Supporting information

CRISPR/Cas12a–based fluorescence immunoassay: combination of efficient signal generation with specific molecule recognition

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1. Chemicals and materials

All DNA molecules in this work were synthesized and HPLC purified by Sangon Biotech Co., Ltd (Shanghai, China). Their sequences of the oligonucleotides are listed in Tables S1. The double-stranded DNA (dsDNA) stock solutions were prepared by using equimolar complementary single-stranded DNA (ssDNA). The equimolar biotin-ssDNA and complementary ssDNA in Tri-HCl buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4) were together heated to 90 °C for 10 min, and cooled slowly down to room temperature (ca. 20 °C), and then the dsDNA formed. Before use, the dsDNA solution was diluted to the desired concentrations with the same Tri-HCl buffer. Engen®20 Lba Cas12a (cpf1) were purchased from NEW ENGLAND BioLabs Inc. (Beijing, China). RNA and RNase-free water were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). Human immunoglobulin G (IgG), mouse anti-IgG, and SA-conjugated antibody were obtained from Sangon Biological Engineering Technology and Services Co., Ltd (Shanghai, China). ELISA kits for human IgG, d-dimer, alpha fetoprotein (AFP), and vascular endothelial growth factor (VEGF) were bought from Neobioscience Company (Beijing, China). All other chemicals were of analytical reagent grade. These serum samples were gifted from Shaanxi Normal University Hospital. The used ultrapure water in whole experiments was obtained from a Millipore Milli Q water

purification system (Billerica, MA).

2. Procedure of fluorescence immunoassay for human IgG

Dilutions of the specific antibody protein (5 μg/mL) in a coating buffer (carbonate-bicarbonate buffer, pH 9.6) were added to the plate (0.1 mL per well), and incubated at 4 °C overnight. Then, the plate was blocked by adding 1% bovine serum albumin (BSA, 0.3 mL per well), and incubated at 37 °C for 1 h. Serial dilutions of antigen (IgG) were added to the plate (0.1 mL per well), and incubated at 37℃ for 1 h. 3.2 μg/mL streptavidinized-antibody and 0.5 μM biotinylated dsDNA were added to the plate (0.1 mL per well), and incubated at 37 °C for 1 h. The non-bound solution was removed, and the wells were washed four times with PBS buffer containing 0.05% Tween-20 between each binding incubation. Finally, 10 μL mixture of Cas12a (250 nM) and crRNA (250 nM), 5 μL Taqman probe (10 μM), 20 μL RNase-free water, and 5 μL Tri-HCl buffer (500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 200 mM $MnCl_2$, 100 μg/mL BSA, pH 7.9) were successively added to each well and reacted at 37 °C for 1 h. After that, 60 μL Tris-HCl (pH 7.4) was added, and the fluorescence intensity of the solution was detected using a FS5 Spectrofluorometer (Edinburgh Instruments Ltd., England) with the excitation wavelength of 480 nm. The fluorescence intensity of the system at 520 nm was used to quantify IgG concentration.

3. Supporting tables

Tables S1 Sequences of the used oligonucleotides in this work

Name	Sequence (5′-3′)
crRNA	UAAUUUCUACUAAGUGUAGAUAAGGUUUGUGUGUUU ACCUG
acDNA	CAGGTAAACACACAAACCTTTAAATGCG
c-DNA	CGCATTTAAAGGTTTGTGTGTTTACCTG
5′-biotin- acDNA	Biotin-TTTTTTTTTTCAGGTAAACACACAAACCTTTAAA TGCG
5′-biotin-c-DNA	Biotin-TTTTTTTTTTCGCATTTAAAGGTTTGTGTGTTTA CCTG
3′-biotin-acDNA	CAGGTAAACACACAAACCTTTAAATGCGTTTTTTTTTT-Biotin
3′-biotin-c-DNA	CGCATTTAAAGGTTTGTGTGTTTACCTGTTTTTTTTTT-Biotin
Taqman probe	FAM-TTATT-BHQ

Tables S2 Results in real human serum by the proposed method

Sample	Initial concentration (ng/mL)	Added (ng/mL)	Found (ng/mL)	Recovery (%)	RSD (n=3, %)
No. 1	101.0	100.0	199	99.0	2.6
No. 2	101.0	100.0	199	99.0	2.6

3. Supporting results

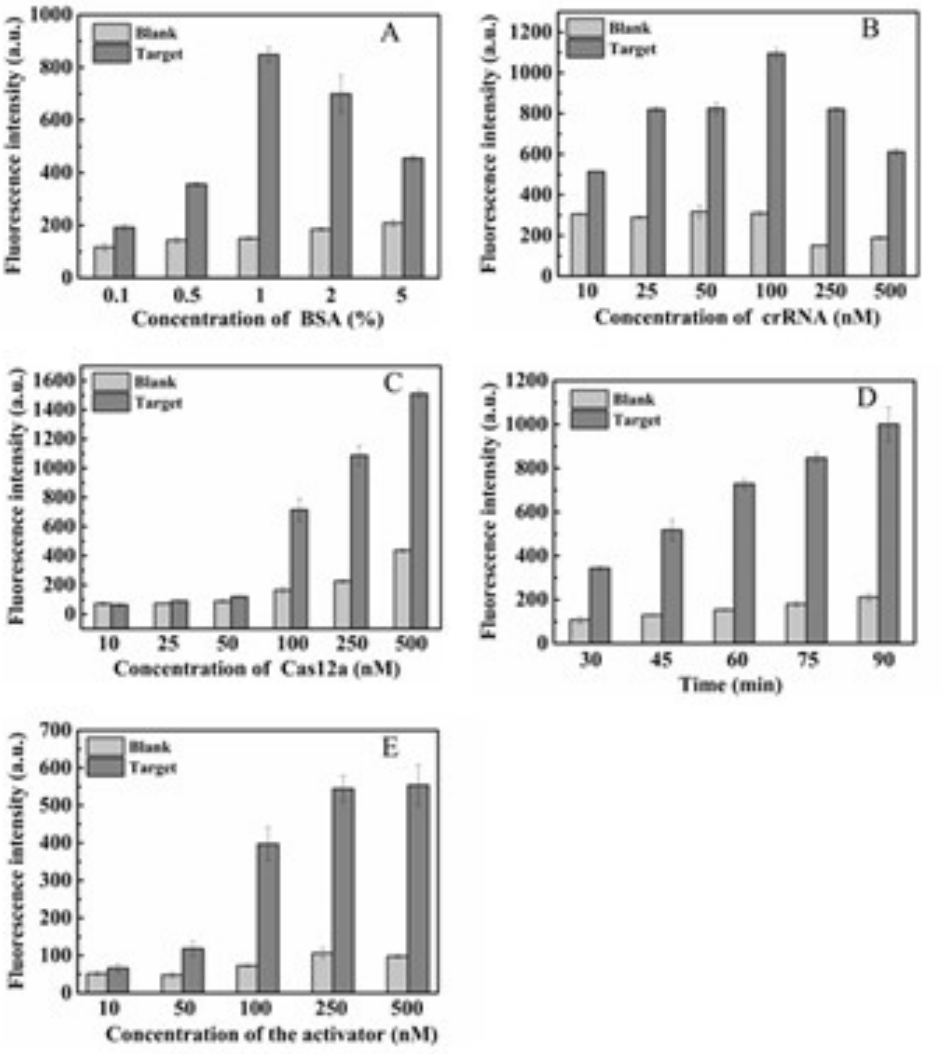

Figure S1. Effect of BSA concentration (A), crRNA concentration (B), Cas12a concentration (C), activator concentration (D), and reaction time (E), and on the fluorescence response of this system.

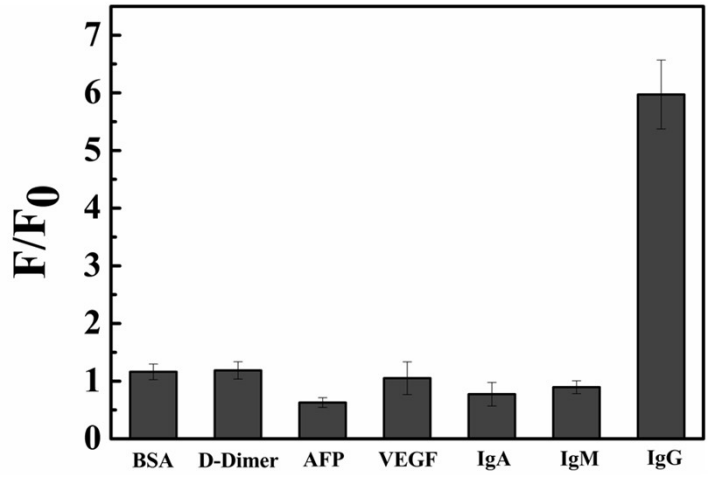

Figure S2. Fluorescence responses of the developed system to human IgG or other control proteins, including bovine serum albumin (BSA), d-dimer, alpha fetoprotein (AFP), vascular endothelial growth factor (VEGF), IgA and IgM. The concentration of human IgG was 100 ng/mL, and the level of other proteins was 200 ng/ mL.