Ultrasensitive, simultaneous detection of two biomarkers with a localized surface plasmon resonance biosensor

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Experimental section

Chemicals and materials

Thrombin, fibrinogen, anti-fibrin antibody (Ab), tris(2-carboxyethyl) phosphine (TCEP), ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and N-hydroxysulfosuccinimide (NHS) were purchased from Sigma (St. Louis, MO, USA). Telomerase was obtained from SenBeiJia Biological Technology Co., Ltd. (Nanjing, China). EnGen® Lba Cas12a and NE Buffer were sourced from New England Biolabs (Ipswich, MA, USA). Deoxyribonucleoside 5'-triphosphate (dNTPs) and T4 DNA polymerase were purchased from Thermo Fisher Scientific (formerly Fermentas, Lithuania). Human HeLa cell lysate, homing peptides, and oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China) and are listed in Table S1.

Human plasma samples obtained from Liaocheng People's Hospital and Hela cell lysate samples bought from Sangon Biotech Co., Ltd.

All other reagents of analytical grade, were purchased from Sinopharm Chemical Reagent Company (Beijing, China). Millipore Milli-Q water (18.2 MΩ cm) was used in all experiments. All 100 μmol L^{-1} DNA solutions (20 mM Tris-HCl, pH 7.4) were heated at 80 °C for 10 minutes and subsequently cooled to room temperature. The DNA solutions were then stored in 4 ºC.

The LSPR signal was collected using Open SPR by Nicoya Lifesciences Inc (Toronto, Canada). UV-visible adsorption spectra were measured on a Hitachi UH4150 UV-VIS-NIR spectrophotometer (Kyoto, Japan).

Measurement of equilibrium dissociation constant (K_D)

Initially, the AuNP sensor chip underwent pretreatment by injecting 10 mM HCl at a flow rate of 150 μL/min. 2.0 μmol L^{-1} CREKA peptide was anchored onto the AuNPs chip surface with the help of EDC (1 mol L^{-1}) and Sulfo-NHS (1 mol L^{-1}). fibrin solutions at varying concentrations (3×10^{-9}, 3×10^{-8}, 3×10^{-7} mol L^{-1}) were introduced onto the chip at a flow rate of 20 μL/min for a duration of 5 minutes. The LSPR sensorgrams were recorded using the Open SPR™ system (Nicoya Lifesciences, Canada). Subsequently, anti-fibrin antibody (Ab) concentrations ranging from 2.3×10^{-9} mol L^{-1} to 2.3×10^{-8} mol L^{-1} were injected onto this chip under the same conditions of 20 μL/min flow rate. Finally, the LSPR sensor was regenerated by injecting 20 mmol L^{-1} NaOH at a flow rate of 150 μL/min. The LSPR sensorgrams were recorded using the Open SPR™ system (Nicoya Lifesciences, Canada). Ultimately, the acquired data were analyzed separately to determine the dissociation constants (K_D) for both CREKA–fibrin and antibody–fibrin interactions, utilizing a one-to-one binding model fitted by TraceDrawer software.

Table S1 The oligonucleotide sequences and homing peptides utilized in the proposed method.

Name	Sequence
P1	5'-NH_2-TTCCTCACCATGTCTGAGGTACTCCTT AAAGGTCGAGCTGGAC-3'
P2	5'-NH_2-TTGTTGAGGTAACCAACTATTTGTTACT GTTGCTTGTGGCCGTTTACGTCGCCGTCCAG-3'
P3	5'-HS-C_6-TTAGGGTTAGGG-3'
crRNA	5'-UAAUUUCUACUAAGUGUAGAUCGUCGCCGU CCAGCUCGACC-3'
CREKA	Cys-Arg-Glu-Lys-Ala
GREKA	Gly-Arg-Glu-Lys-Ala
CREKK	Cys-Arg-Glu-Lys-Lys
CRKEA	Cys-Arg-Lys-Glu-Ala
CAREK	Cys-Ala-Arg-Glu-Lys
ERCKA	Glu-Arg-Cys-Lys-Ala
CGLIIQK	Cys-Gly-Leu-Iie-Iie-Gln-Lys-Asn-Glu-Cys

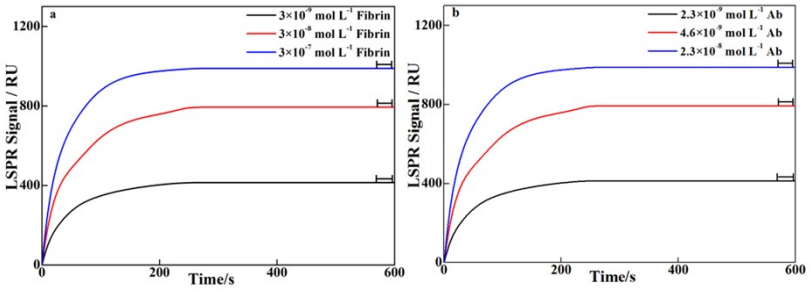

Fig. S1 Measurement of binding affinity between fibrin and CREKA (a), Aband fibrin (b) by LSPR assay. Experimental conditions: 2.0 μmol L^{-1} CREKA, fibrin (3×10^{-9} mol L^{-1}, 3×10^{-8} mol L^{-1}, 3×10^{-7} mol L^{-1}), Ab (2.3×10^{-9} mol L^{-1} , 4.6×10^{-9} mol L^{-1}, 2.3×10^{-8} mol L^{-1}), and PB buffer (10 mmol L^{-1}, pH=7.4).

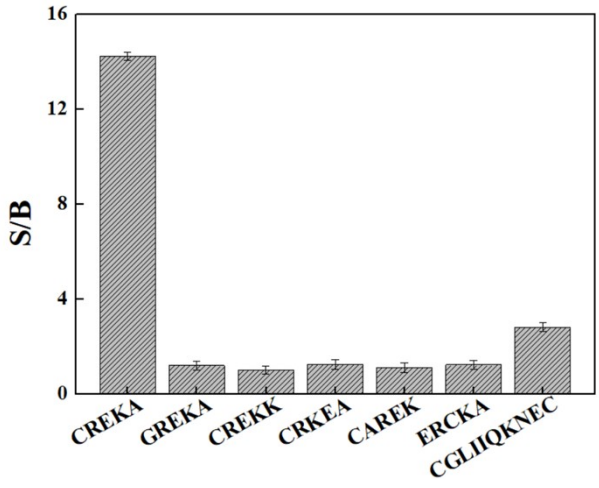

Fig. S2 The impact of diverse peptides on the detection of the fibrin system. Experimental conditions: 0.1 μmol L^{-1} P3, 1×10^{-6} IU mL^{-1} telomerase, 2.0 pmol L^{-1} P1-CREKA, 0.2 pmol L^{-1} P2-Ab, 0.01 U μL^{-1} T4 polymerase, 3×10^{-10} mol L^{-1} fibrin, 0.25 μmol L^{-1} crRNA, 0.025 μmol L^{-1} Cas12a and PB buffer (10 mmol L^{-1}, pH=7.4).

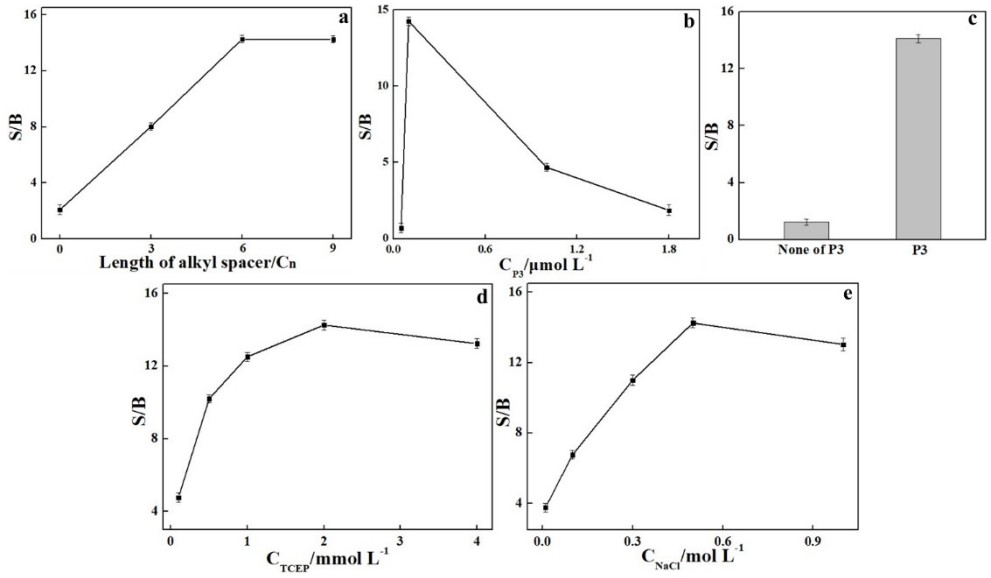

Fig. S3 The influence of length of alkyl spacer(a) and P3 concentration(b) on the detection of the fibrin system. The influence of P3 in cycle on the detection of telomerase (c). The influence of TCEP concentration(d) and NaCl concentration(e), and length of alkyl spacer(e) on the detection. Experimental conditions: 0.1 μmol L^{-1} P3, 1×10^{-6} IU mL^{-1} telomerase, 2.0 pmol L^{-1} P1-CREKA, 0.2 pmol L^{-1} P2-Ab, 0.01 U μL^{-1} T4 polymerase, 3×10^{-10} mol L^{-1} fibrin, 0.25 μmol L^{-1} crRNA, 25 nmol L^{-1} Cas12a and PB buffer (10 mmol L^{-1}, pH=7.4).

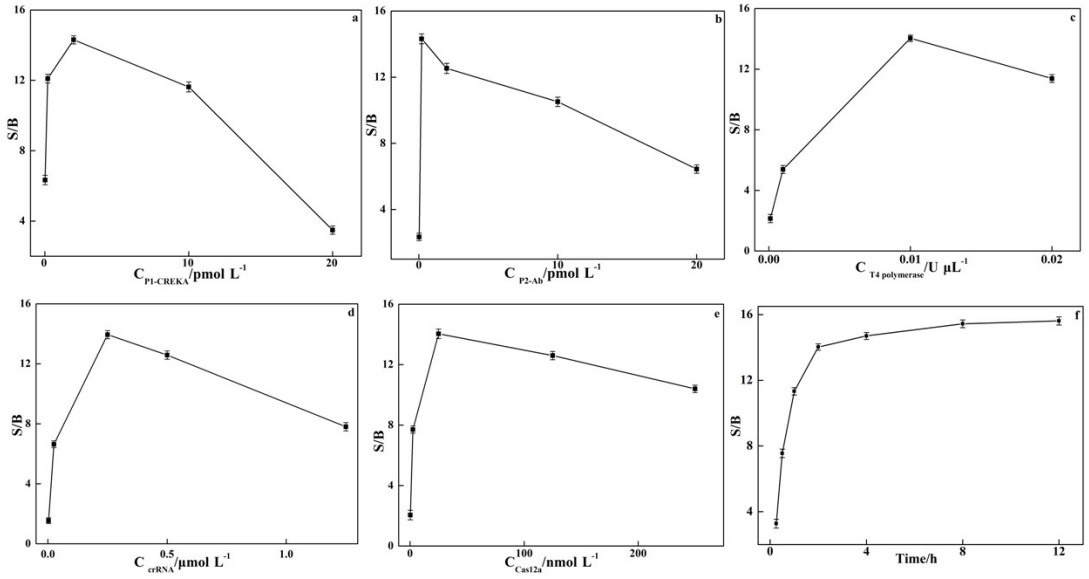

Fig. S4 The influence of P1-CREKA concentration (a), P2-Anti-fibrin concentration (b), T4 DNA polymerase concentration (c), crRNA concentration (d), Cas12a concentration (e) and cutting time (f) on the detection of the fibrin system.

Experimental conditions: 0.1 μmol L^{-1} P3, 1×10^{-6} IU mL^{-1} telomerase, 2.0 pmol L^{-1} P1-CREKA, 0.2 pmol L^{-1} P2-Ab, 0.01 U μL^{-1} T4 polymerase, 3×10^{-10} mol L^{-1} fibrin, 0.25 μmol L^{-1} crRNA, 0.025 μmol L^{-1} Cas12a and PB buffer (10 mmol L^{-1}, pH=7.4).

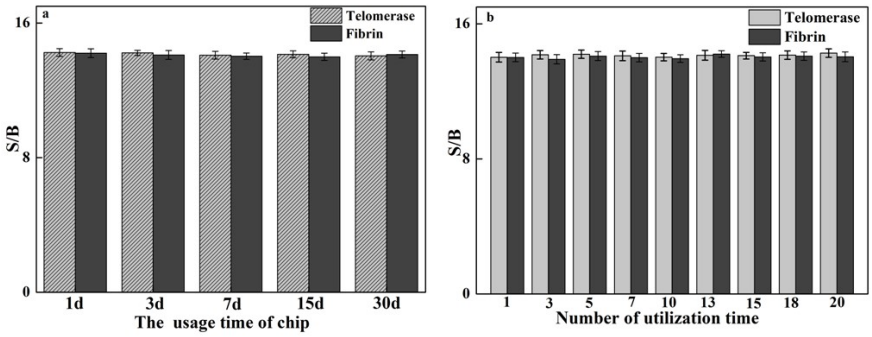

Fig. S5 The impact of the usage time of chip (a) and the regeneration number (b) on the S/B of the system. Experimental conditions: 0.1 μmol L^{-1} P3, 1×10^{-6} IU mL^{-1} telomerase, 2.0 pmol L^{-1} P1-CREKA, 0.2 pmol L^{-1} P2-Ab, 0.01 U μL^{-1} T4 polymerase, 3×10^{-10} mol L^{-1} fibrin, 0.25 μmol L^{-1} crRNA, 0.025 μmol L^{-1} Cas12a and PB buffer (10 mmol L^{-1}, pH=7.4).