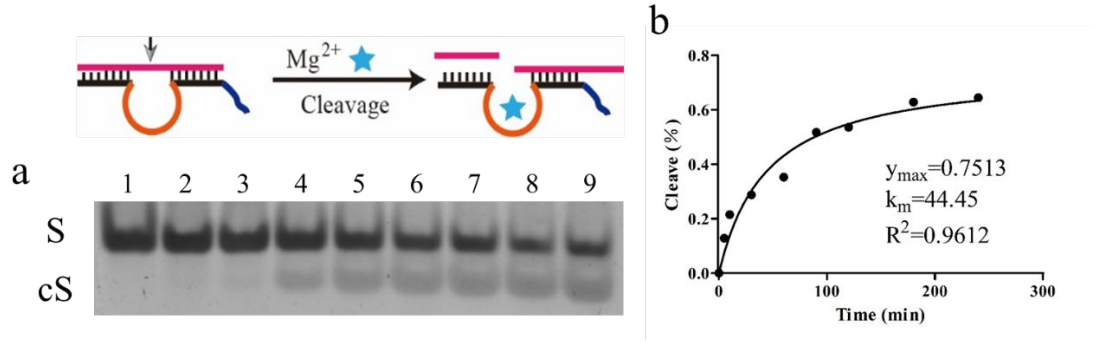

Figure S8. PAGE analysis of the cleavage of Stimulus by DNAzyme in the presence of Mg^{2+}. S: stimulus; cS: cleaved stimulus. Line 1-9: cleaved Stimulus upon the addition of DNAzyme for different time (0 min, 5 min, 10 min, 30 min, 60 min, 90 min, 120 min, 180 min and 240 min). The concentration is 10 μM for Stimulus, 3.2 μM for DNAzyme and 10 mM for Mg^{2+}. The cleavage rate is defined as the cleaved Stimulus (lane 2-9) compared with intact Stimulus (lane 1). Kinetic plot of DNAzyme was performed with GraphPad Prism 5. The black line is the fit using a simple model for Michaelis-Menten model.

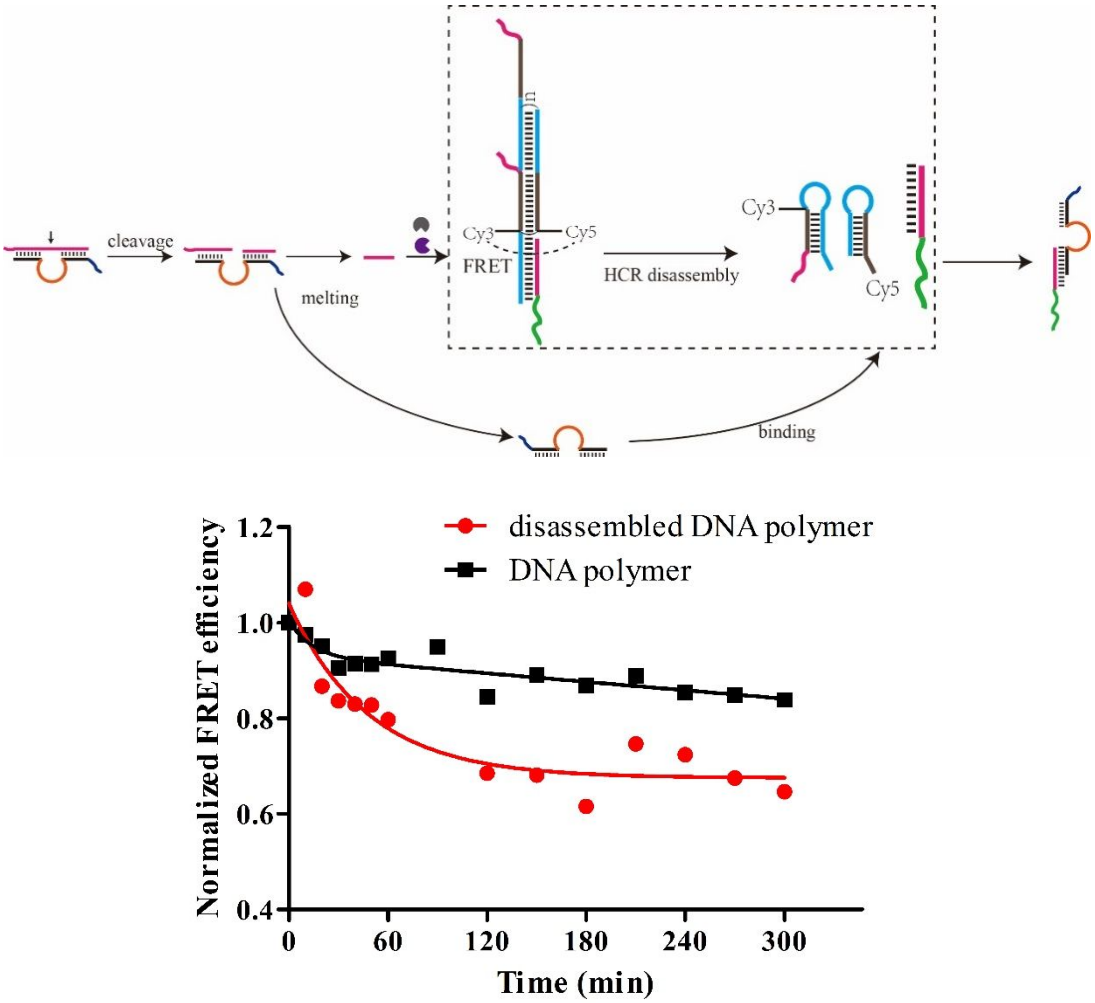

Figure S9. FRET efficiency between DM1-Cy3 and DM2-Cy5 upon the disassembly of DNA polymer (red line). Intact DNA polymer was used as control (black line). The concentration is 0.4 μM for Trigger, 1.5 μM for DM1-Cy3 and DM2-Cy5, 2.5 μM for stimulus, 0.8 μM for DNAzyme, 10 mM for Mg^{2+}, dNTP (1 mM), ATP (1 mM), 1×reaction buffer A for T4 PNK (containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 5 mM DTT, and 0.1 mM spermidine), 0.2 U μL^{-1} for T4 PNK, and 0.16 U μL^{-1} for Bsm DNA polymerase. The presented line is the fit using a model for two phase decay.

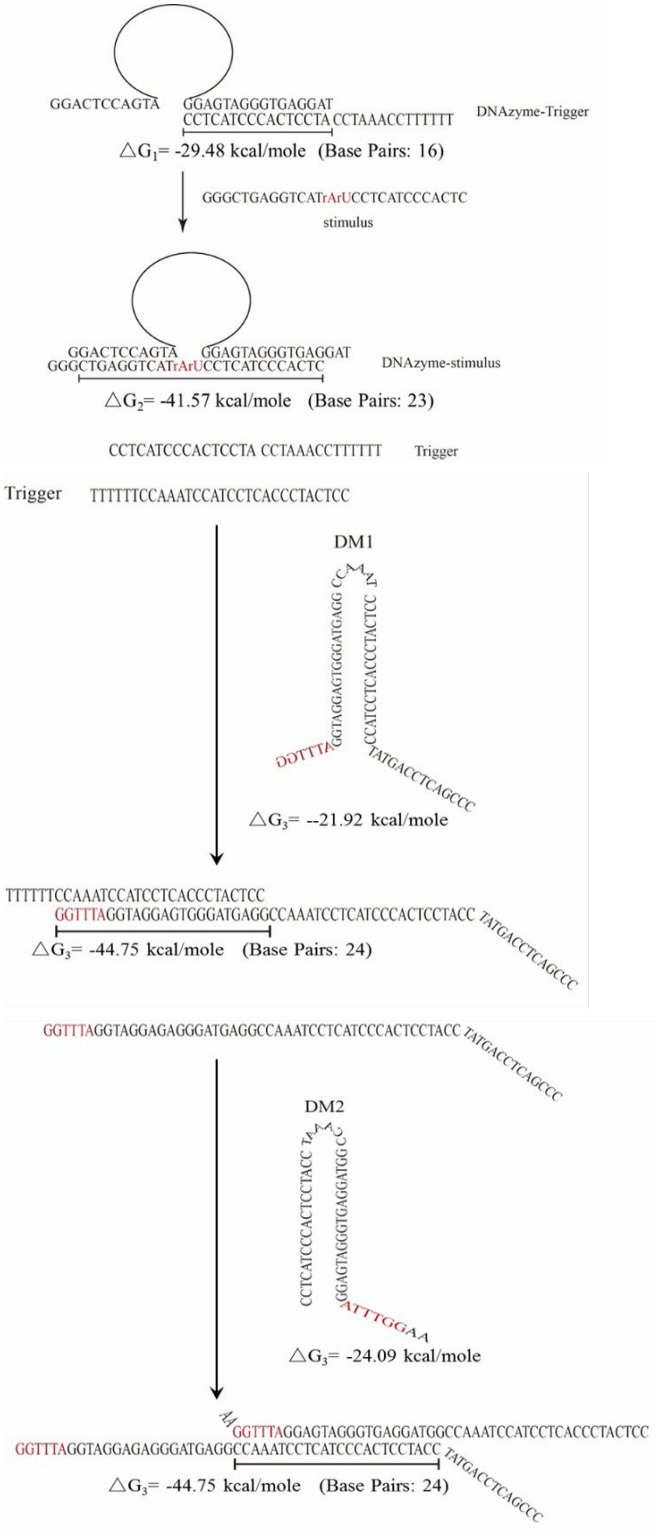

Figure S10. The ΔG values of the different structures of DNA were calculated with oligonucleotides analyze tool from Integrated DNA Technologies, Inc. (https://www.idtdna.com/calc/analyzer) under the condition of $[Mg^{2+}]$ = 10 mM, $[Na^{+}]$ = 100 mM, and at the temperature of 37 °C.

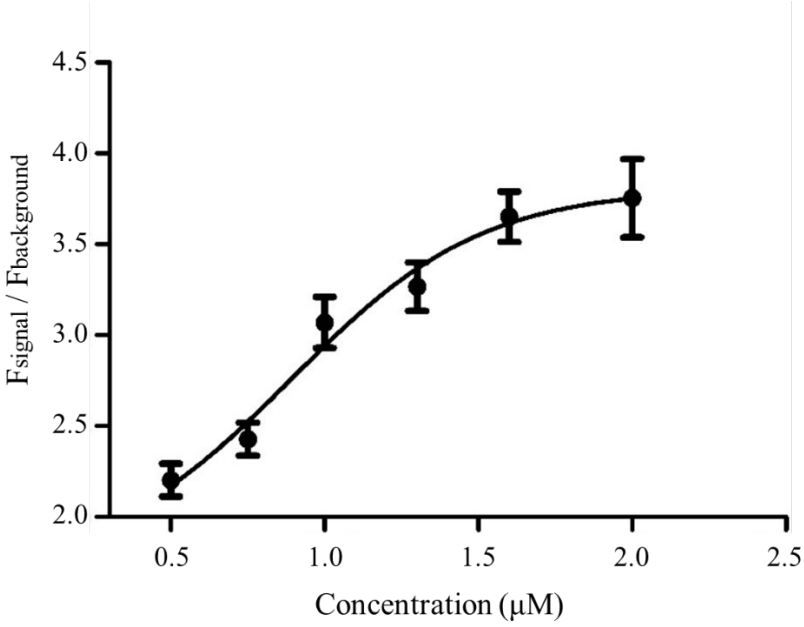

Figure S11. Fluorescence intensity of GMVs responding to different concentration of stimulus ranging from 0.5-2.0 μM. By comparing the fluorescence intensity on membrane surface and in solution (F_{in} / F_{out}), the sensitivity of this DNA-based system was estimated to be 1 μM for the value of F_{in} / F_{out} is above 3.

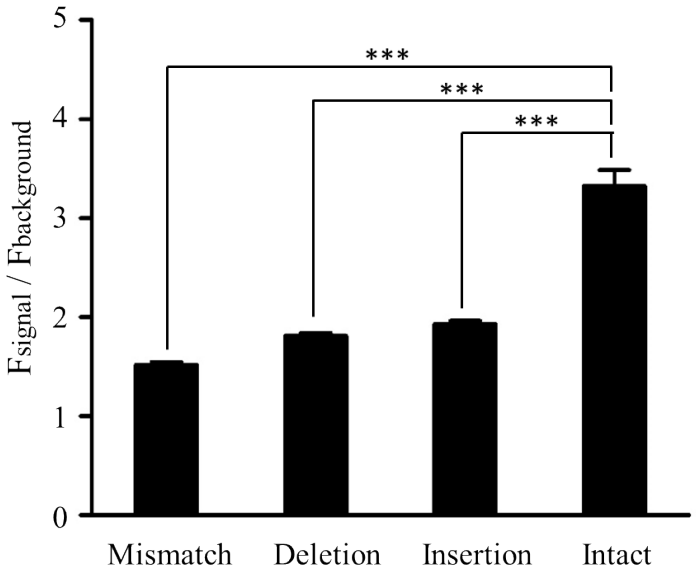

Figure S12. Selectivity of DASsys for Stimulus. The value of F_{in} / F_{out} is below 3 for the sequence of stimulus with mismatch, deletion and insertion, which indicated the high precision of this DASsys in responding to Stimulus. The statistical significance of the observed differences was analyzed by t tests. Statistical significance was set at $p < 0.05$ (*P < 0.05, **P < 0.01, ***P < 0.001).

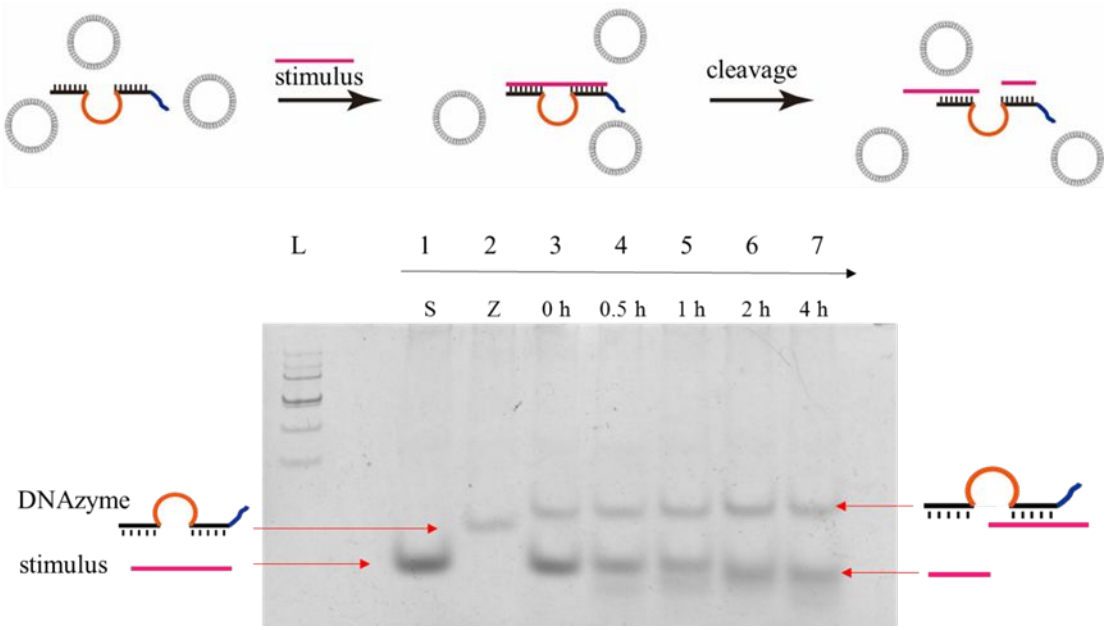

Figure S13. Cleavage of stimulus in the presence of GMVs. PAGE results indicated that the cleavage of stimulus could be accomplished in the presence of GMVs. L: 50-bp DNA ladder; Line 1: stimulus; Line 2: DNAzyme; Line 3-6: different reaction time upon the addition of stimulus (0 h, 0.5 h, 1 h, 2 h, and 4 h).

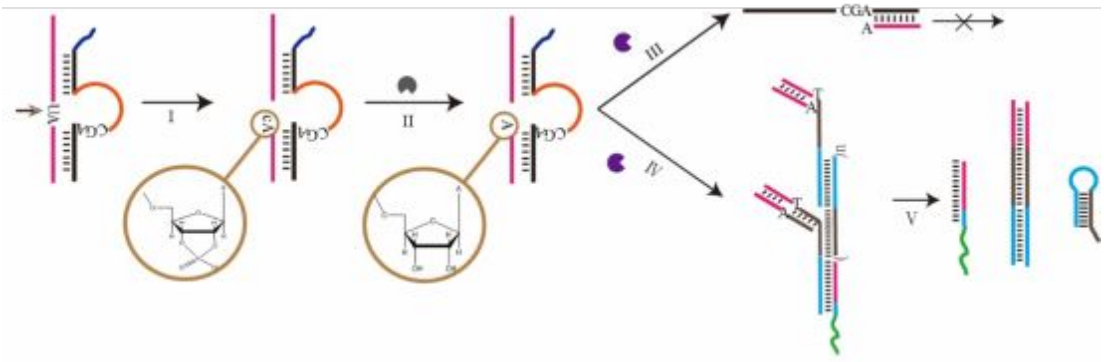

Figure S14. Enzyme-dependent DNA polymer disassembly. Stimulus was cleaved by DNAzyme to yield a forward RNA fragment with 2′, 3′ cyclic phosphate group on the 3′-end (I); PNK enzyme removed the cyclic phosphate group (II); DNA polymerase cannot replicate the stimulus primer with a mismatch at the 3′-end (III); stimulus primer extended by DNA polymerase on DNA polymer's toehold (IV); DNA polymer was disassembled (V).

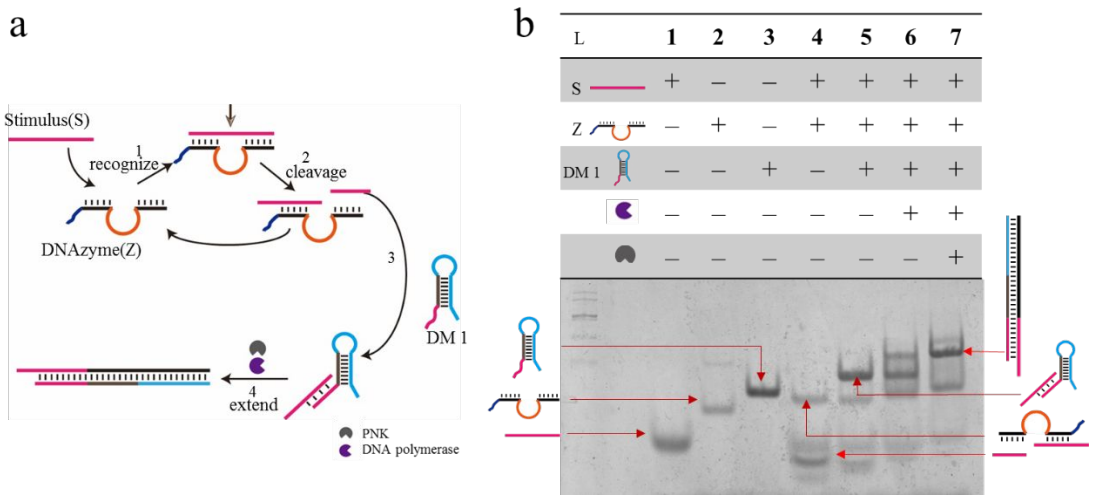

Figure S15. Schematic illustration of extension of cleaved stimulus. (a) Step 1: addition of stimulus. Step 2: cleavage of stimulus by DNAzyme. Step 3: cleaved stimulus binds with DM1's toehold. Step 4: the cyclic phosphate group of cleaved stimulus was removed by PNK enzyme, and the DNA strand was extended by DNA polymerase. (b) Polyacrylamide gel electrophoresis (PAGE) analysis verified the cleavage and extension of cleaved stimulus. L: 50-bp DNA ladder; Line 1: stimulus; Line 2: DNAzyme; Line 3: DM1; Line 4: cleavage of stimulus by DNAzyme; Line 5: PAGE analysis of steps 2-3; Line 6: PAGE analysis of steps 2-4 (without T4 PNK enzyme); Line 7: PAGE analysis of steps 2-4.

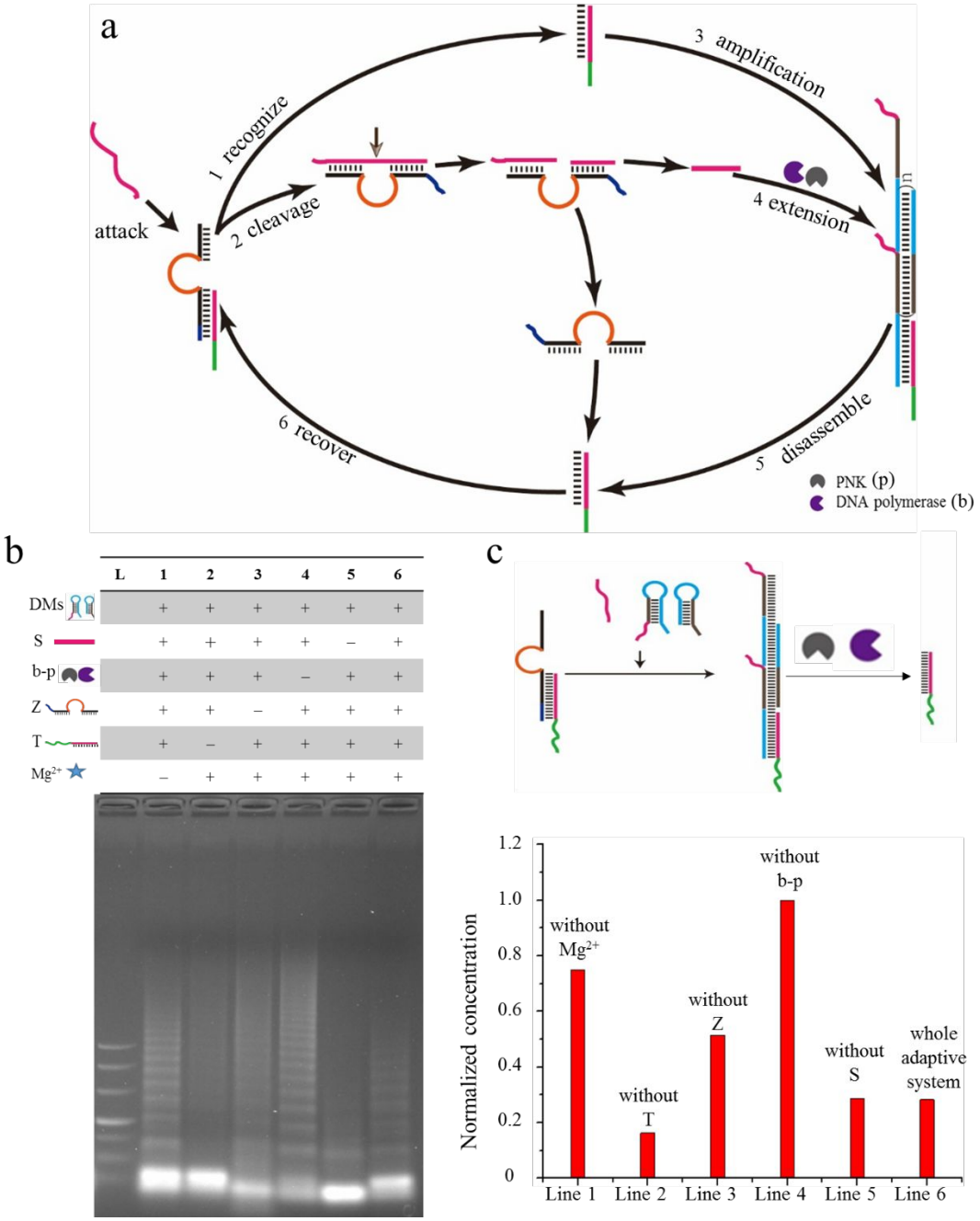

Figure S16. (a) Schematic design of DASsys. Step 1: Upon the addition of stimulus (S), trigger (T) was released; Step 2: cleavage of stimulus by DNAzyme (Z); Step 3: formation of DNA polymer by HCR; Step 4: cleaved stimulus binds with the toehold on DNA polymer, and extension occurred by PNK enzyme and Bsm DNA polymerase (b-p); Step 5: DNA polymer was disassembled, and the trigger was released. Step 6: free DNAzyme binds with trigger to form sensor for the next incoming stimulation. (b) Agarose gel electrophoresis analysis of DNA polymer formation and dissociation at different conditions. L: 50-bp DNA ladder; Line 1: DNA polymer could not be

disassembled in the absence of ion Mg^{2+}; Line 2: DNA polymer could not form in the absence of trigger; Line 3: DNA polymer could not be disassembled in the absence of DNAzyme; Line 4: DNA polymer could not be disassembled in the absence of enzymes; Line 5: DNA polymer could not form in the absence of stimulus; Line 6: disassembled DNA polymer for whole adaptive system. (c) The amount of produced DNA polymer counted from the image of agarose gel electrophoresis confirmed the feasibility of DASsys.

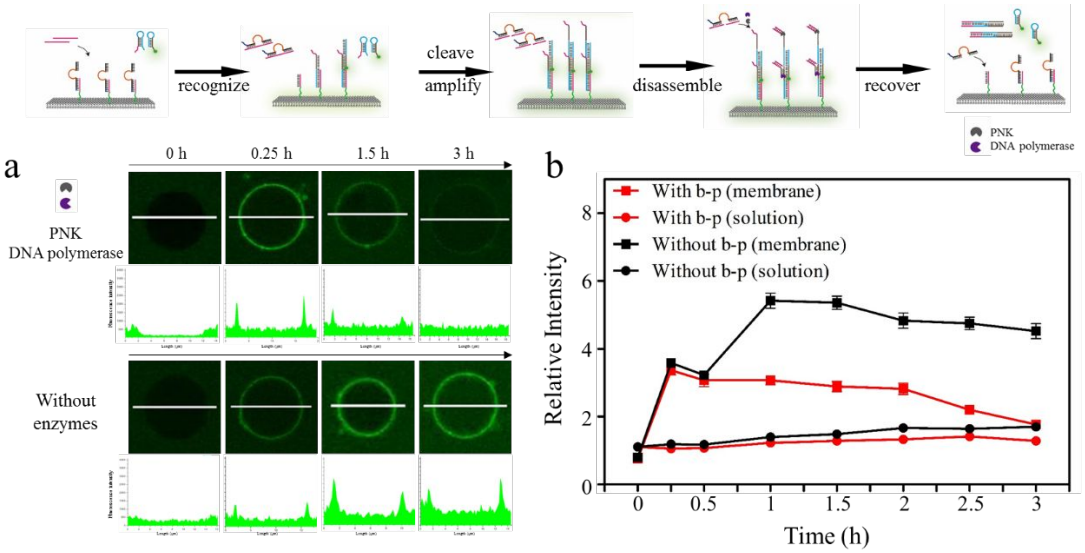

Figure S17. Construction of DASsys anchored on membrane surface. (a) Confocal imaging of DNA polymer assembly and disassembly in the presence of PNK and DNA polymerase (b-p) (first panel). For the absence of enzymes, DNA polymer was anchored on membrane (second panel). Scale bar is 5 μm. All samples were prepared by using 200 μL GMVs solution, and the incubation was carried out at 37 °C. (b) Relative mean fluorescence intensity of FAM-labeled DM2 on membrane surface (membrane) and in solution (solution) during the whole process. Red line: in the presence of Bsm polymerase and PNK enzymes; Black line: without Bsm polymerase and PNK enzymes. Square indicated the relative fluorescence intensity on membrane, and circle indicated the relative fluorescence intensity in solution. The error bars stand for the standard deviation from 20 GMV events at each time point.

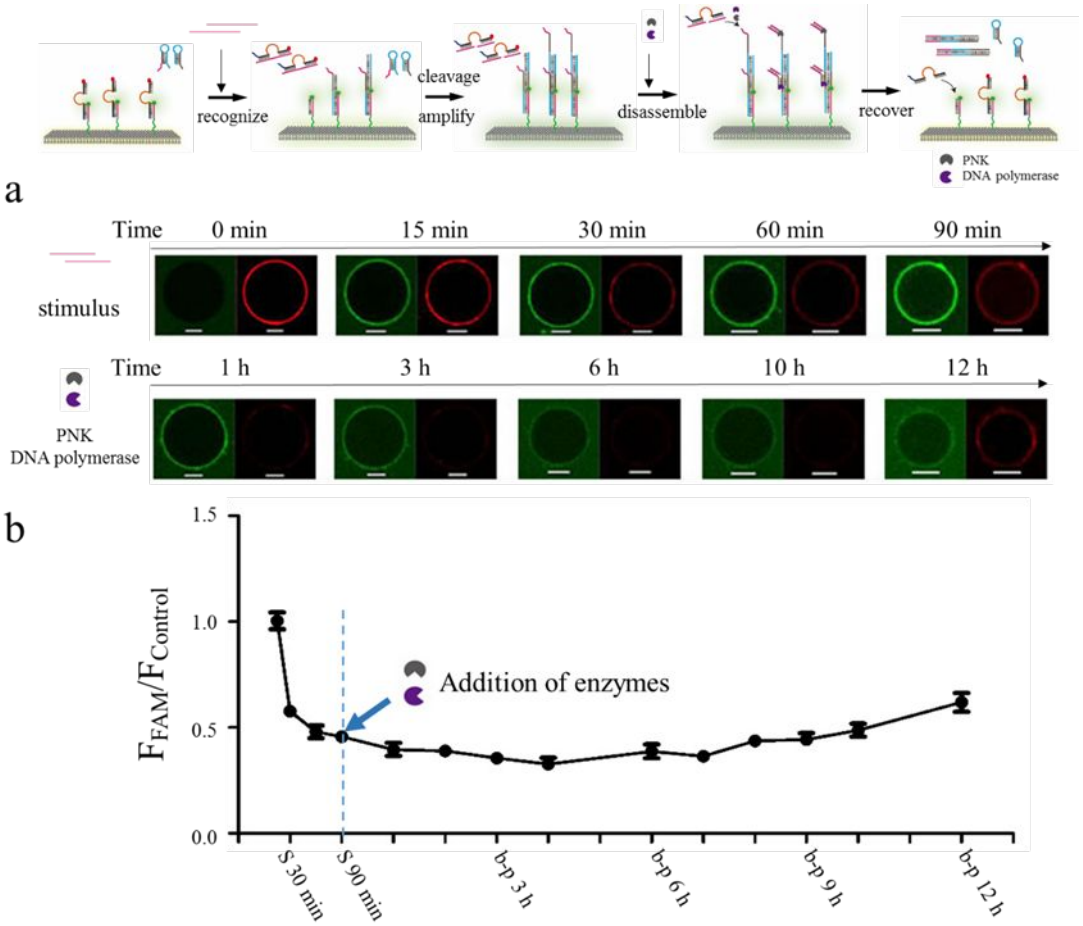

Figure S18. Schematic representation of DNA polymer assembly and disassembly on the membrane surface of GMVs. (a) Confocal imaging of DNA polymer formation on membrane surface triggered by stimulus (first panel). Afterwards, disassembly of DNA polymer upon the addition of Bsm polymerase and PNK enzymes (b-p) (second panel). Scale bar is 5 μm. (b) The fluorescence intensity of FAM on the membrane surface in the presence of stimulus (F_{FAM}) or without stimulus ($F_{control}$) was measured, and the value of F_{FAM} / $F_{control}$ was calculated. Fluorescence of FAM was monitored by confocal microscopy, and the intensity was calculated by ImageJ software. All samples were prepared by using a 200 μL GMVs solution, and the incubation was carried out at 37 °C.