Supporting Information

The Design of Anti-fouling and Anti-hydrolysis Cyclic Peptides for Accurate Electrochemical Antigen Testing in Human Blood

Rui Han, Wenjie Hou, Yang Li, Min Chen, Caifeng Ding*, Xiliang Luo*

Key Laboratory of Optic-electric Sensing and Analytical Chemistry for Life Science, MOE; Shandong Key Laboratory of Biochemical Analysis; College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China

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1. Supplementary Note

1. Instrument

A three-electrode electrochemical system was constructed to perform electrochemical measurements such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and amperometric i-t curve, on a CHI 660E electrochemical workstation (Shanghai Chenhua Instruments Co., Ltd., China), which consists of a working glassy carbon electrode (GCE), a reference saturated calomel electrode and an auxiliary platinum electrode. The structures of the cyclic and linear peptides were confirmed by 1H-NMR spectroscopy with a Bruker AV 600 NMR spectrometer and electronic circular dichroism spectra (CD) were recorded utilizing J-1100 instrument (Jasco, Japan) with wavelengths of 190-260 nm. Zeta potentials of peptides were investigated by ZEN3600 zeta potential analyzer (Malvern, U.K.). Scanning electron microscopy (SEM) was performed with a Hitachi S4800 scanning electron microscope (Hitachi, Japan). The water contact angles of all modified surfaces were measured by means of a JC2000D1 meter (Shanghai, China). X-ray photoelectron spectroscopy (XPS) was performed using the Thermo Fisher Scientific XPS System. TCS-SP5 laser

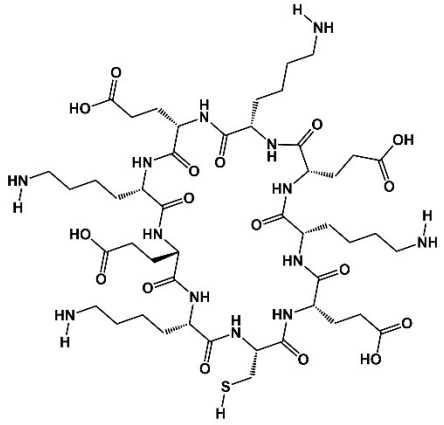
confocal microscopy (Leica, Germany) was used for fluorescent analysis of proteins, cells and bacteria adsorption.

2. Optimization of the incubation time for the RBD

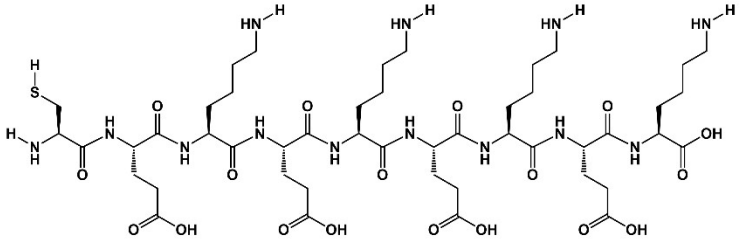
To determine the optimal incubation time for the target, the biosensor was incubated with the RBD solution (100.0 ng mL^{-1}) for different time periods, and the DPV signal was recorded accordingly. It was clearly found that the response signal increased with the extension of incubation time and reached a maximum at 60 min, as shown in Fig. S9. Therefore, the incubation time for target detection was selected as 60 min.

2. Supplementary Figures

(A) Cyclic peptide (cPep): c(CEKEKEKEK)



(B) Linear peptide 1 (lPep 1): CEKEKEKEK



(C) Linear peptide 2 (lPep 2): CPPPPEKEKEKEK

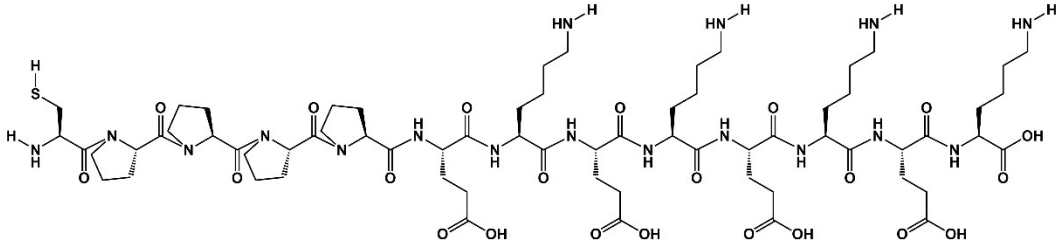


Fig. S1 The molecular structure of cyclic peptide (A), linear peptide 1 (B) and 2 (C).

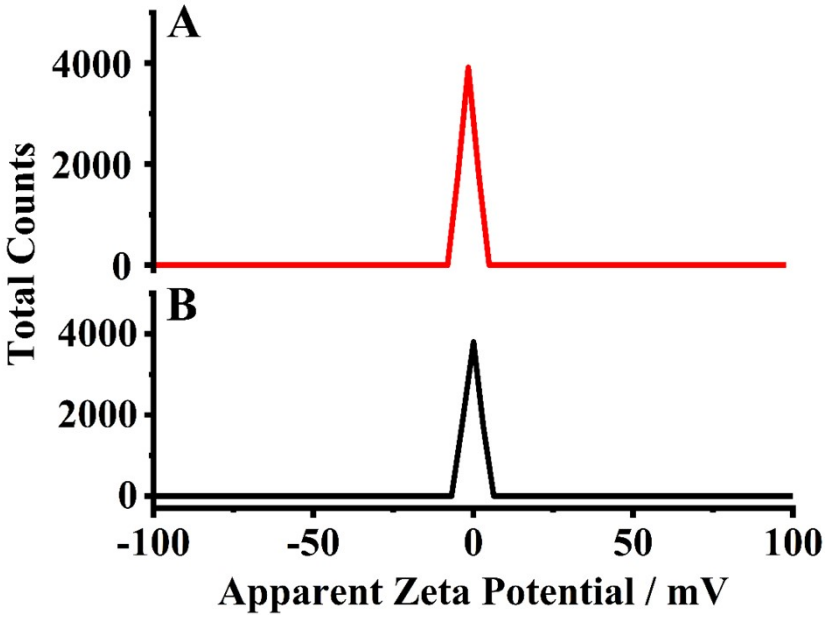


Fig. S2 Zeta potentials of the cyclic peptide (A) and linear peptide 1 (B).

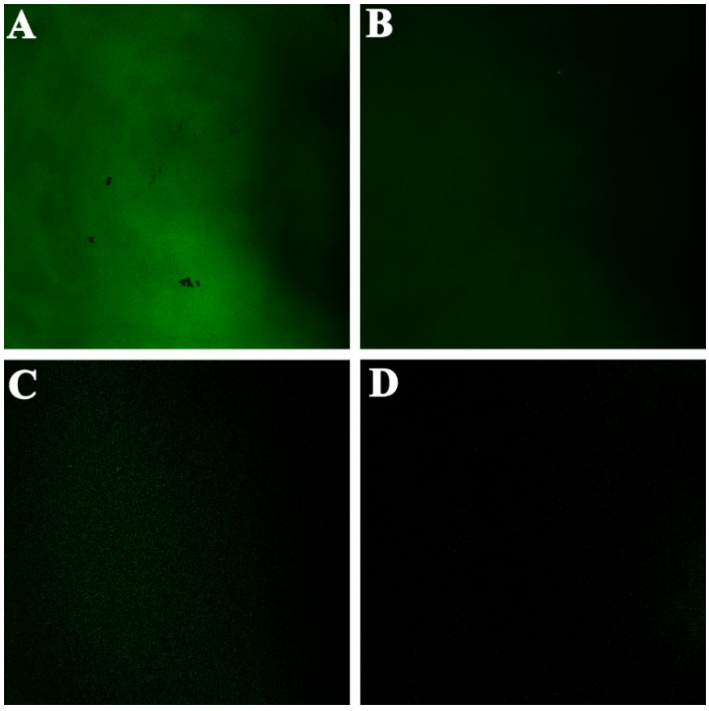
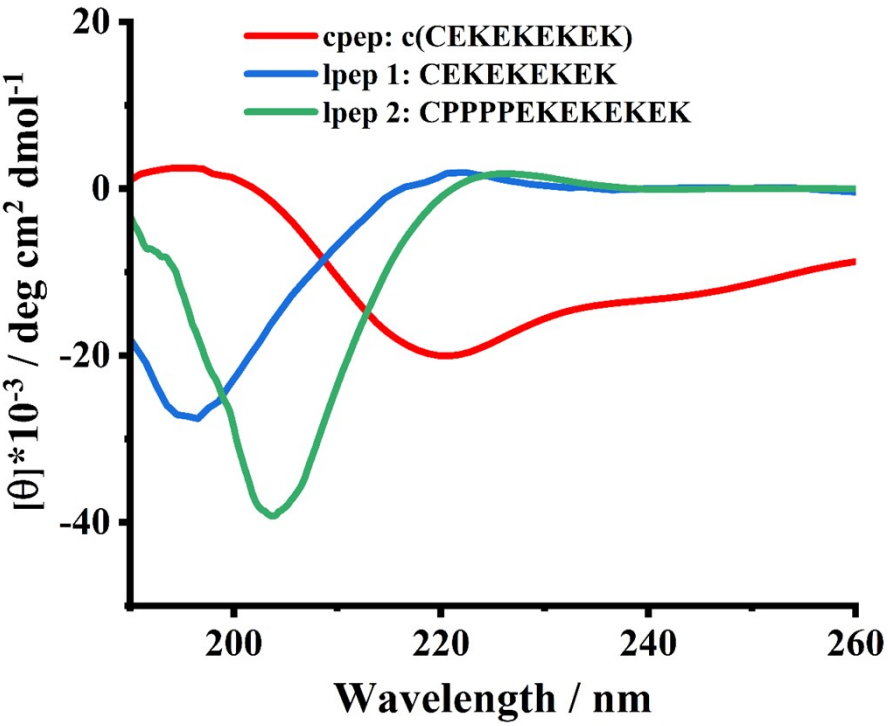


Fig. S3 Fluorescent images of FITC-BSA (10.0 mg mL^{-1}) adsorption on peptide-free surface (A), and lPep1 (B), lPep2 (C) and cPep (D) modified surfaces.



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58 **Fig. S4** CD spectra of aqueous solutions of 0.2 M cyclic peptide (red line), linear

59 peptide 1 (blue line) and linear peptide 2 (green line).

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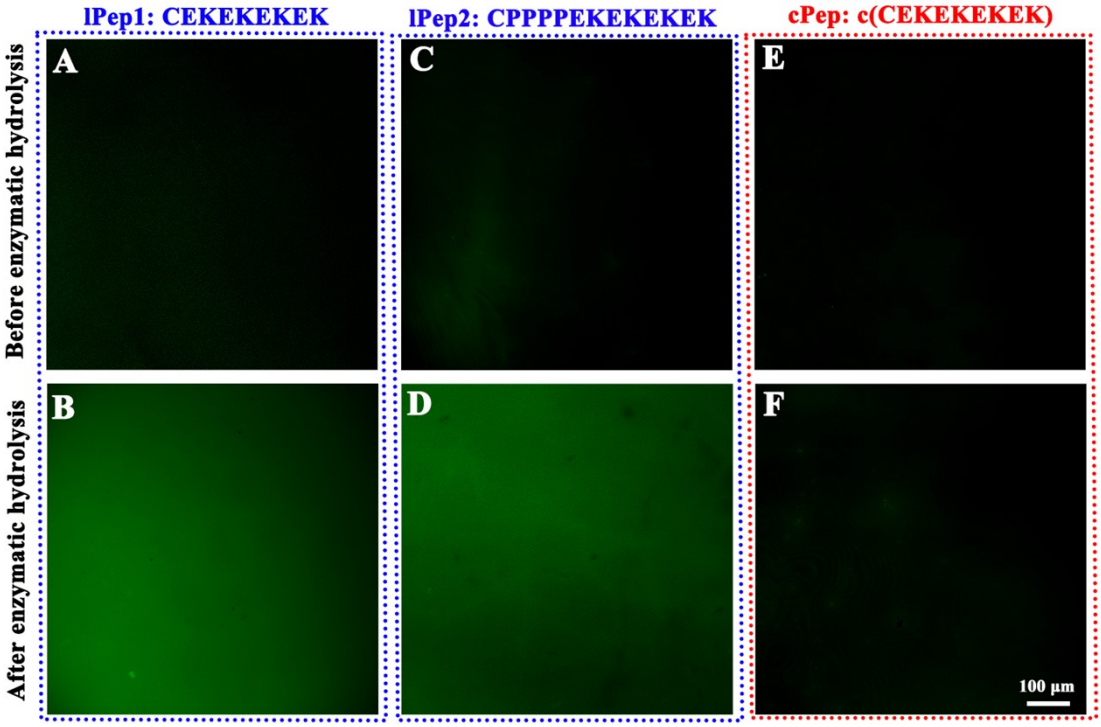


Fig. S5 Fluorescent images of FITC-BSA adsorption on lPep1 (A, B), lPep2 (C, D) and cPep (E, F) modified surfaces before (A, C, E) and after (B, D, F) enzymatic hydrolysis of cPB. Scale bar = 100 μm.

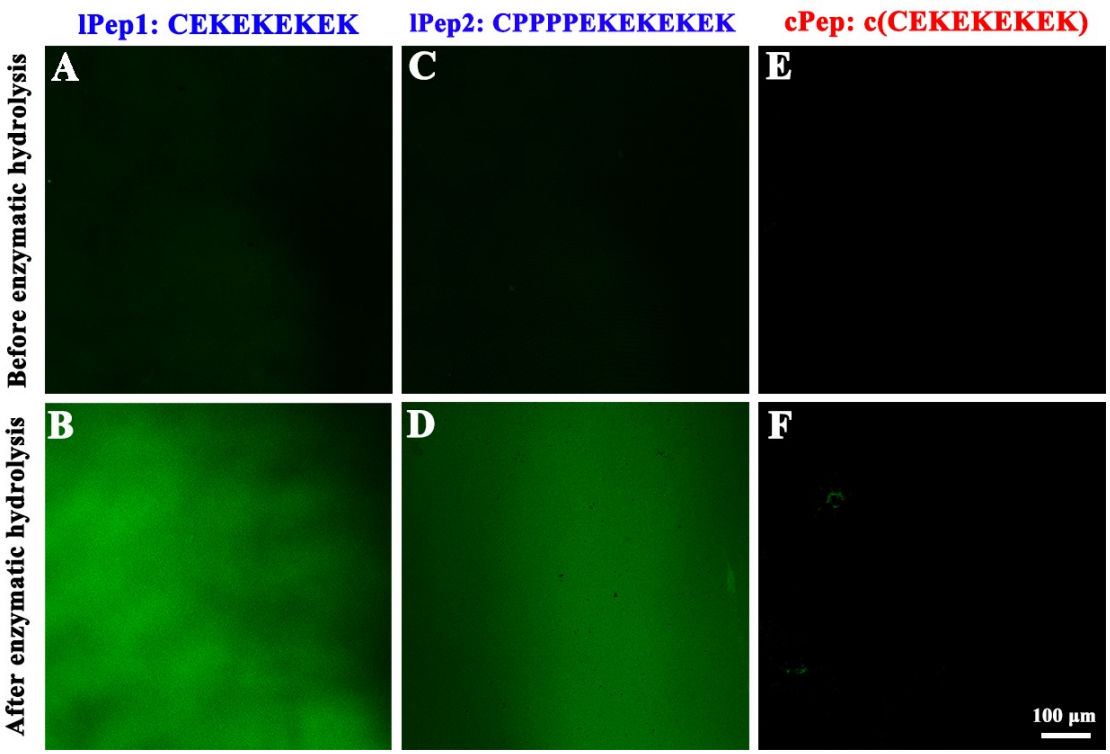


Fig. S6 Fluorescent images of FITC-BSA adsorption on lPep1 (A, B), lPep2 (C, D) and cPep (E, F) modified surfaces before (A, C, E) and after (B, D, F) enzymatic hydrolysis of Ap. Scale bar = 100 μm.

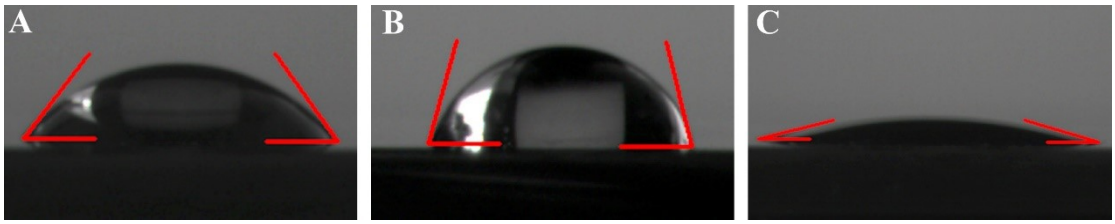


Fig. S7 Water contact angle images of the PEDOT/GCE (A), AuNP/PEDOT/GCE (B), and cPep/PEDOT/GCE (C) surfaces.

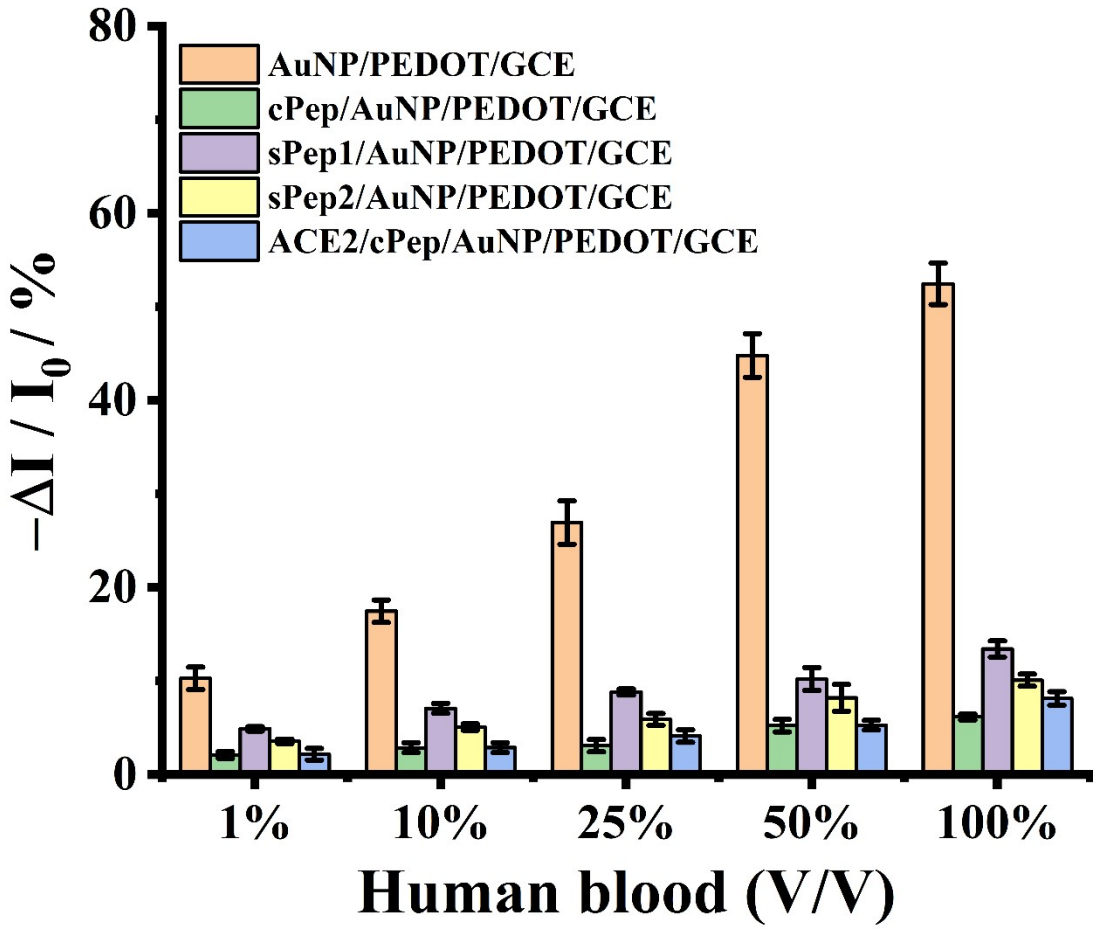


Fig. S8 Comparative antifouling characteristics of various modified electrodes after soaking in human blood with different concentrations (V/V) for 30 min. Error bars represent the standard deviations of three parallel detections.

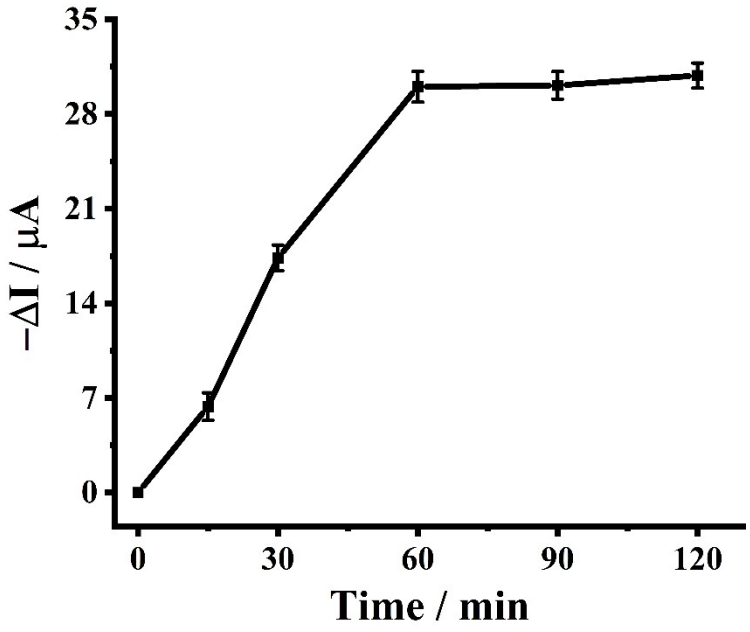


Fig. S9 The effect of RBD (100.0 ng mL^{-1}) incubation time on the DPV response. Error bars represent the standard deviations of three repeated determinations.

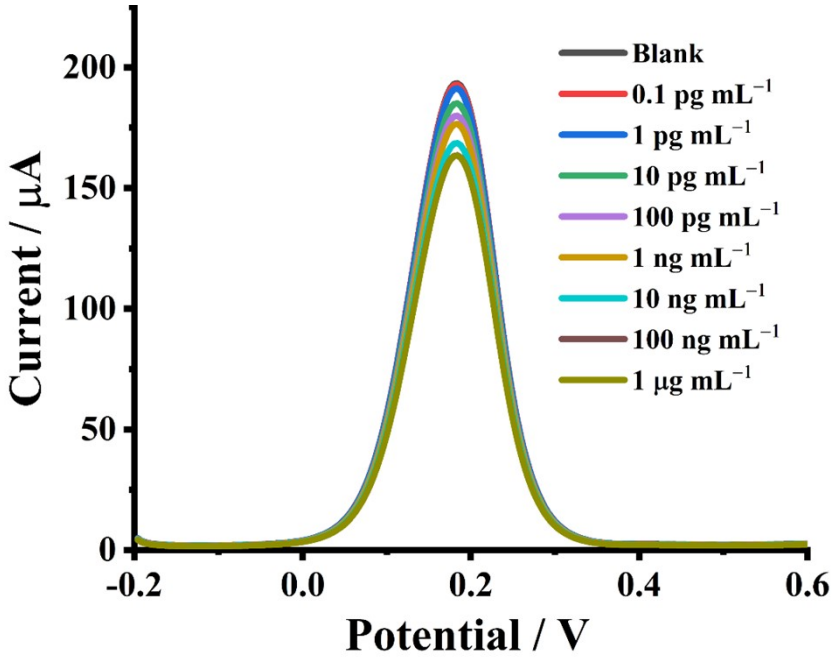


Fig. S10 DPV curves of the cyclic peptide-based biosensor at different concentrations of RBD.

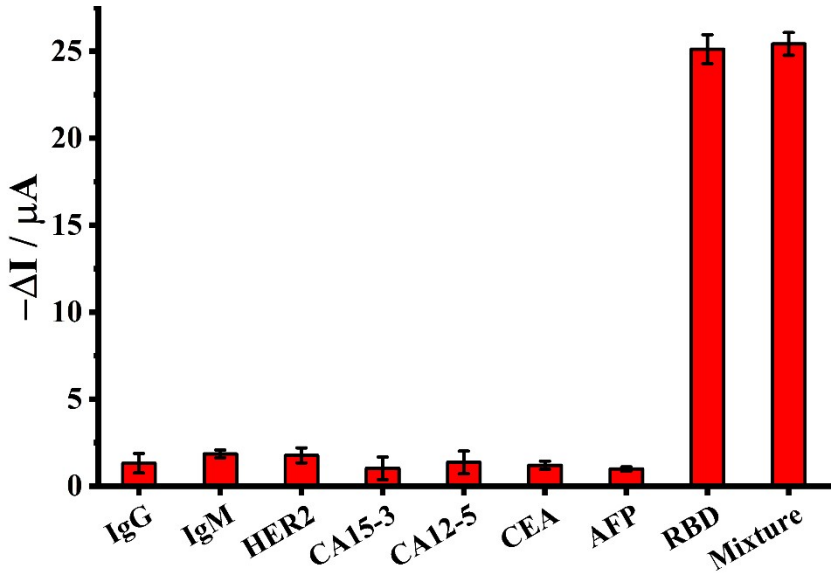


Fig. S11 Responses of the biosensor to 10.0 ng mL^{-1} target (RBD), nonspecific proteins (IgG, IgM, HER2, CA15-3, CA12-5, CEA, AFP) with higher concentrations (1.0 μg mL^{-1}), and mixture solution of above proteins. Error bars represent the standard deviations of three parallel detections.

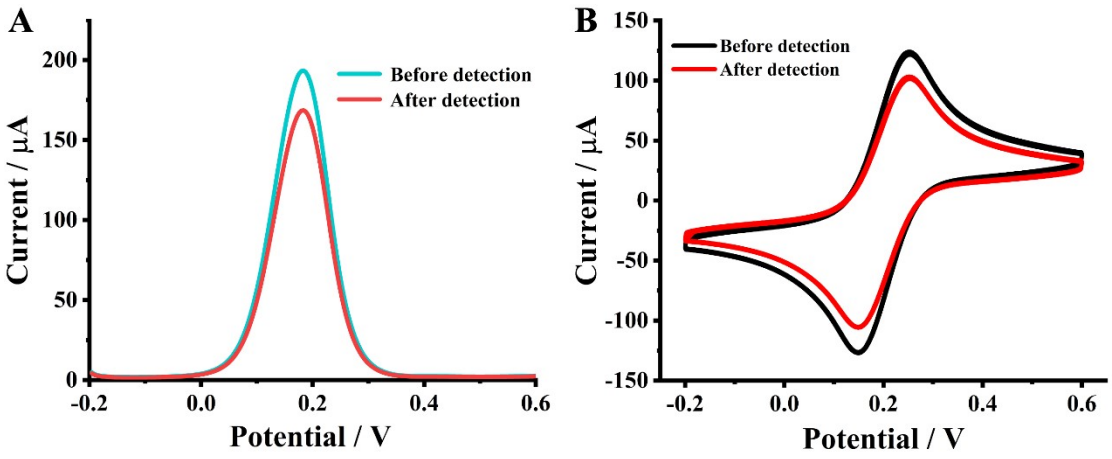


Fig. S12 (A) Seven DPV scans in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution of the biosensor before and after the detection of 10.0 ng mL^{-1} RBD. (B) 50 CV scans in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution of the biosensor before and after the detection of 10.0 ng mL^{-1} RBD.

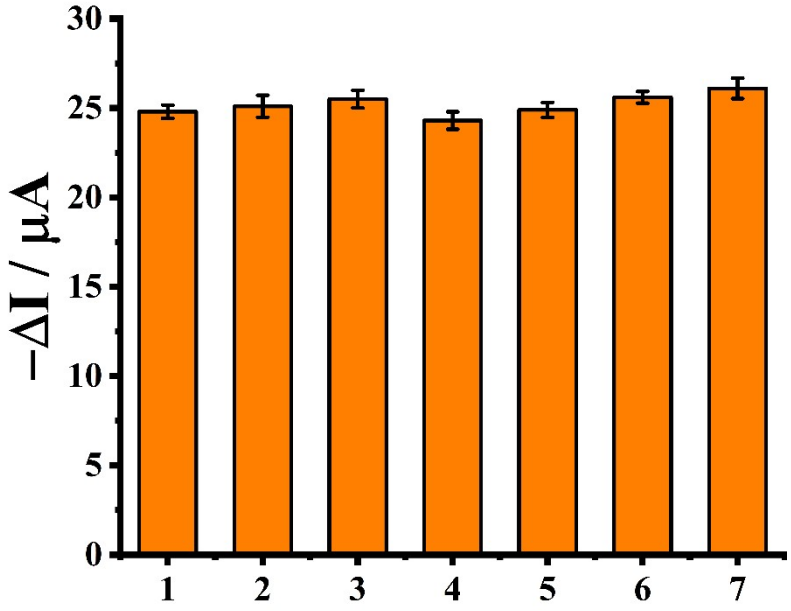
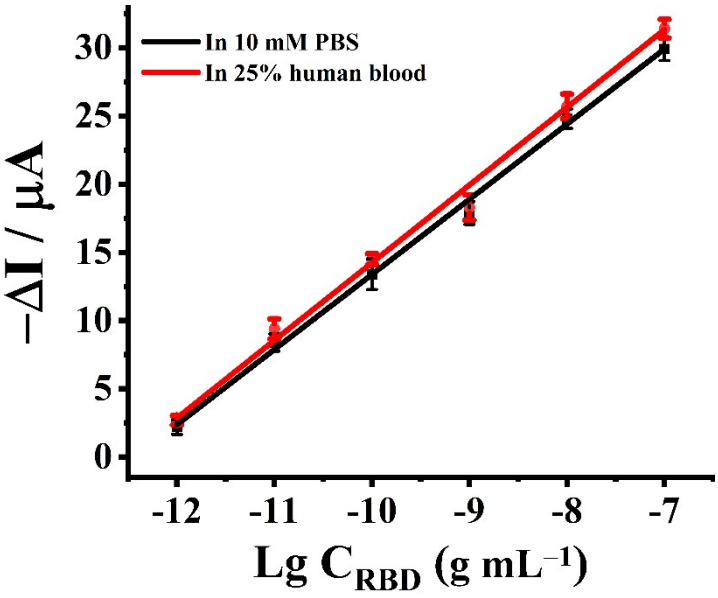


Fig. S13 The reproducibility for the detection of 10.0 ng mL^{-1} RBD with seven independently prepared biosensors. Error bars represent the standard deviations of three parallel detections.



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124 **Fig. S14** Calibration curves of the biosensor for the detection of RBD in 10.0 mM PBS

125 and 25% human blood (V/V). Error bars represent the standard deviations of three

126 parallel detections.

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Supplementary Table

Table S1 Performance comparison of different approaches for the antigen detection of COVID-19.

Detection method	Linear range	Detection limit	Ref.
Electrochemical analysis	2.0-40.0 pg mL^{-1}	0.7 pg mL^{-1}	[1]
Electrochemical analysis	0.002-100.0 pg mL^{-1}	0.577 fg mL^{-1}	[2]
Electrochemical analysis	0.1-20.0 ng mL^{-1}	16.9 fg mL^{-1}	[3]
Electrochemical analysis	0.05-1.0 μg mL^{-1}	18.2 ng mL^{-1}	[4]
SERS immunoassay	1.0-6.0 fg mL^{-1}	4.7 fg mL^{-1}	[5]
Electrochemical analysis	1.0 pg mL^{-1}-100.0 ng mL^{-1}	0.45 pg mL^{-1}	This work

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