Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for:

Installing CRISPR-Cas12a sensors to a portable glucose meter for point-of-care detection of analytes

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Table of Contents

Section A: Materials and reagents

All oligonucleotides used in this study were synthesized by Shanghai Sangon Biological Engineering Co., Ltd. (Shanghai, China) and purified by HPLC. Their sequences are shown in Table S1. For stock solution preparation, all DNA sequences were dissolved and diluted with Millipore water to a final concentration of 20 μM. After annealing at 90 °C for 5 min, the resulting solutions were cooled down to room temperature and stored at 4 °C until use.

Engen® Lba Cas12a (Cpf1) (M0653T), T4 DNA ligase (M0202S), 10X T4 DNA ligase buffer, Low MW DNA ladder (N3233) and 6×gel loading dye (B7025) were purchased from New England Biolabs (Beijing, China) Ltd. Cardiac troponin I antibodies (Mouse monoclonal antibody, L3C00401 and L3C00402), and recombinant Human Troponin I (L4C00102) were purchased from Shanghai Linc-Bio Science Co., Ltd.(Shanghai, China). Tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP, A600974-0001), and streptavidin immunomagnetic beads (D110557) were provided by Shanghai Sangon Biological Engineering Co., Ltd. (Shanghai, China). The commercial glucose meter and blood glucose test strips were purchased from Abbott Diabetess Care Ltd.(UK). The commercial glucose meter and blood glucose test strips were purchased from Abbott Diabetess Care Ltd.(UK). A prototype of portable glucose meter capable of measuring glucose down to 26.0 μM of glucose (Figure S2A) using commercially available Abbott Precision Xtra® glucose test strip has been provided by GlucoSentient, Inc (USA). The prototype was developed using a Cypress PSoC Prototyping Kit and glucose measurement was achieved using chronoamperometry. Artificial human serum were provided by Kejing Biological Technology Co., Ltd.(Jiangsu, China). Other reagents (analytical grade) were purchased from Sinopharm Chemical Reagent Co.(Shanghai, China). All solutions were prepared with Millipore water (18.25 $M\Omega \cdot cm^{-1}$).

Buffers used in this work:

PBS buffer: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4.

PBST buffer: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 0.05% Tween-20, pH 7.4.

Reporter solution: 0.1 M KOH, 2 M sucrose, pH 4.2.

0.5X TBE buffer: 4.5 mM Tris, 4.5 mM boric acid, 0.1 mM EDTA, pH 8.2.

1 M PBS buffer: 1370 mM NaCl, 27 mM KCl, 100 mM Na_2HPO_4, 20 mM KH_2PO_4, pH 5.0.

10X NE buffer 2.1: 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/ml BSA, pH 7.9

Buffer A: 0.1 M NaCl, 0.1 M sodium phosphate buffer, pH 7.3.

Section B: Table S1. Sequences of DNA oligonucleotides used in this work[a]

Name	Sequence (5'-3')
Probe 1 (P1)	AGTACTGACAGGATCCTCATCGACTACTGATAGCGCGACTACATA
Probe 2 (P2)	P-ACGATAAGGATGCGGTGTATGTTGTGGATCCCTACAGTAGCGATG
Probe 3 (P3)	P-ATCAGTAGTCGATGGCTTCCACAACATACAC
Biotin-modified Probe 4 (P4-Biotin)	Biotin-TTTTTTTATTTTATTTTATTTTTTTCATCGCTACTGTAGCCAATCCTGTCAGTACT
Thiol-Probe 5 (P5-SH)	SH-TTATTTTATTCATCGCTACTGTAGCCAATCCTGTCAGTACT
Probe 6 (P6)	P-ACGATAAGGATGCGGTGTATGTTGTGGATCCATTGACGAGAGAGG
Probe 7 (P7)	P-ACACGATTGACGACCCTCATCGACTACTGATAGCGCGACTACATA
Connector	P-CGCATCCTTATCGTCCTCTCTCGTCAATCCAGTCGTCAATCGTGTTATGTAGTCGCGCT
Kras gene	TTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTG
Mismatched target DNA 1 (MT1)	TTGTGTTAGTTGGAGCTGGTGGCGTAGGCAAGAGTG
Mismatched target DNA 2 (MT2)	TTGTGTTAGTTCGAGCTGGTGGCGTAGGCAAGAGTG
Mismatched target DNA 2 (MT2)	TTGTGTTAGTTCGAGCTCGTGGCGTAGGCAAGAGTG
Random Target Analogue (RTA)	TTGACTTTGAACGACCTCGTGACGTCCAGAACCTTG
Proximity Probe A (PPA)	SH-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGAACGCTGAAGC
Proximity Probe B (PPB)	P-GCTGGGGGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-SH
crRNA for *Kras* gene (crRNA-*Kras*)	UAAUUUCUACUAAGUGUAGAUCACUCUUGCCUACGCCACCAGCUCCAACUACCACAA
crRNA for cTnI protein (crRNA-cTnI)	UAAUUUCUACUAAGUGUAGAUCCCCCAGCGCUUCAGCGUUC
F-Q Reporter	FAM-TTATT-BHQ1
DNA for MAbA	SH-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGAACGCTGAAGC
DNA for MAbB	P-GCTGGGGGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-SH
Connector-cTnI	TTTACCCCCAGCGCTTCAGCGTTC

[a]The bases in red indicate the mutated bases in target *Kras* gene.

Section C: Experimental details

C1. Procedure for *Kras* gene detection

C1.1 Preparation and characterization of the protein-DNA conjugates

The protein-DNA conjugates were synthesized by the maleimide-thiol reaction using a heterobifunctional linker sulfo-SMCC.[1] Briefly, 90 μL of 100 μM thiol-DNA 5 (P5-SH) was first mixed with 6 μL of 1 M PBS buffer (pH 5.0) and 6 μL of 30 mM TCEP. After vortexing for 30 s, the solution was placed on a shaker for 60 min at 37 °C. Then, the thiol-DNA was purified by Amicon-3K using Buffer A for 8 times, and dispersed in 200 μL of Buffer A. For invertase conjugation, 400 uL of 6.7 mg/mL invertase in Buffer A was first mixed with 0.34 mg of sulfo-SMCC. After vortexing, the solution was placed on a roller for 60 min at room temperature. The mixture was then purified by Amicon-100K using Buffer A for 8 times, and dispersed in 200 μL of Buffer A, and then mixed with the above solution of TECP-activated thiol-DNA. The resulting solution was kept at 4 °C for 2 hours. To remove un-conjugated thiol-DNA, the solution of P5-Invertase was purified by Amicon-100K for 8 times using Buffer A. The concentration of purified protein-DNA conjugate was quantified using a NanoDrop Onec (Thermo Fisher), and the final stock solution of P5-Invertase was 20 μM.

The P5-Invertase conjugate was characterized using 10% native PAGE with the following experimental conditions: voltage (80 V), 0.5X TBE buffer, 90 min. Low MW DNA ladder was used as the marker.

C1.2 Preparation and characterization of the poly-invertase-immobilized magnetic beads (poly-invertase-MBs)

The poly-invertase-MBs were prepared as follow: (1) Firstly, the annealed building units were prepared by mixing equimolar amounts of P1, P2, and P3 (667 nM) in 60 μL of 1X T4 DNA ligase buffer (50 mM

Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, pH 7.5), followed by heating at 95 °C for 5 min and then gradually cooling to 30 °C. (2) The biotin-modified building units were synthesized by incubating equimolar quantities of annealed building units and P4-Biotin at 37 °C for 60 min. Similarly, the invertase-modified building units were synthesized by incubating equimolar quantities of annealed building units and P5-Invertase at 37 °C for 60 min. The basic building units were prepared by mixing equimolar amounts of P6, P7, and P3 (667 nM) in 60 μL of 1X T4 DNA ligase buffer, followed by heating at 95 °C for 5 min and then gradually cooling to 30 °C. (3) Then, 9 μL of Connector (6.67 μM) and 1 μL of T4 DNA ligase (400 U) were added to 90 μL of T4 DNA ligase buffer containing three building units (biotin-modified building units, invertase-modified building units and basic building units with a ratio of 1:1:4) at 16 °C for 60 min to form the polyinvertase immobilized DNA nanospheres.[2] (4) 10 μL of streptavidin immunomagnetic beads were washed using PBST buffer for 5 times, and then mixed with the 100 μL of poly-invertase immobilized DNA nanosphere. The resulting solution was kept at roon temperature for 60 min to form the poly-invertase-MBs. To remove un-reacted poly-invertase immobilized DNA nanosphere, the poly-invertase-MBs were purified by magnetic separation for 5 times using a PBST buffer and dispersed in 100 μL of PBS. Finally, the poly-invertase-MBs was stored at 4 °C until use.

The step by step assembly of DNA nanosphere were characterized using 10% native PAGE with the following experimental conditions: voltage (80 V), 0.5X TBE buffer, 90 min. Low MW DNA ladder was used as the marker.

The poly-invertase-MBs was characterized by testing its catalytic activity of the conversion of sucrose to glucose using a portable glucose meter. Briefly, 10 μL of the poly-invertase-MBs (5 mg/mL) was mixed with 40 μL of sucrose reporter solution, and incubated at 37 °C for 30 min. After that, 5 μL of the resulting

solution was dropped on a glucose strip, and then tested using our portable glucose meter. An equimolar amount of P5-Invertase, free invertase and PBS buffer were used as control samples.

C1.3 Particle size distribution analysis of streptavidin-MBs and poly-invertase-MBs

For hydrodynamic diameter measurement, 20 μL of streptavidin-MBs or poly-invertase-MBs (with the same concentration as MBs of 0.5 mg/mL) was added to 980 μL of PBS. After mixing thoroughly, the dynamic light scattering (DLS) measurement was measured on Malvern Instruments Zetasizer HS III (Malvern, UK) at room temperature.

C1.4 Design of CRISPR/Cas12a-based assay with PGM readout

The CRISPR/Cas12a-PGM assay contains three key components: (1) an CRISPR/Cas12a system containing a pre-assembled Cas12a/crRNA ribonucleoprotein and *Kras* gene as an activator for Cas12a/crRNA; (2) a reporter system consisting of a pre-assembled poly-invertase-MBs and a reporter solution containing 2 M sucrose; (3) a protable PGM used to measure glucose concentration in the above solution.

The pre-assembled Cas12a/crRNA ribonucleoprotein containing Cas12a and crRNA was prepared by mixing 97 μL of 1X NE buffer 2.1, 1 μL of Engen® Lba Cas12a (10 μM in 1X NE buffer 2.1) with 1 μL of crRNA-*Kras* (10 μM in DEPC-treated water) at 37 °C for 15 min.

In a typical CRISPR/Cas12a-based detection experiment, 99 μL of pre-assembled Cas12a/crRNA ribonucleoprotein in 1X NE buffer 2.1 was incubated with 1 μL of different concentrations of target *Kras* at 37 °C for 15 min to activate Cas12a/crRNA system. Then, 10 μL of 5 mg/mL poly-invertase-MBs was added and further incubated at 37 °C for 30 min. After magnetic separation, 20 μL of supernatant solution was collected, and incubated with 30 μL of reporter solution at 37 °C for 30 min. After that, 5 μL of the resulting solution was dropped on a glucose strip, and then tested using our portable glucose meter.

C1.5 Fluorescent monitoring of the activation of Cas12a/crRNA system using *Kras* gene

To evaluate whether the Cas12a/crRNA ribonucleoprotein can be activated by *Kras* gene, a fluorecent cleavage assay was designed using a ssDNA fluorescence reporter (F-Q reporter). Briefly, 99 μL of 101 nM of pre-assembled Cas12a/crRNA ribonucleoprotein was first mixed with 1 μL of different concentrations of *Kras* gene at 37 °C for 15 min. After that, 5μL of 10 μM of F-Q reporter was added, and further incubated at 37 °C for 30 min. Finally, the fluorescent signal of the resulting solution was measured using a F-320 (TIANJIN GANGDONG SCI.&TECH. CO,. Ltd. China). The excitation wavelength was 488 nm, and the fluorescence spectra were collected from 500 nm to 650 nm.

C1.6 Characterization of the CRISPR/Cas12a-PGM system

To characterize the CRISPR/Cas12a-PGM system, a typical CRISPR/Cas12a-PGM experiment was performed by using 1 μL of target *Kras* gene (1 μM in Millipore water) in the presence of the Cas12a/crRNA ribonucleoprotein and the poly-invertase-MBs (Section C1.2). Negative control experiments were performed in the absence of either *Kras* gene, Cas12a or crRNA-*Kras*, respectively.

C1.7 Sensitivity test for *Kras* gene detection

For sensitivity analysis, 1.0 μL of different concentrations of kras gene was prepared in Millipore water, and tested according to the typical CRISPR/Cas12a-PGM experiment (Section C1.4).

C1.8 Detection of *Kras* gene in human serum using CRISPR/Cas12a-PGM system

For *Kras* gene detection in human serum, 1.0 μL of different concentrations of *Kras* gene was prepared in 100% human serum, and tested according to the typical CRISPR/Cas12a-PGM experiment (Section C1.4).

C1.9 Selectivity test for *Kras* gene detection using CRISPR/Cas12a-PGM system

For selectivity analysis, 1.0 μL of 5 nM *Kras* gene, one mismatched base-contained target DNA (MT1), two mismatched bases-contained target DNA (MT2), three mismatched bases-contained target DNA (MT3) and random target analogue (RTA) were prepared in Millipore water, and tested according to the typical CRISPR/Cas12a-PGM experiment, respectively.(Section C1.4).

C2. Procedure for the detection of cTnI protein using an antibody-based CRISPR/Cas12a-PGM assay

C2.1 Preparation and characterization of the antibody-DNA conjugates

The antibody-DNA conjugates were synthesized by the maleimide-thiol reaction using heterobifunctional linker sulfo-SMCC. Briefly, 200 μL of 150 μg/mL cTnI antibody A (MAbA) or cTnI antibody B (MAbB) in PBS buffer was mixed with 200 μL of 1.5 mg/mL sulfo-SMCC. After vortexing for 30 s, the solution was placed on a shaker for 30 min at room temperature. The mixture was then purified by Amicon-50K using PBS buffer for 8 times, and dispersed in 200 μL of PBS buffer. In parallel, a certain amount of thiol-DNA (molar ratio: 10 fold to corresponding protein) in 200 μL Millipore was mixed with 10 μL of 1 M PBS buffer (pH 5), and 10 μL of 30 mM TCEP, and then incubated at 37 °C for 1 hour. The thiol-DNA was then purified by Amicon-3K using PBS buffer for 8 times, dispersed in 200 μL of PBS, and then mixed with the above solution of sulfo-SMCC-activated cTnI antibodies. The resulting solution was kept at 4 °C for 2 hours.

To remove unconjugated thiol-DNA, the solution for MAbA-DNA or MAbB-DNA was purified by Amicon-50K using PBS buffer for 8 times. The concentrations of purified antibody-DNA conjugates were quantified using a NanoDrop Onec (Thermo Fisher), and the final stock solution of MAbA-DNA and MAbB-DNA was 500 nM, respectively.

The antibody-DNA conjugates were characterized using 12% native PAGE with the following experimental conditions: voltage (80 V), 0.5X TBE buffer, 90 min. Low MW DNA ladder was used as the marker.

C2.2 Detection of cTnI protein using antibody-based CRISPR/Cas12a-PGM assay

For cTnI protein detection, an antibody-based CRISPR/Cas12a-PGM assay was designed, which contains two key components: (1) two antibody-modified proximity ligation probes (MAbA-DNA and MAbB-DNA) that both consists of an antibody for antigen recognition, ligation region, and spacer region to minimize the structural steric hindrance during the assembly; (2) an ssDNA connector as the template to assists the ligation by hybridization with two proximity probes at the ligation region.

In a typical antibody-based CRISPR/Cas12a-PGM assay, 36 μL of reaction solution containing 1 nM MAbA-DNA, 1 nM MAbB-DNA and 1 μM Connector in 1X T4 DNA ligase buffer was prepared, and mixed with 4 μL of cTnI protein (0, 20 ng/mL, 50 ng/mL, 200 ng/mL). After incubating at 37 °C for 30 min, 10 μL of 0.005X T4 DNA ligase was added, and further incubated at 37 °C for 5 min to obtain a solution A. In parallel, a solution B was prepared by mixing 44 μL of Millipore water, 5 μL of 10X NE buffer 2.1, 0.5 μL of Engen® Lba Cas12a (10 μM) with 0.5 μL of crRNA-cTnI (10 μM in DEPC-treated water) at 37 °C for 30 min. After that, the above solution A and B was mixed with 10 μL of poly-invertase-MBs (Section C1.2), and incubated at 37 °C for 60 min. After magnetic separation, 20 μL of supernatant solution was collected, and incubated with 30 μL of 2 M of sucrose reporter solution at 37 °C for 30 min. After that, 5 μL of the resulting solution was dropped on a glucose strip, and then tested using our portable glucose meter.

C2.3 Fluorescent monitoring of the activation of Cas12a/crRNA system using cTnI protein

To evaluate whether the Cas12a/crRNA ribonucleoprotein can be activated by the cTnI-based ligation product, a fluorecent cleavage assay was designed using a ssDNA fluorescence reporter (F-Q reporter). Briefly, 47.5 μL of 105 nM of pre-assembled Cas12a/crRNA ribonucleoprotein, 2.5 μL of 10 μM of F-Q reporter in 1X NE buffer 2.1 was mixed with 50 μL of ligation products (Section C2.2). After incubating at 37 °C for 30 min, the fluorescent signal of the resulting solution was measured using a F-320 (TIANJIN GANGDONG SCI.&TECH. CO,. Ltd. China). The excitation wavelength was 488 nm, and the fluorescence spectra were collected from 500 nm to 650 nm. Negative control experiments were performed in the absence of T4 DNA ligase in antibody-based CRISPR/Cas12a-PGM assay.

C2.4 Selectivity test for cTnI protein detection using antibody-assisted CRISPR/Cas12a-PGM system

For selectivity analysis, 4 μL of 200 ng/mL cTnI protein and other competing proteins were prepared in PBS, and tested according to the typical antibody-based CRISPR/Cas12a-PGM experiment (Section C2.2).

Section D: Supporting Figures

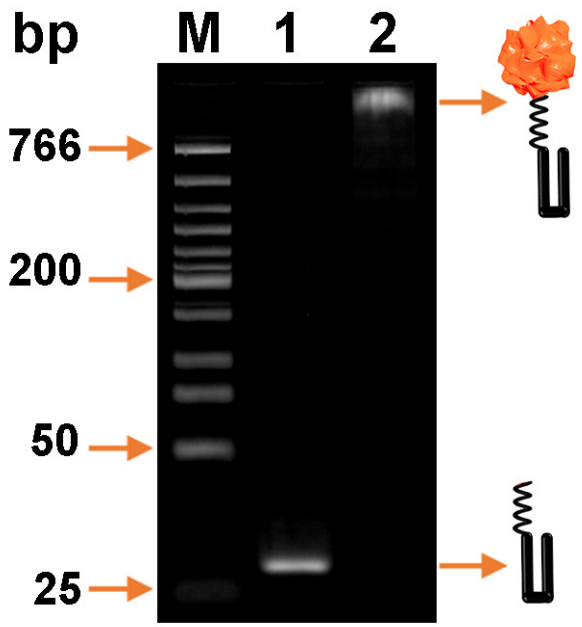

Fig. S1 Native PAGE (10%) analysis of the P5-invertase conjugates. Lane 1: P5-SH; Lane 2: P5-Invertase conjugate; M: Low molecular weight DNA ladder was used as the marker.

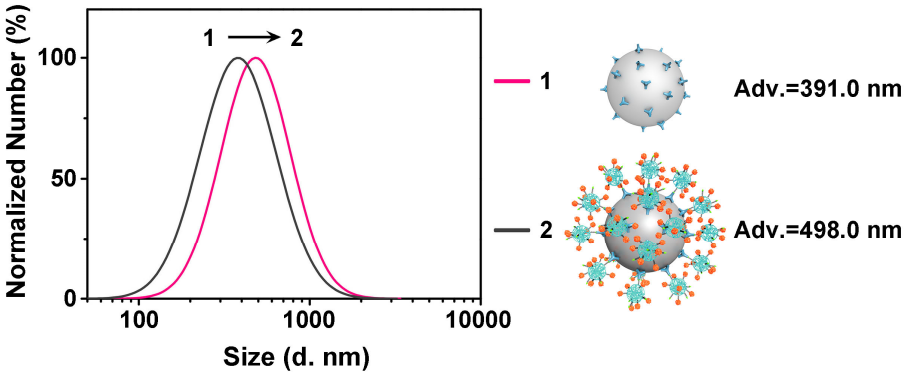

Fig. S2 Hydrodynamic diameter of streptavidin-MBs (1) and poly-invertase-MBs (2), respectively.

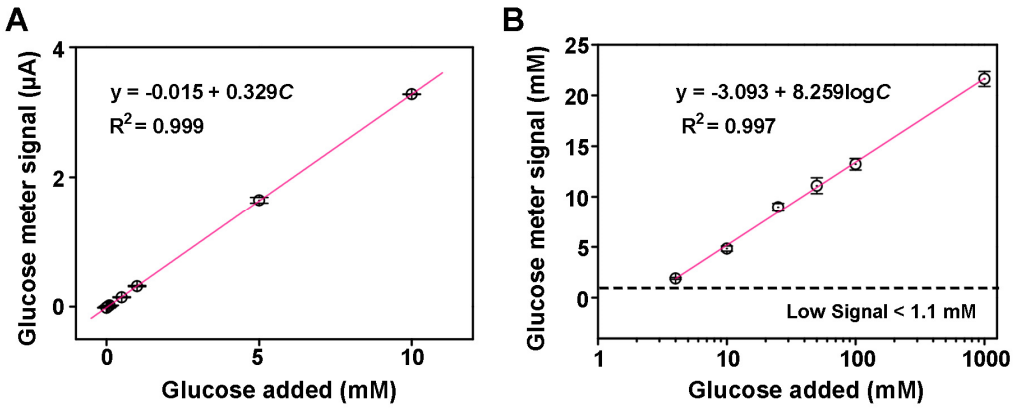

Fig. S3 Comparison of the sensitivity of our home-made portable glucose meter (A) with a commercially available personal glucose meter (B) using the same test strips. Different concentrations of glucose were added in the reporter solution, and tested using the above two meters. Error bars represent the standard deviations of three independent measurements. The signal below the dash line represents < 1.1 mM and shows as “Low” in the PGM.

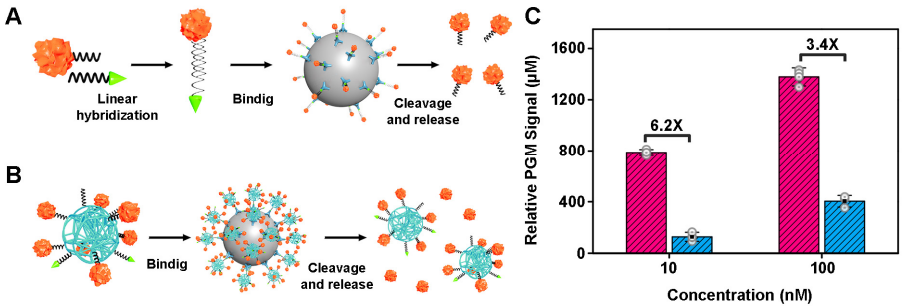

Fig. S4 Graphic illustration of the detection of *Kras* gene by CRISPR/Cas12a-based PGM system with a nonpolymeric invertase immobilized magnetic beads reporter (A) and poly-invertase immobilized MBs reporter (B). (C) Comparison of PGM signals for different concentrations of *Kras* gene using poly-invertase-MBs (red) and nonpolymeric invertase-MBs (blue), measured by CRISPR/Cas12a-PGM system.

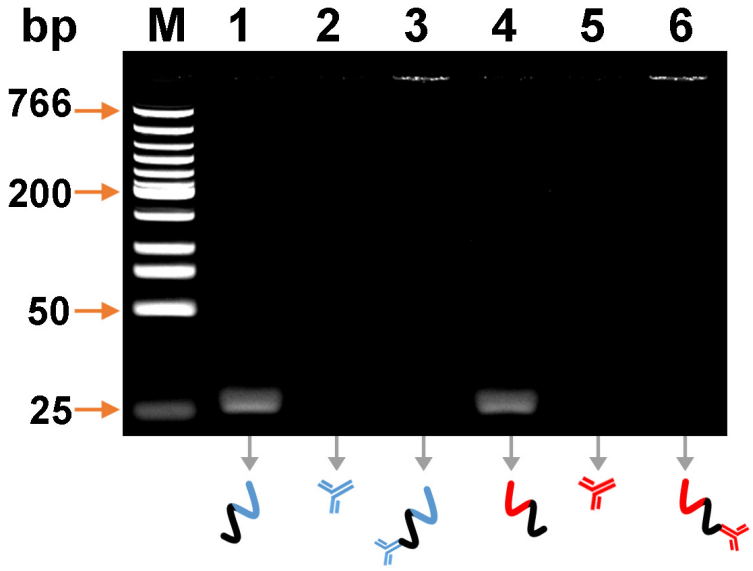

Fig. S5 Native PAGE (12%) analysis of the MAbA-DNA and MAbB-DNA conjugates. Lane 1: thiol-DNA for MAbA; Lane 2: MAbA; Lane 3: MAbA-DNA conjugate; Lane 4: thiol-DNA for MAbB; Lane 5: MAbB; Lane 6: MAbB-DNA conjugate; M: Low molecular weight DNA ladder was used as the marker.

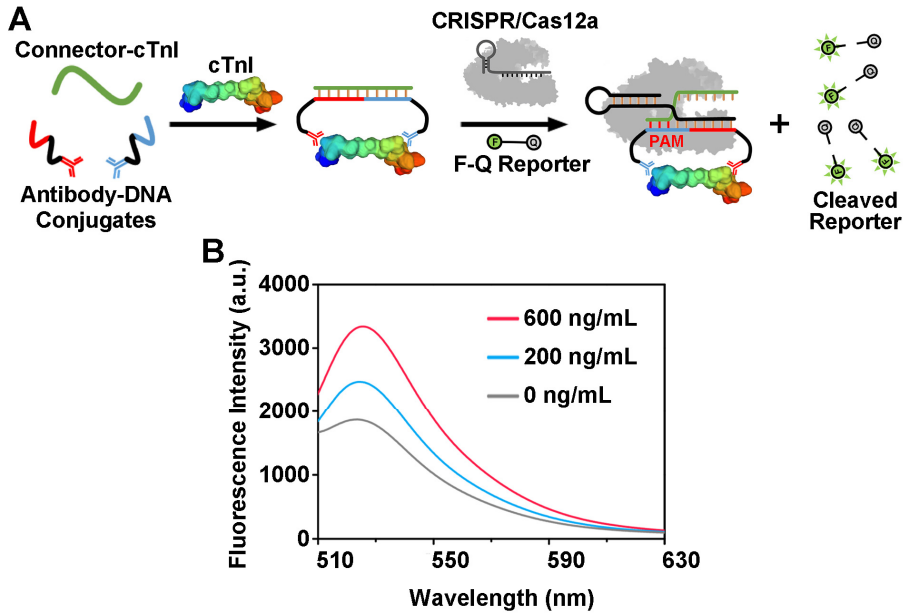

Fig. S6 Graphic illustration (A) and fluorescence spectrum measurement (B) of cTnI antigen by the antibody-assisted CRISPR/Cas12a system with a single-stranded DNA fluorescence reporter.

References:

1 J. Zhang, Y. Lu, *Angew. Chem., Int. Edit.* 2018, **57**, 9702-9706.

2 S. Bi, Y. Dong, X. Jia, M. Chen, H. Zhong, B. Ji, *Nanoscale* 2015, **7**, 7361-7367.