Electronic Supplementary Information

Building the anti-interfering DNAzyme-powered micromachine against the inhibition of biological matrices

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CONTENTS

1 Materials and Instrumentation

1.1 Materials

The synthesized oligonucleotides were provided by Sangon Biotech. Co., Ltd. (Shanghai, China). And the sequences were listed in the table 1. Stock solutions of the oligonucleotides were diluted using ultrapure water (>18.2 MΩ cm^{-1}) and stored at -20°C. Streptavidin (SA) coated magnetic beads (300 nm, 10 mg mL^{-1}) were purchased from BioMag Scientific Inc. (Wuxi, China). Gold nanoparticles were ordered from Ted Pella (Redding, CA), NaCl, $MgCl_2.6H_2O$, acetic acid (HAc) and tween-20 were purchased from Kelong Chemical Reagent Co. (Chengdu, China). Tris(hydroxymethyl)aminomethane (Tris) and Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-2Na) were purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). Thrombin, bovine serum albumin (BSA), streptavidin (SA), myoglobin (MYO), immunoglobulin (IgG), human serum albumin (HSA), hemoglobin (HEMO), cytochrome C (Cyt-C) were ordered from Sigma-Aldrich (St. Louis, MO, USA). Exonuclease I (E.coli) was purchased from New England Biolabs (New England Biolabs Ltd., Beijing, China) Human serum and blood were obtained from West China Hospital. Diluted human serum and blood were prepared by adding varying volumes of reaction buffer (25 mM Tris-HAc, 100 mM NaCl, 10 mM $MgCl_2$, 0.05% tween-20, pH = 8.2). Without other special mentions, the regents were at least analytical grade.

1.2 Instrumentation

The fluorescence assays were conducted by F-7000 spectrofluorometer (Hitachi, Japan) Zeta potential test was achieved by the Zetasizer Nano ZS (Malvern, UK). The

confocal fluorescence images were pictured by Nikon N-SIM super-resolution microscope system.

2 Experimental section

2.1 Preparation of MB track

The streptavidin coated magnetic beads (SA-MBs) was obtained from BioMag Scientific Inc. (Wuxi, China). The synthesis step are included in this section: in order to crosslink free carboxylic acid on the surface of Fe_3O_4@SiO_2-COOH with amine-containing streptavidin (SA) via carbodiimide chemistry, Fe_3O_4@SiO_2-COOH (1 mg) was added into a 1.5 mL centrifuge tube, and washed three times with 500 μL of 25 mM MES buffer (pH 5.5). Then, freshly prepared EDC (50 μL, 50 mg mL^{-1}) and NHS (50 μL, 50 mg mL^{-1}) were added with gentle shaking for 15 min. After magnetic separation of the MBs from the excess EDC and NHS, the MBs were washed with MES buffer, and then 100 μg of SA (100 μL, 1 mg mL^{-1}) was added into the EDC and NHS activated Fe_3O_4@SiO_2-COOH, and incubated for 6 h at 23 °C with gentle shaking. Afterwards, the SA-MBs was incubated with 50 mM Tris-HCl (pH 8.0) with continuous shaking for 30 min at room temperature to quench the unreacted activated carboxylic acid groups. Finally, the SA-MBs were washed with 1×PBST buffer three times, and then resuspended in 1×PBST buffer for further use.

After washing the streptavidin coated magnetic beads (10 mg mL^{-1},100 μL) for 3 times using 300 μL binding buffer (10 mM Tris-HCl, 1 M NaCl, 1 mM EDTA, 0.05% tween-20, pH=7.4), 200 μL binding buffer was added into the tube to resuspend the magnetic beads. Then probe-1 or probe-3 (100 μM, 2 μL), substrate-FAM or

substrate-ROX (50 μM, 6 μL), control DNA (100 μM, 2 μL) were added into the beads solution, mixed and shaken at 37°C for 1 hour. At last, the excessive DNA strands were removed by magnetic separation and rinsed 3 times with 600 μL binding buffer and the magnetic beads was stored at 4°C and immersed in 3D DNA nanomachine reaction buffer (25 mM Tris-HAc, 100 mM NaCl, 10 mM $MgCl_2$, 0.05% tween-20, pH=8.2) containing 0.1% BSA.

2.2 Preparation of thiol-functionalized DNA nanomachine (gold nanoparticle as moving track)

The protocol is modified from our reported method[s1]. Briefly, Probe-1-thrombin and GNP-Substrate-ROX were mixed at 1:10 molar ratio. The mixture containing 0.05% Tween 20 was then incubated with 1 mL 20 nm AuNPs at room temperature for overnight. Then, 16.5 μL 3 M NaCl was utilized for six times to maximize the loading amount. After salting procedure, the mixture was incubated at room temperature for 24 hr. To reduce the interaction between DNA oligo and AuNP surface, we added certain concentration of DNA spacer (Ctrl DNA-SH) and obtain the mixture contained substrate, Probe-1-thrombin and spacer with 10:1:1 molar ratio. After incubation at room temperature for another 24 hr, the mixed solution was washed by centrifuge at 11, 000 g for 30 min. Finally, the functional AuNPs was re-suspended in 1×PBS (pH 7.4) at a concentration of 2.3 nM.

NOTE: For thiol-functionalized DNA nanomachine DNA nanomachines, 20 nm AuNP served as the moving track, other components, including sub-FAM, probe-1 and probe-2 were same as MBs-based DNAzyme-powder micromachines, but the AuNP

track was constructed by thiol-functionalized crosslinking.

2.3 Characterizations of the MB track

Zeta potential assays were conducted by the Zetasizer Nano ZS system (Malvern, UK). The MB track (5 mg mL^{-1}, 10 μL) and streptavidin coated magnetic beads (5 mg mL^{-1}, 10 μL) were added into the 1mL ultrapure water respectively. Because the pI value of streptavidin protein is about 6.0, the magnetic beads in water is negative charge. When the DNA was attached on the surface of magnetic beads, the zeta potential value would be greater in negative direction owing to the DNA strands with negative charge. The micromachine was observed under Nikon N-SIM super-resolution microscope system. The dye excited with a 480 nm laser line and detected with a 520 nm (±10 nm) band pass filter. The fluorescence images were presented after processing by ImageProplus 6.0 software.

2.4 The operation of anti-interfering DNAzyme-powered micromachine

In order to operate the prepared anti-interfering DNAzyme-powered micromachine, varying concentrations of HCV target (or targets containing varying concentrations of human serum or blood) was added into the reaction solution, including 0.25 mg mL^{-1} micromachine, 85 μL reaction buffer (25 mM Tris-HAc, 100 mM NaCl, 10 mM $MgCl_2$, 0.05% tween-20, pH = 8.2). After incubation for 20 minutes, it was washed by adding 100 μL reaction buffer for twice. Then varying concentrations of Probe-2 was mixed and incubated at room temperature for 3 hours. Subsequently, the mixture was separated by the magnet, the upper solution was transferred into quartz cuvettea

and its signal intensity was detected by the F-7000 spectrofluorometer using the following parameters. FAM (HCV target): ex: 480 nm, em: 500- 700 nm, ex slit: 10 nm, em slit: 10 nm. ROX (thrombin protein): ex: 570 nm, em: 595- 700 nm, ex slit: 10 nm, em slit: 10 nm.

2.5 The operation of thiol-functionalized DNA nanomachine in serum

In order to operate the prepared thiol-functionalized DNA nanomachine, 5 μL of various concentrations of thrombin proteins containing 50% serum were added into the reaction solution, including 100 pM nanomachine, 85 μL reaction buffer. After incubation for 20 minutes, it was washed by adding 100 μL reaction buffer for twice. Then 50 nM Probe-2-thrombin was mixed and incubated at room temperature for 3 hours. Without separation, the solution was collected in quartz cuvette and the signal intensity was detected by the F-7000 spectrofluorometer using the following parameters. ROX: ex: 570 nm, em: 595- 700 nm, ex slit: 10 nm, em slit: 10 nm.

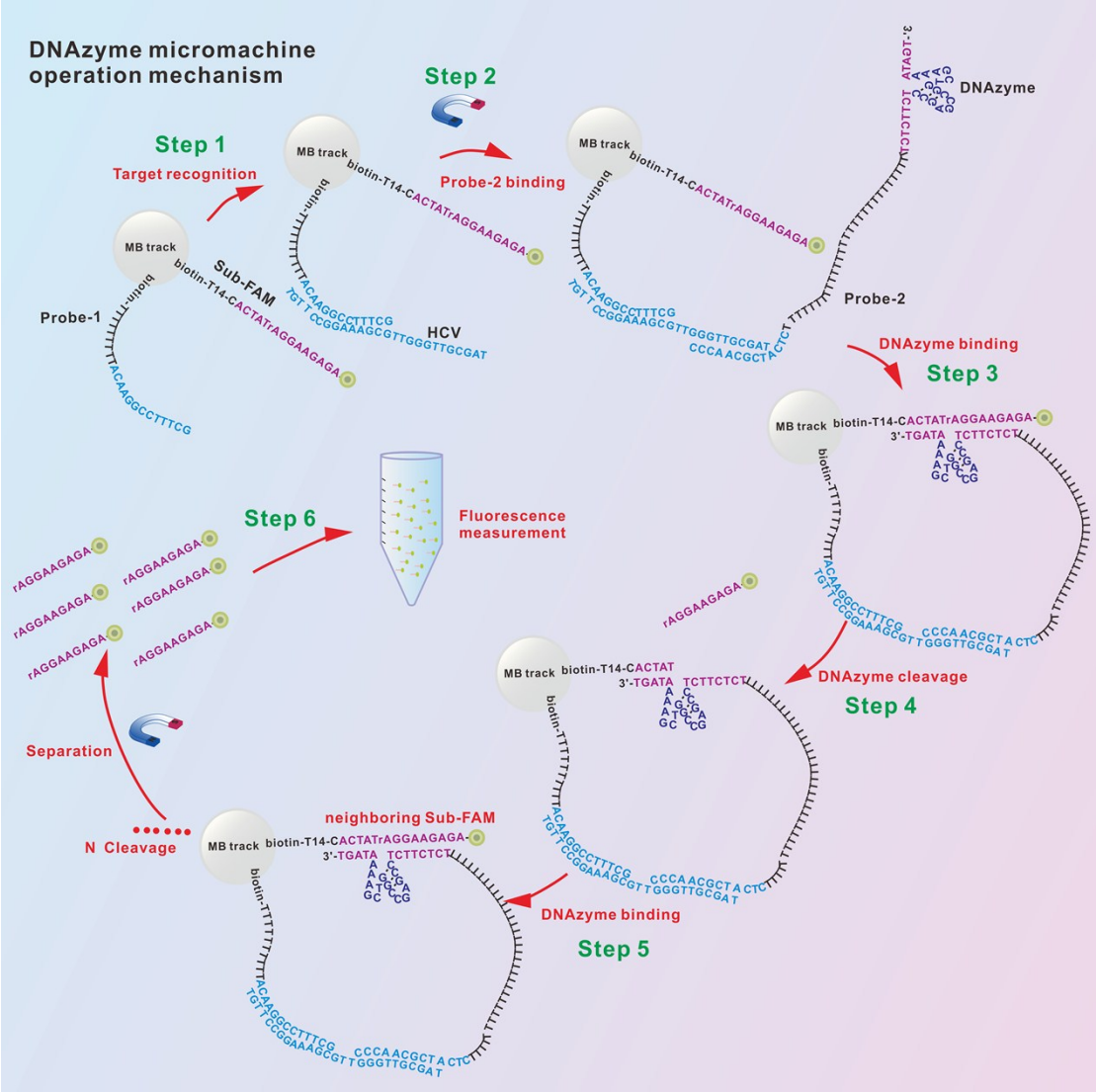
2.6 Ethics statement

The authors declared that all experiments were performed with the approval of the Committee on Biomedical Ethics of West China Hospital (CBEWCH) of Sichuan University. We have informed the consent on this study and human serum and blood samples for this study were treated as the guidelines of CBEWCH.

Table S1. DNA sequences used in the study

Oligonucleotides	**Sequence（5’→3’）**
Probe-1-HCV	Biotin-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTACAAGGCCTTTCG Probe-1 for HCV recognition
Probe-2-HCV	Probe-2 for HCV recognition Swing arm CCCAACGCTACTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTGTCTCTTCTCCGAGCCGGTCGAAATAGT Catalytic core
HCV target	TAGCGTTGGGTTGCGAAAGGCCTTGT
Substrate-FAM	Biotin-TTTTTTTTTTTTTTCACTAT/rA/GGAAGAGAT-FAM
Probe-1-Thrombin	Biotin- TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAGTCCGTGGTAGGG CAGGTTGGGGTGACT 29-mer aptamer for thrombin recognition
Probe-2-Thrombin	15-mer aptamer to thrombin Swing arm GGTTGGTGTGGTTGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTG TCTCTTCTCCGAGCCGGTCGAAATAGT Catalytic core
Substrate-ROX	Biotin-TTTTTTTTTTTTTTCACTAT/rA/GGAAGAGAT-ROX
Control-DNA	Biotin-TTTTTTTTTTTTT
GNP-probe-1	SH- TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAGTCCGTGGTAGGG CAGGTTGGGGTGACT 29-mer aptamer to thrombin
GNP-Substrate-ROX	SH-TTTTTTTTTTTTTTCACTAT/rA/GGAAGAGAT-ROX
Ctrl-DNA-SH	SH-TTTTTTTTTTTTT
HCV-SNP1	TAGCG**A**TGGGTTGCGAAAGGCCTTGT

HCV-SNP2	TAGCG**A**TGGGTTGCGAAAGGC**G**TTGT
HCV-SNP3	TAGCG**A**TGGGT**A**GCGAAAGGC**G**TTGT



Scheme S1. The mechanism of DNAzyme-powered micromachine operation. Step-1: In the presence of HCV nucleic acids, the Probe-1 attached on MB track can capture the HCV target via base pairing interaction. Step-2: After removing the sample matrix by washing and magnetic separation, free probe-2 was introduced and capture the HCV, bringing the the DNAzyme onto the surface of MBs track, dramatically increasing the local effective concentrations of DNAzyme and its substrates. Step-3: According to the principle of bind-induced DNA assembly, the stability of duplex of DNAzyme-substrate was enhanced. Step-4: With the help of cofactor Mg2+, the DNAzyme was folded into active structure and cleaved substrates, releasing the

fluorescent fragment of substrate. Step-5: This DNAzyme is also liberated from hybridization due to the instability of the duplex. The movement of DNAzyme can be accomplished from one to the next substrate on the surface of MBs by using free energy from hydrolysis of cleavage. Step-6: After magnetic purification, the cleaved fluorescent fragment of substrate can be transferred into fluorometer for quantification. Thus, DNAzyme-powdered mircomachines can be used for amplified detection of specific target.

3. Results and discussion

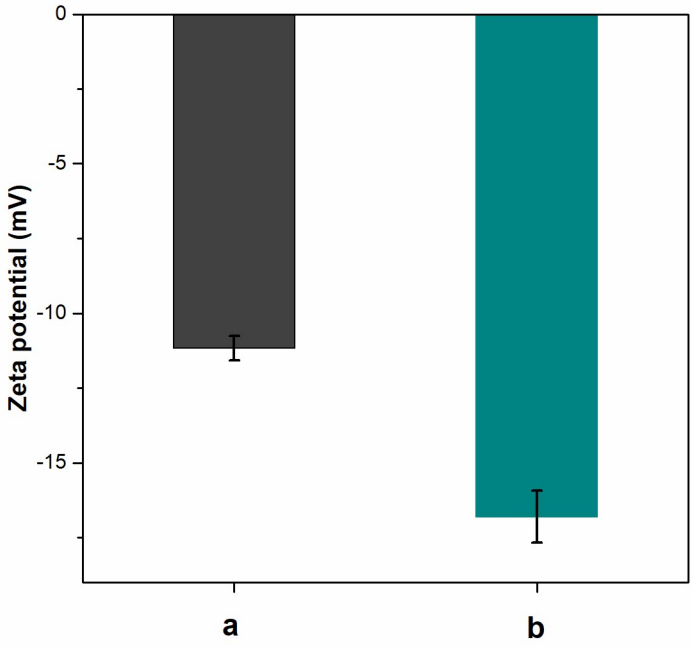


Fig. S1 Zeta potential test of MB track. (a) 0.05 mg mL^{-1} streptavidin coated magnetic beads, (b) 0.05 mg mL^{-1} MBs track. The streptavidin coated magnetic beads in water is negative charge, resulting in the streptavidin pI value is about 6.0. When the DNA was attached on the surface of magnetic beads, the zeta potential value would be shifted to negative direction due to the negative charge of DNA strands. Each error bar represents one standard deviation from triplicated analyses.

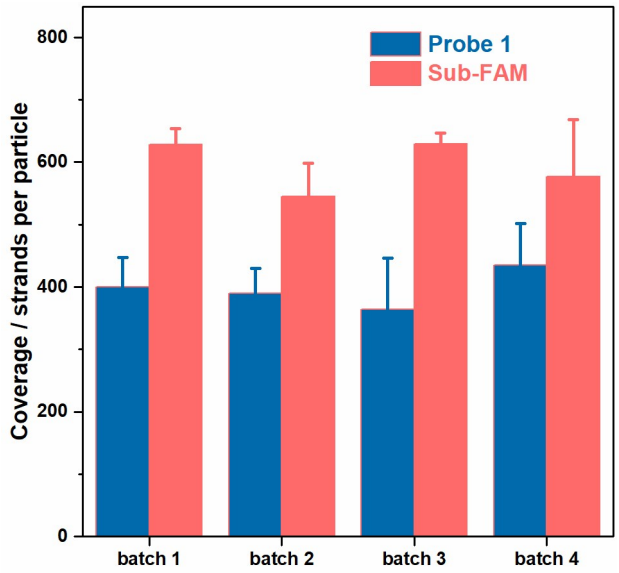


Fig. S2 Coverage detection of Probe-1 and Sub-FAM on magnetic beads. In this assay, Probe 1 was labelled with ROX flurophore for signal indication, Sub-FAM still was tagged with FAM flurophore. They were mixed with control DNA at the same preparation described in ESI 2.1 MB track preparation. Then, 0.025 mg mL^{-1} MB track (about 3.645×10^8 particles, from the manufactur) was incubated with 0.2 U μL^{-1} Exo I at 37°C for 3 hours. Such mixtures were separated by magnet and the solutions were collected for fluorescence measurement. For ROX labelled Probe-1, ex: 570 nm, em: 595- 700 nm, ex slit: 10 nm, em slit: 10 nm. For Sub-FAM, ex: 480 nm, em: 500-700 nm, ex slit: 10 nm, em slit: 10 nm. The detailed calculation was originated from the reported work. [S1] The produce of functionalized MBs track with low batch-to-batch variation, ensured the reproducibility of operation of the DNAzyme-based micromachines. Each error bar represents one standard deviation from triplicated analyses.

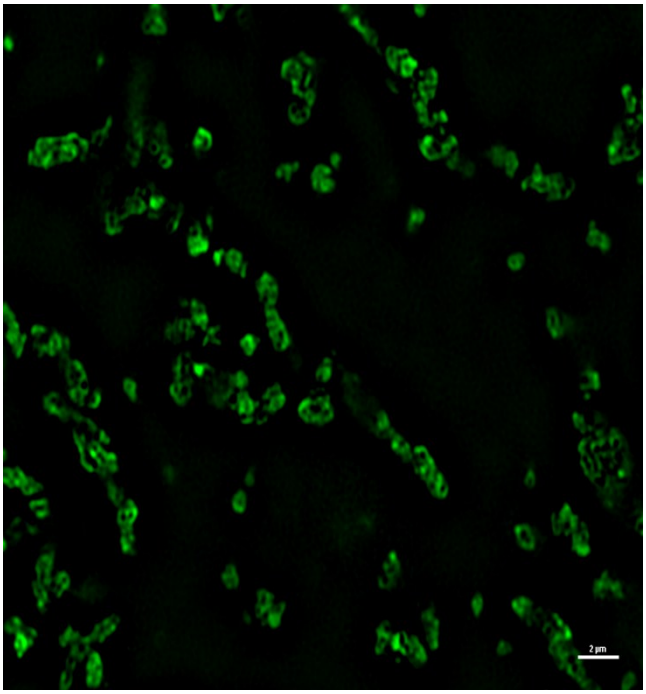


Fig. S3 Confocal fluorescence microscope image of MB tracks. The conjugated MB tracks were purified and dispersed in 1X PBS buffer (pH=7.4). Once excited, the obvious green fluorescence signal could be observed in the imaging, suggesting the MBs track was successfully decorated with substrate strand. The ruler is 2 μm.

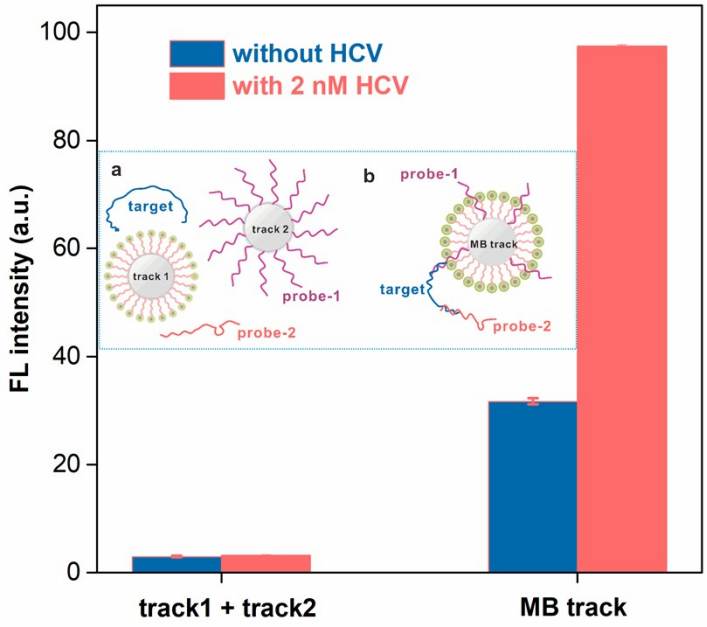


Fig. S4 Testing the cross cleavage between neighboring micromachine. The test principle was illustrated in Fig. S4a. The track 1 (0.1 mg mL^{-1}), track 2 (0.1 mg mL^{-1}) and all the components were incubated together, resulting in a low FL intensity signal. But operation occurring at our designed MB track (0.25 mg mL^{-1}) is significant, which indicates the DNAzyme cleavage reaction is carried out on one individual magnetic bead without inner-particle cross cleavage. Each error bar represents one standard deviation from triplicated analyses.

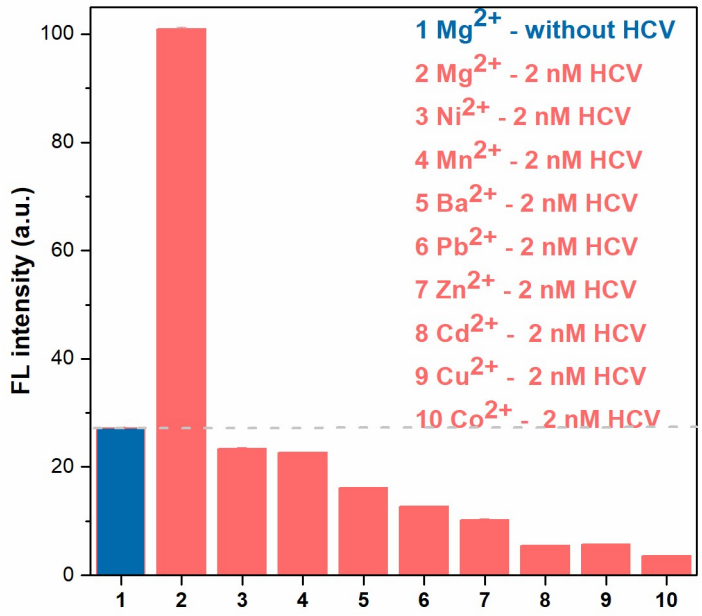


Fig. S5 Evaluation of DNAzyme cofator. Because of while various metals existed in human serum and DNAZyme in this study was truncated. There was a requirement to study the specificity of cofactor activity. Commonly used dicovalent ions were conducted for this assays. As presented in the Fig. S5, the micromachine only can be trigered at the present of Mg^{2+}. [MB-Track] = 0.25 mg mL^{-1}, [HCV] = 2 nM, [probe-2] = 30 nM, It took 20 min for target binding. After separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours.

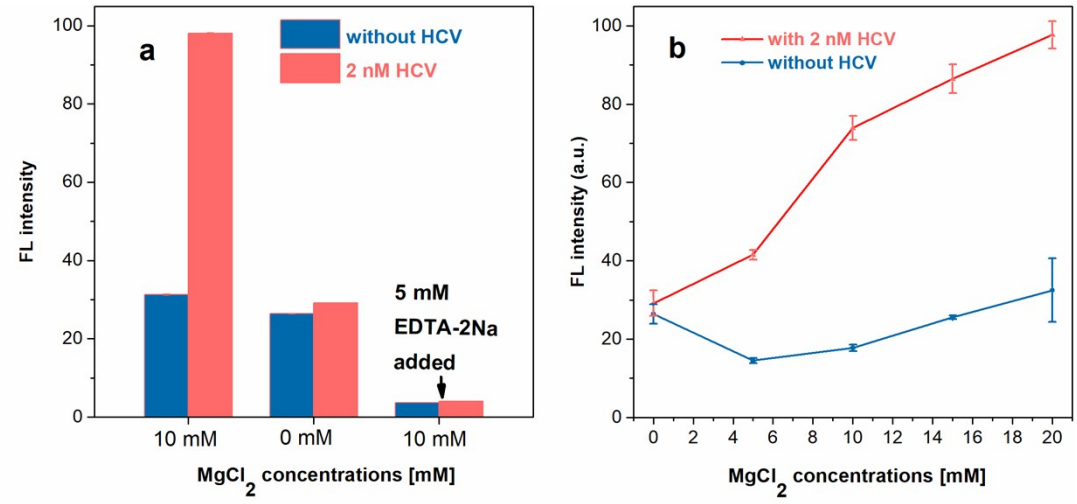


Fig. S6 Micromachine was triggered using different concentrations of cofactor Mg^{2+}. As shown in Fig. S6a, there is no obvious signal increasing, indicating no cleavage of substrate without cofactor Mg^{2+}. Meanwhile, coordination reagent EDTA was added deactivate the DNAzyme cleavage activity, the fluorescence signal is much lower than other group. The Mg^{2+} not only is the cofactor for assisting the DNAzyme folding, but also it is a key parameter for DNAzyme and substrate hybridization. Therefore, a higher background was generated at a higher concentration of Mg^{2+}, due to the undesired hybridization and cleavage. For the best ratio of signal to background, 10 mM Mg^{2+} was chosen for following assays (Fig. S6b). [MB-Track] = 0.25 mg mL^{-1}, [HCV] = 2 nM, [probe-2] = 30 nM. After separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

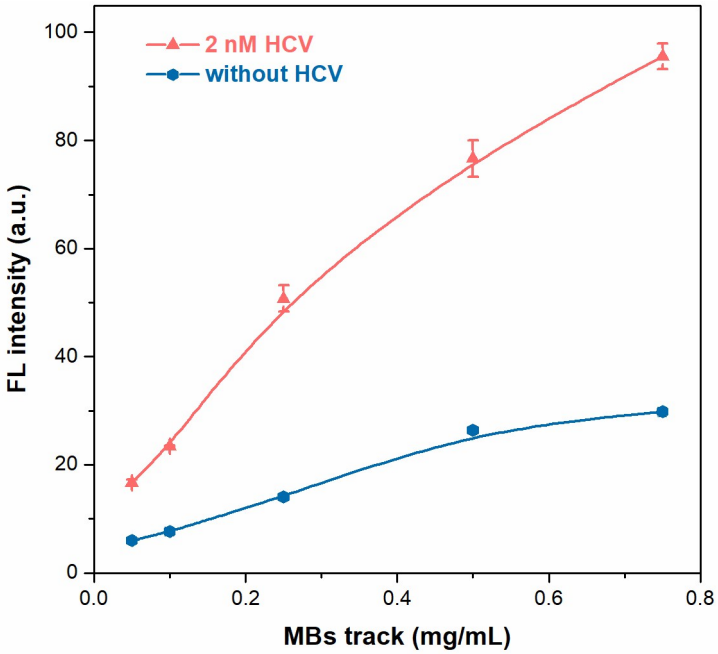


Fig. S7 HCV sensing at different concentrations of anti-interfering DNAzyme-powered micromachine. The more micromachine lead to more targets binding events. However, in crowed environment, the nonspecific cleavage of substrate or absorption, would cause the higher background. Hence, 0.25 mg mL^{-1} DNAzyme-powered micromachine tracks of gave rise to the optimal ratio. [HCV] = 2 nM, [probe-2] = 30 nM. After separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

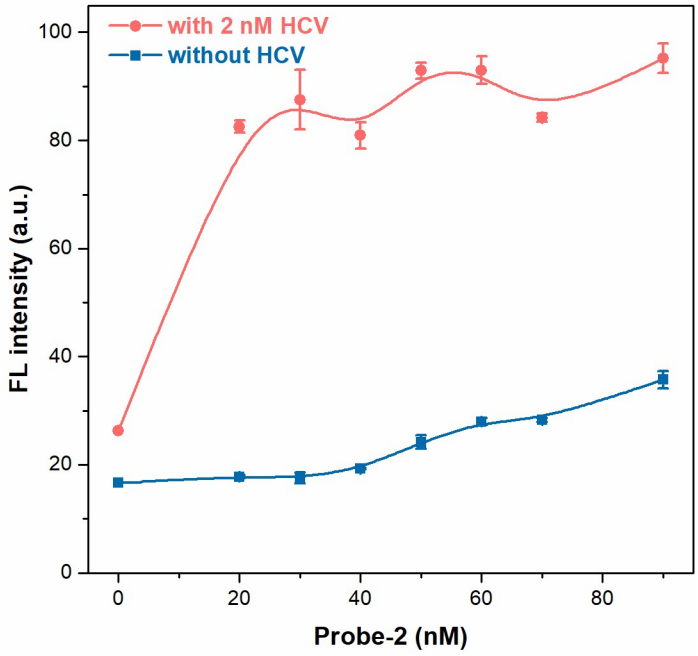


Fig. S8 The operation curve of anti-interfering DNAzyme-powered micromachine under different concentrations of probe-2. We hypothesised that redundant probe-2 would cuase a severe background due to independent hybridization between DNAzyme and its substrates. Therfore, it is necessary to optimise the concentrations of probe-2. As shown in the Fig. S8, 30 nM of probe-2 generated higher signal and lower backgroud than that of other concentrations. [MB-Track] = 0.25 mg mL^{-1}, [HCV] = 2 nM. After separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

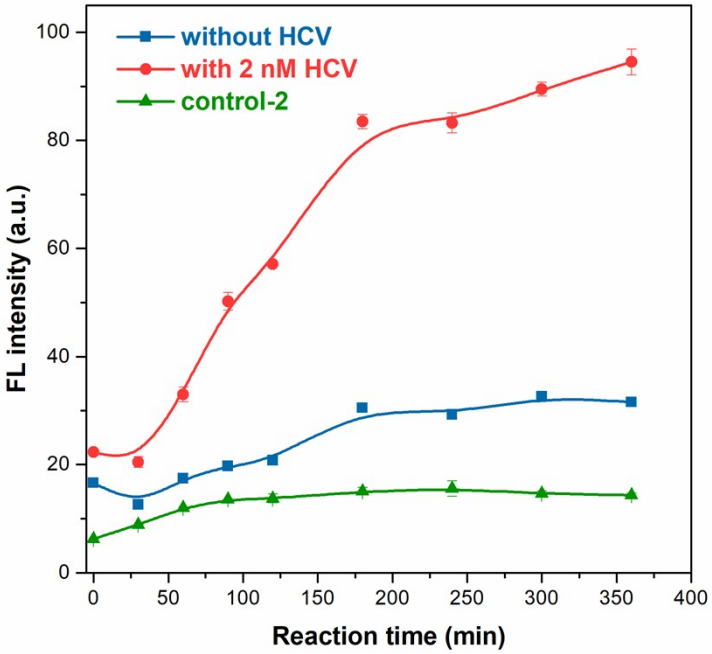


Fig. S9 The anti-interfering DNAzyme-powered micromachine operation at different reaction time. Owing to the DNAzyme cleavage is a kinetic process, the cleavage rate was time-dependent manner. As shown in the Fig. S9, the fluorescent signal is increased along with the reaction time, indicating that the more splited substrates are released from the magnetic tracks. We chose the 3 hours as reaction time. [MB-Track] = 0.25 mg mL^{-1}, [HCV] = 2 nM, [probe-2] = 30 nM. Control-2 represented MB track conjugated with substrates and probe-1-HCV only. After separation, the DNAzyme cleavage reactions were carried out at room temperature for varying time. Each error bar represents one standard deviation from triplicated analyses.

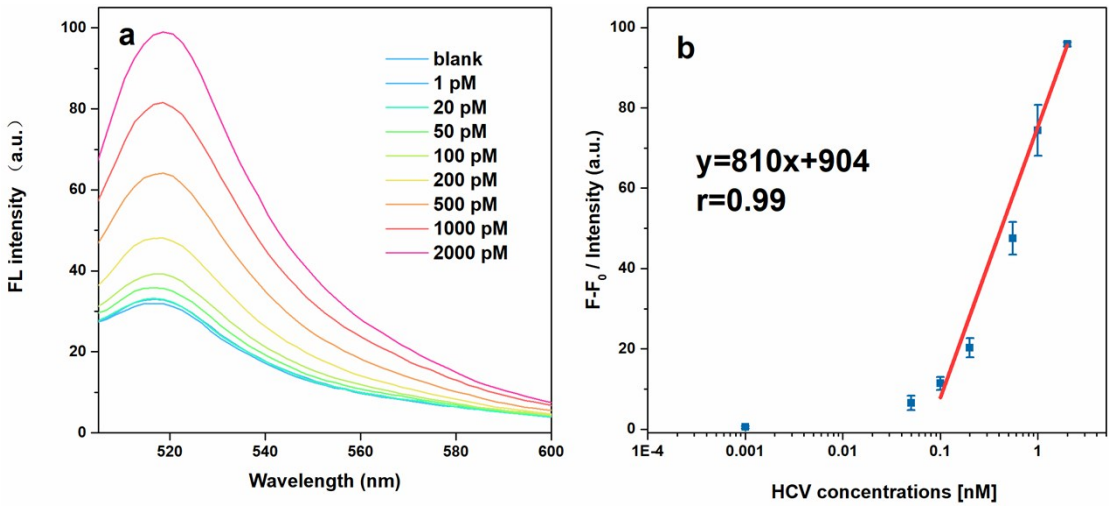


Fig. S10 Analytical performance of sensing **HCV** using the anti-interfering DNAzyme-powered micromachine in buffer solution. a) Various concentrations of HCV target activated the micromachine and the results were shown as in the Fig S10(a), its detection limit is 1 pM. b) Dynamic range of the HCV target. [MB-Track] = 0.25 mg mL^{-1}, [probe-2] = 30 nM, varying concentrations of HCV target. It took 20 min for target binding. After separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

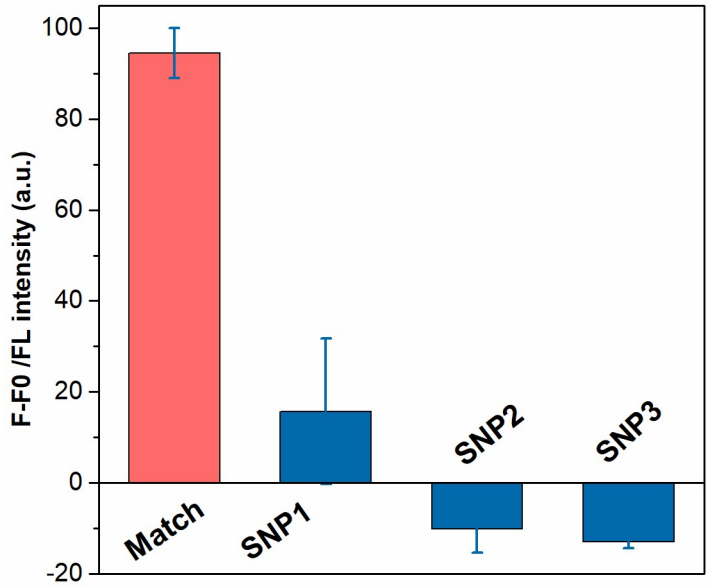


Fig. S11 Single Nucleotide Polymorphism assay. Match DNA strand is 1 nM. The mutation sites of SNP1, SNP2 and SNP3 are highlighted in the table S1. The concentrations of DNA in this assay are 5 nM, respectively. [MB-Track] = 0.25 mg mL^{-1}, [probe-2] = 30 nM, It took 20 min for target binding. After separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

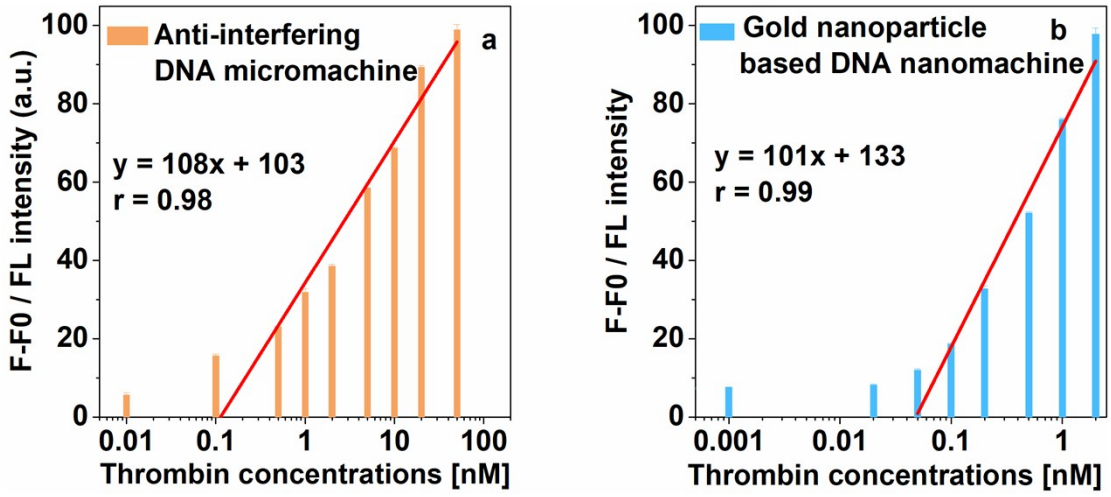


Fig. S12 Comparison performance of sensing thrombin protein using anti-interfering DNAzyme-powered micromachine and gold nanoparticle based DNA nanomachine in buffer solution. a) Various concentrations of thrombin protein activated the anti-interfering DNAzyme-powered micromachine. Its detection limit is 10 pM. b) Various concentrations of thrombin protein activated the gold nanoparticle based nanomachine. [AuNP-Track] = 100 pM, [probe-2-thrombin] = 50 nM, After target recognition, for the anti-interfering DNA micromachine, after separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours. For the gold nanoparticle based DNA nanomachine, without separation, and it was carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

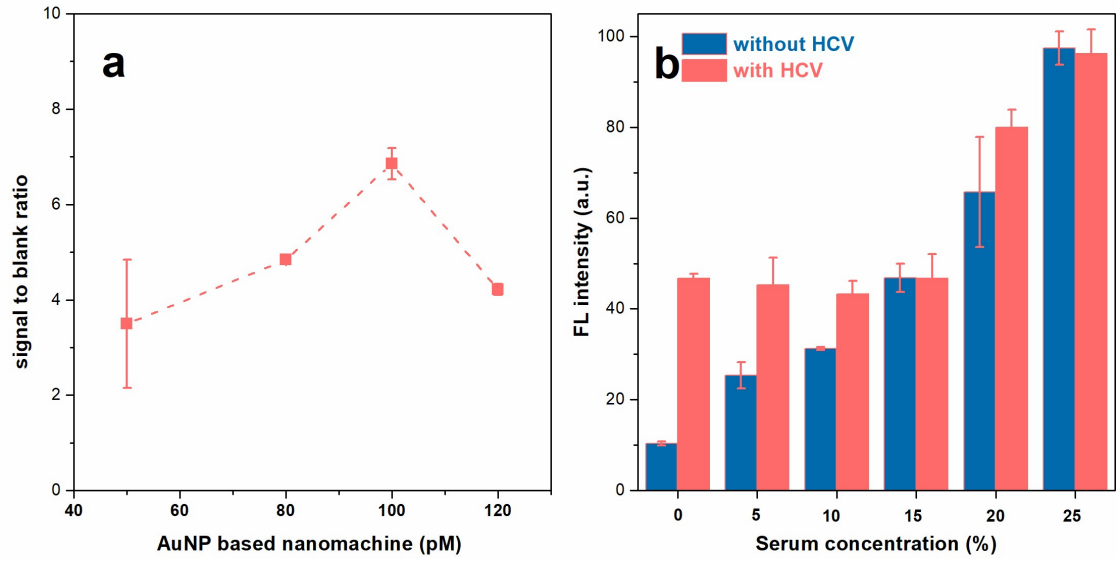


Fig. S13 (a) AuNP track concentration optimization. Optimized concentration for AuNP-based DNA nanomachine is 100 pM. (b) Analytical performance of sensing HCV using the AuNP based nanomachine in varying concentraions of serum. [HCV] = 0.5 nM, [AuNP-Track] = 100 pM, [probe-2] = 10 nM. The reactions were carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

NOTE: For AuNP-based DNA nanomachines, 20 nm AuNP served as the moving track, other components, including sub-FAM, probe-1 and probe-2 were same as MBs-based DNAzyme-powder micromachines, but the AuNP track was constructed by thiol-functionalized crosslinking.

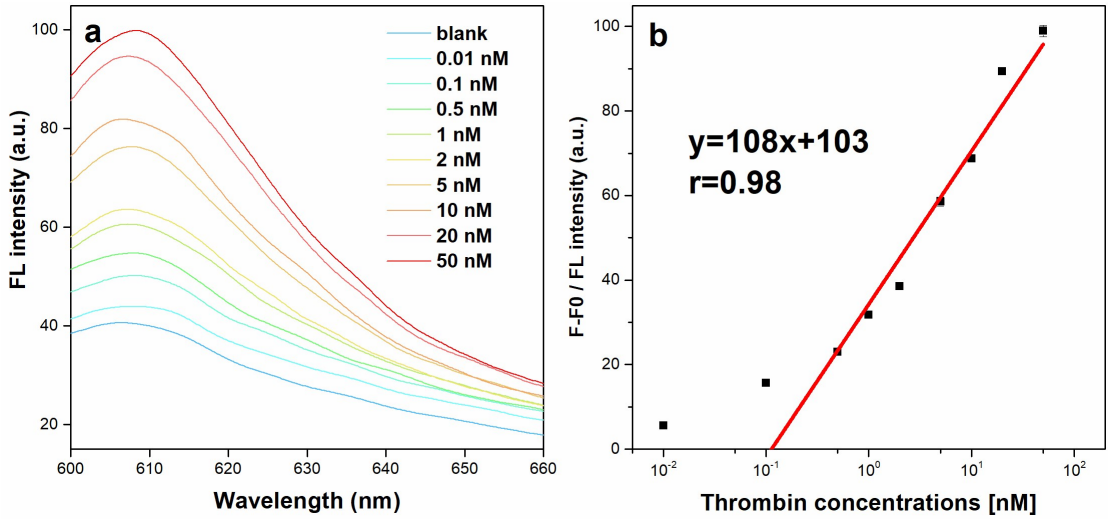


Fig. S14 Analytical performance of sensing **thrombin protein** using the anti-interfering DNAzyme-powered micromachine in buffer solution. a) Various concentrations of thrombin protein activated the anti-interfering DNAzyme-powered micromachine. Shown as in the Fig S14(a), its detection limit is 10 pM. b) Dynamic range of the thrombin protein. [MB-Track] = 0.25 mg mL^{-1}, [probe-2-thrombin] = 50 nM, It took 20 min for target binding. After separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

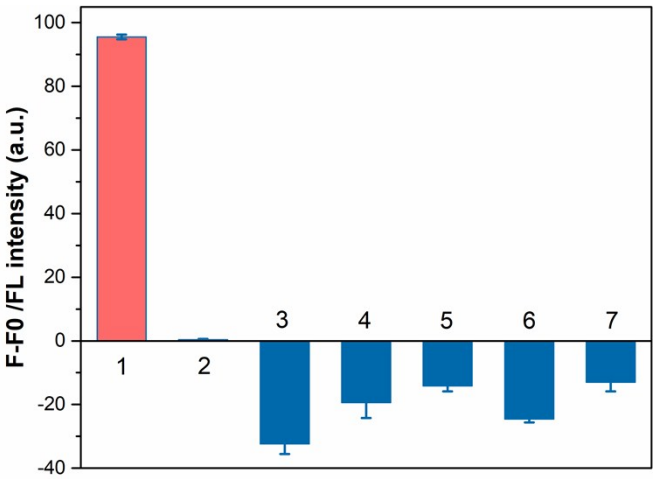


Fig. S15 Investigation of specificity of anti-interfering DNAzyme-powered micromachine. (1) Thrombin is 2 nM, (2) SA, (3) HSA, (4) MYO, (5) HEMO, (6) Cyt-C, (7) IgG are 10 nM, respectively. [MB-Track] = 0.25 mg mL^{-1}, [probe-4] = 50 nM, It took 20 min for target binding. After separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

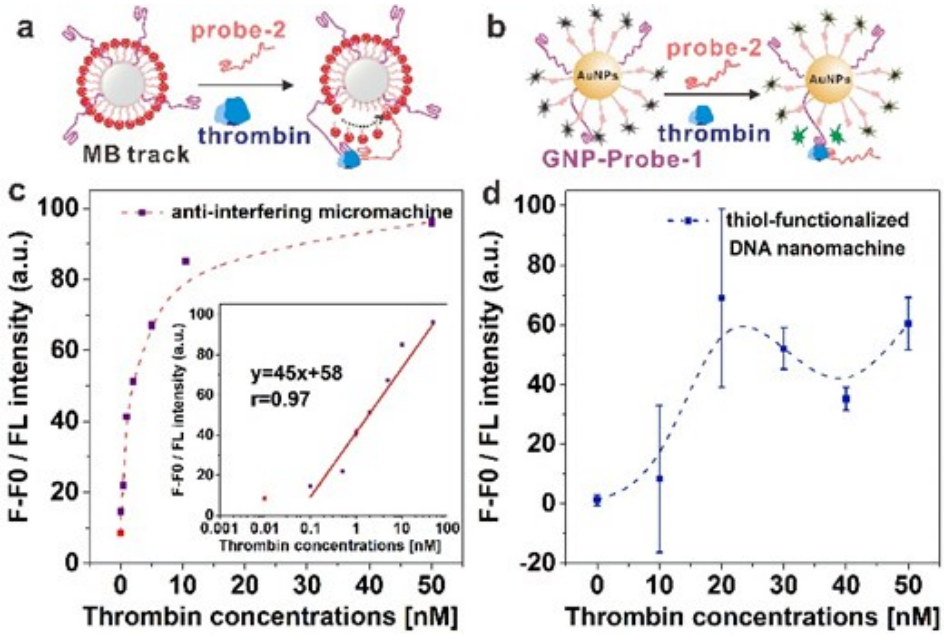


Fig. S16 Comparison of anti-interfering capability between anti-interfering micromachine (a, c) and thiol-functionalized DNA nanomachine (b, d). [MB-Track] = 0.25 mg mL^{-1}, [AuNP-track] = 100 pM, [Target] = 2 nM, [probe-2- thrombin] = 50 nM, 50% serum was presented in reaction solution. It was carried out at room temperature for 3 hours. Each error bar represents one standard deviation from triplicated analyses.

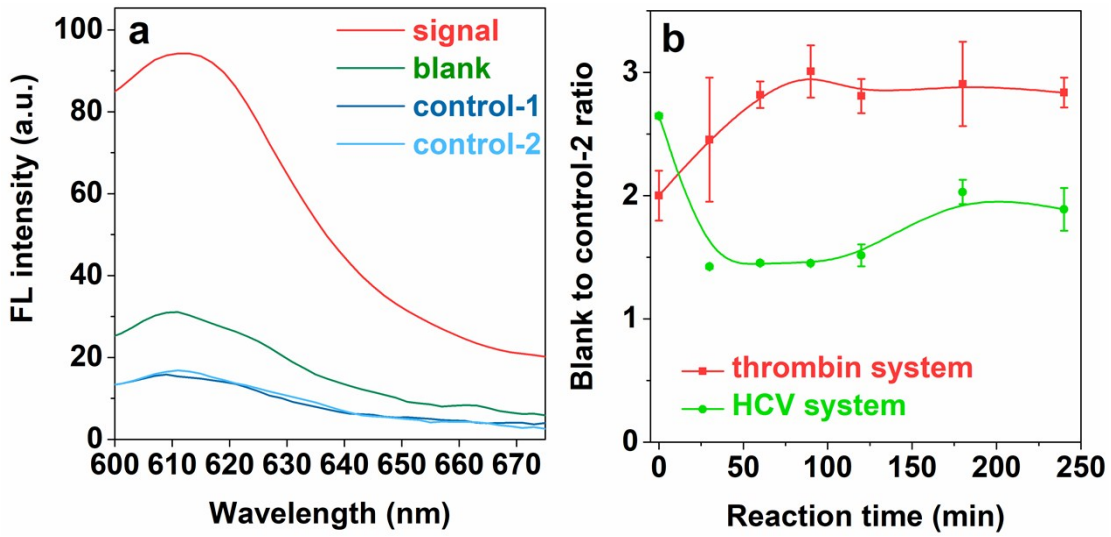


Fig. S17 Investigation of the background generation caused by unexpected DNAzyme cleavage from intermolecular formation of divided thrombin aptamer for thrombin detection. **(a)** Typical curve for the anti-interfering DNAzyme-powered micromachine for thrombin detection. The target group contained all components. Blank group meant without thrombin only. **Control-1** group represented combination with thrombin and MB track. **Control-2** represented MB track only. **(b)** Blank to control-2 ratio at varying reaction time. [MB track] = 0.25 mg mL^{-1}, [probe-2-thrombin] = 50 nM, [thrombin] = 20 nM. [probe-2-HCV] = 30 nM The DNAzyme cleavage reactions were carried out at room temperature for varying time (0, 30, 60, 90, 120, 180, 240 min). **Note:** In Fig. S16a, we intentionally use 20 nM thrombin as signal to compare the signal to blank ratio with that of 2 nM HCV. The value of signal to blank ratio for thrombin detection (Signal to blank ratio for thrombin: 3.3) was still lower than that of HCV (Signal to blank ratio for HCV: 3.7) detection (Fig.1). In Fig. S17b, the blank to control-2 ratio of thrombin sensing system was higher than that of HCV sensing system. These results indicated intermolecular G-quadruplex formation of divided thrombin aptamer (15 n.t. and 29 n.t.) probably induced the background.

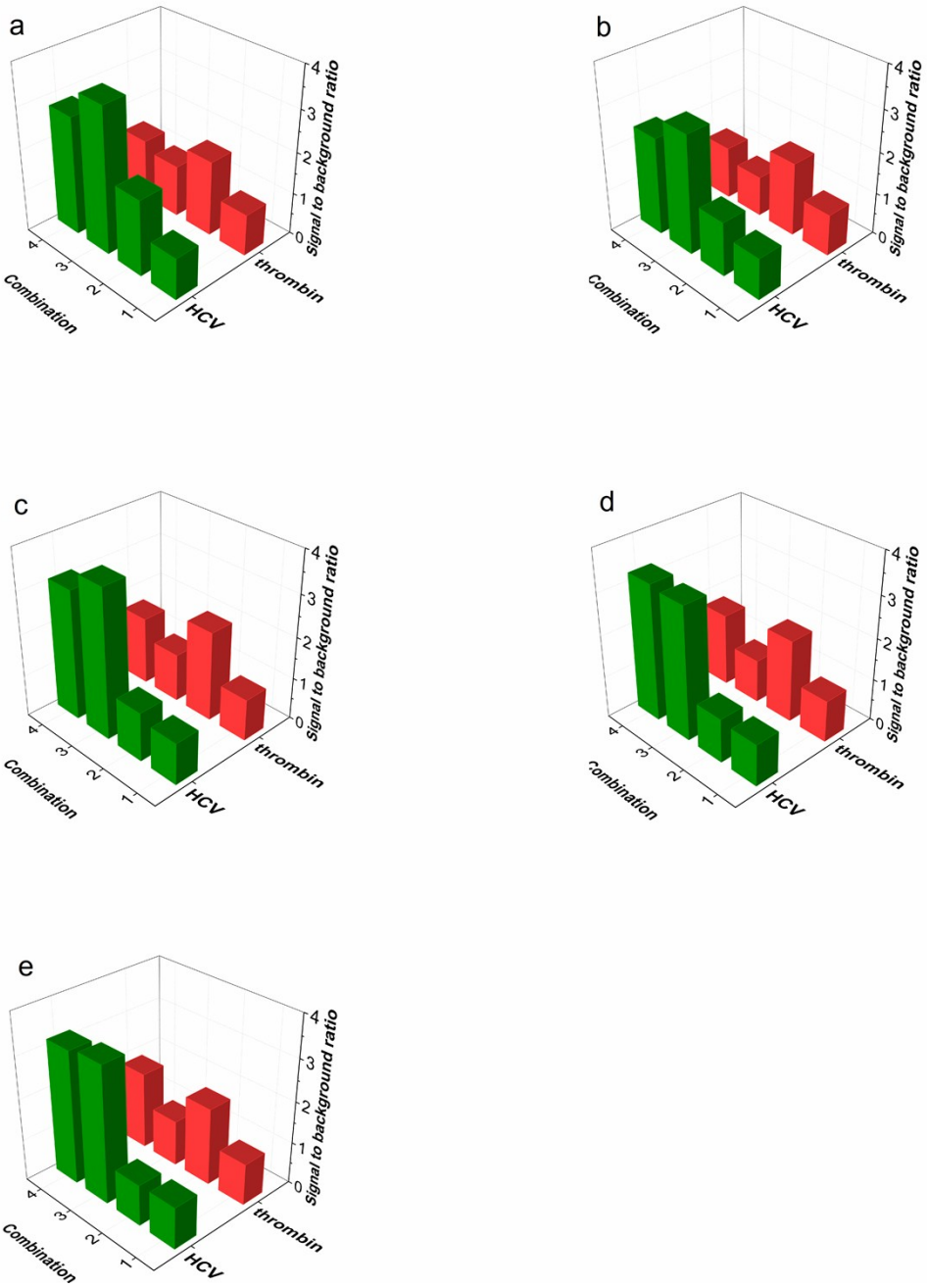


Fig. S18 Varying concentrations of MB track were employed to study the analytical performance on simultaneous determination of HCV target and thrombin protein. The concentrations of MB-Track were 0.05 mg mL^{-1}(a), 0.1 mg mL^{-1}(b), 0.2 mg mL^{-1}(c), 0.4 mg mL^{-1}(d) and 0.5 mg mL^{-1} (e) respectively. (**Note**: the total concentrations of MB track were composed of 50% of FAM labelled MB track for HCV and 50% of ROX labelled MB track for thrombin respectively.) [probe-2-HCV] = 5 nM, [probe-2-thrombin] = 20 nM, [HCV] = 2 nM, [thrombin] = 10 nM. The DNAzyme cleavage reactions were carried out at room temperature for 3 hours. **Combination 1-4 represented blank, only thrombin, only HCV and mixture of thrombin and HCV**, respectively.

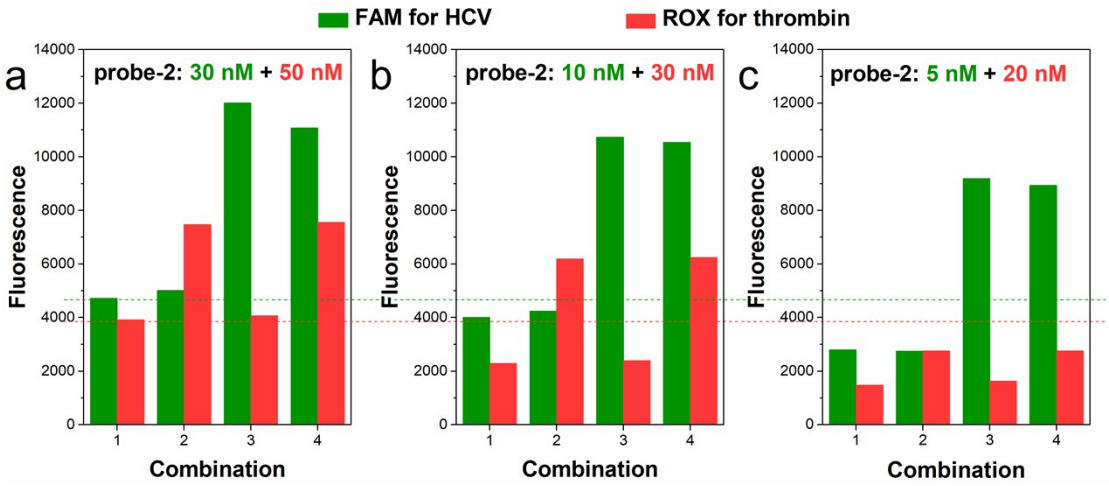


Fig. S19 Varying concentrations of probe-2 were employed to study the analytical performance on simultaneous determination of HCV target and thrombin protein. (a) [probe-2-HCV] = 30 nM, [probe-2-thrombin] = 50 nM, (b) [probe-2-HCV] = 10 nM, [probe-2-thrombin] = 30 nM, (c) [probe-2-HCV] = 5 nM, [probe-2-thrombin] = 20 nM. (**Note**: FAM labelled MB track for HCV and ROX labelled MB track for thrombin is 0.25 mg mL^{-1} and 0.25 mg mL^{-1} respectively) [HCV] = 2 nM, [thrombin] = 10 nM. The DNAzyme cleavage reactions were carried out at room temperature for 3 hours. **Combination 1-4 represented blank, only thrombin, only HCV and mixture of thrombin and HCV**, respectively.

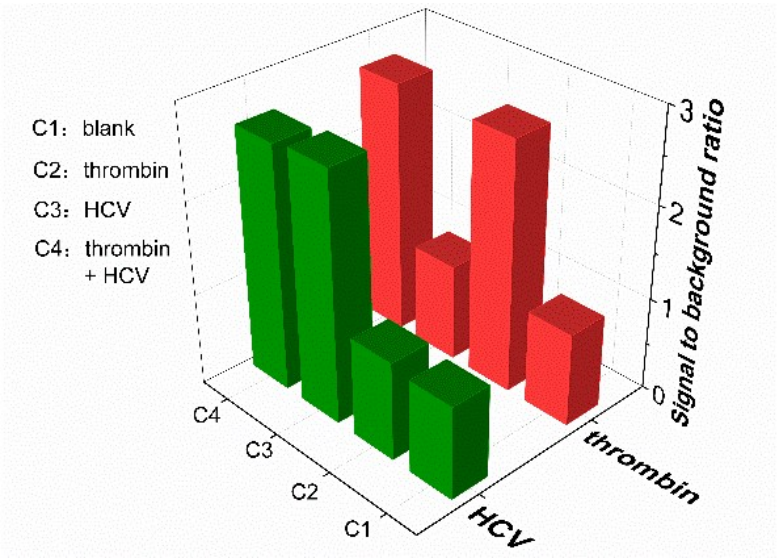


Fig. S20 Anti-interfering DNAzyme-powered micromachine simultaneously response to HCV and thrombin. Two types of DNAzyme-powered micromachines were activated by 2 nM HCV and 10 nM thrombin protein in the same tube. [MB-Track] = 0.5 mg mL^{-1}, [probe-2-HCV] = 10 nM, [probe-2-Thrombin] = 30 nM. After separation, the DNAzyme cleavage reactions were carried out at room temperature for 3 hours.

Notes and references

[S1] J. Chen, A. Zuehlke, B. Deng, H. Peng, X. Hou and H. Zhang, *Anal. Chem.*, 2017, **89**, 12888-12895.