Electronic Supplementary Information

Antifouling biosensors for reliable protein quantification in serum based on designed all-in-one branched peptides

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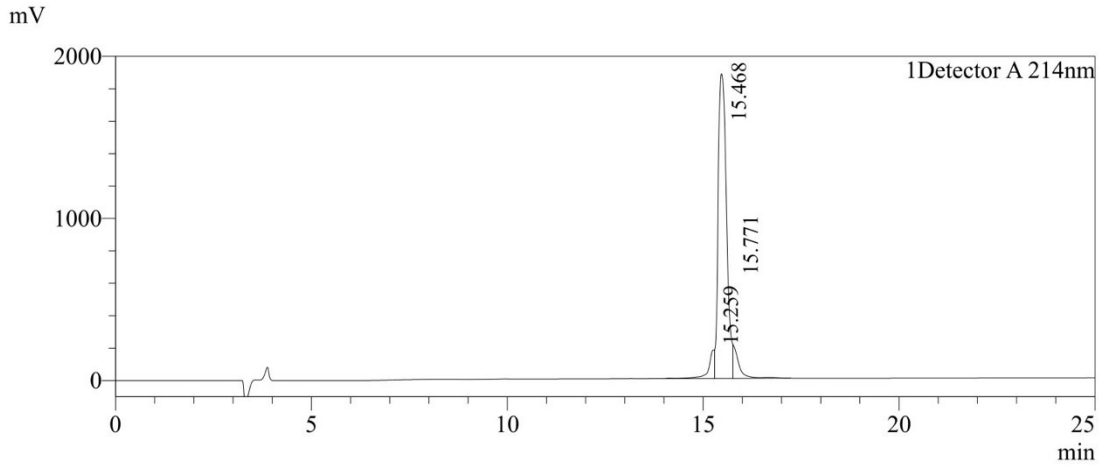
Experimental section

Reagents

We designed the all-in-one branched peptide (Pep, (AVWGRWHD)4(KE)2KEPPPPDDDC), the synthesis and purification were conducted by the Hefei Bankpeptide Biological Technology. The 3,4-ethylenedioxythiophene (EDOT) monomer and hydrogen tetrachloroauric (III) acid ($HAuCl_4 \cdot 3H_2O$) were ordered from the Shanghai Aladdin Chemistry. Alpha fetoprotein (AFP) was ordered from Sangon Biotech. (Shanghai, China). Other biological reagents were purchased from Beijing Bo Yang Hongda. The chromatogram and mass spectra data of the designed peptides are shown in Fig.S1.



Peak Table

Peak #	Ret. Time	Area	Height	Area %
1	15.259	1857856	175846	5.866
2	15.468	27668943	1878930	87.358
3	15.771	2146383	206054	6.777
Total		31673182	2260830	100.000

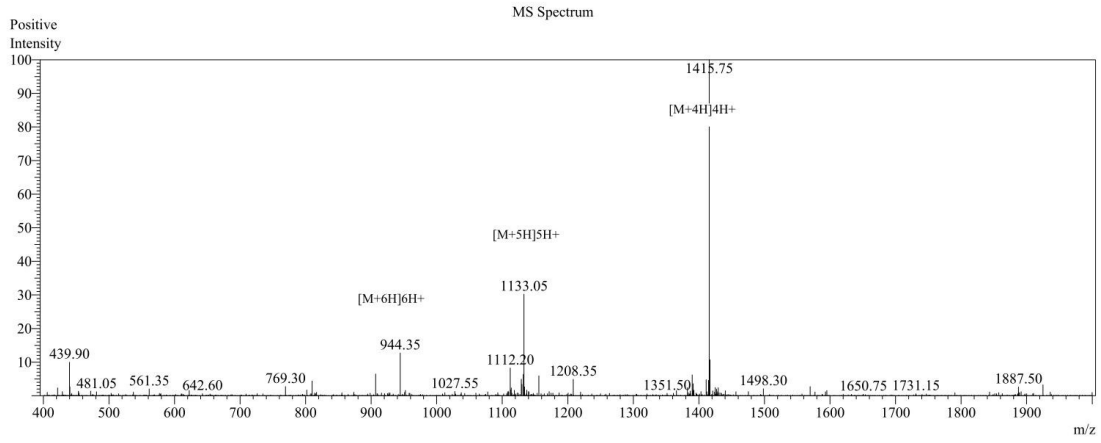


Fig. S1 Chromatogram and MS Spectrum of the all-in-one branched peptide.

The Eighth People's Hospital of Qingdao provided clinical human serum samples for actual sample testing, authorized by the hospital's Review Committee, and obtained informed consent for the use of human serum, and conducted experiments in accordance with relevant laws/regulations and provided operating guidelines. Analytical grade and above were the purity of other chemicals used in this experiment purity. Deionized water (DI water, >18 MΩ cm) was purified using a Milli-Q system (Millipore Co., Bedford, MA, USA) and used to prepare all aqueous solution.

Apparatus

JEOL JSM-7500F SEM instrument was used to take scanning electron microscope (SEM) (Hitachi High-Technology, Japan). ESCALAB 250Xi spectrometer was used to test X-ray photoelectron spectroscopy (XPS) under monochromatic Al Kα X-ray source ($h\nu$ = 15 kV, Thermo Fisher Scientific, USA). We used Nano-ZS Zetasizer ZEN3600 (Malvern, U.K.) to determine the zeta potential (ζ) of the designed peptide. Jasco J-810 CD spectropolarimeter was used to test circular dichroism (CD) spectra. Surface wettability of different electrodes were conducted with the water contact angle (WCA) using a JC2000D1 instrument bought by Shanghai Zhongchen Instrument Co. (Shanghai, China). The fluorescence image was carried out using TCS SP5 confocal laser microscope (Leica, Germany). Electrochemical experiments were measured by a Gamry Interface Reference 3000 potentiostat/galvanostat/ZRA (Gamry Instruments, Warminster, PA, US) equipped with a three-electrode setup. All electrochemical measurements were performed in an electrolyte solution containing 0.1 M KCl, 5.0 mM $[Fe(CN)_6]^{3-/4-}$, and pH 7.4 10.0 mM phosphate

buffered saline (PBS). The potential window of CV scan is -0.2 to 0.6 V (scan rate: 100 mV/s). And the potential window of DPV is also -0.2 to 0.6 V with 25 mV pulse size.

Characterization of the designed peptides

Theoretical calculation and zeta potential measurement were used to obtain the electrical properties, and zeta potential was monitored in 1.0 mg/mL designed peptide solution. We used CD spectroscopy to measure the secondary structure of the designed peptide, and subtracted the buffer spectrum from the peptides sample spectrum. Hydrophilicity capabilities were performed by a WCA measurement with the droplet volume of 10 μL.

Preparation of antifouling sensing interface

Prior to fabrication procedure of the antifouling interface, the bare GCEs were polished with alumina slurries successively, and then washed with absolute alcohol, H_2O, ultrasonically for 1 min, respectively. Gold nanoparticles (AuNPs) were electrodeposited onto the bare GCE by continuous CV scanning (from -0.9 to 0.5 V) for 5 cycles in a deposition electrolyte containing 0.5 M KNO_3 and 1.0 mM $HAuCl_4$. Designed all-in-one zwitterionic branched peptides were immobilized onto the AuNPs/GCE by soaking in PBS buffer containing 0.2 mg/mL peptides for 6 h. Afterward, 20 mM EDOT monomers were ultrasonicated and then used to electropolymerize PEDOT onto Pep/AuNPs/GCE at 1.0 V constant voltage for 40 s. Finally, PEDOT/Pep/AuNPs/GCE (gold nanoparticles-peptide-PEDOT modified electrode, GPPE) with an antifouling sensing interface was successfully prepared.

Antifouling assessments

Complex biological sample fetal bovine serum (FBS) and fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA) was used to measure the antifouling performance of the modified electrode. Nonspecific adsorption was monitored by DPV technique and fluorescence microscopy before and after soaking in complex biological samples.

Sensing of IgG

The modified electrodes (GPPE) were incubated in IgG solutions with various concentrations for 75 min, and then washed the unspecific-bounded molecules with PBS. The signal suppression ($\triangle I/I_0$) of the constructed biosensor was monitored by DPV technology in the range of -0.2 to 0.6 V.

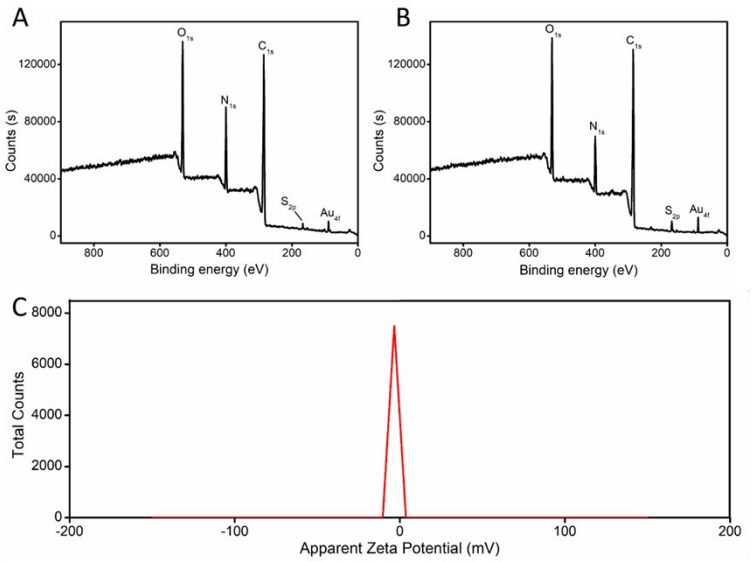


Fig. S2 XPS spectra of electrode surfaces modified with (A) Pep/AuNPs, (B) PEDOT/Pep/AuNPs, respectively. (C) Zeta potential of the zwitterionic branched peptide.

Table S1. Static water contact angles of the different surfaces.

Test points	Contact angle (°)		
	Bare GCE	AuNPs/GCE	Pep/AuNPs/GCE
1	55.43	71.93	32.18
2	55.19	71.54	32.58
3	54.76	71.36	32.61
Average	55.13 ± 0.34	71.61 ± 0.29	32.46 ± 0.24

Optimization of the experimental conditions.

To fabricate the optimal antifouling sensing protocol, several key experimental parameters, such as the effect of the deposition time of conducting polymer PEDOT on antifouling and sensing condition, the effect of the peptides concentration on antifouling performance and the effect of IgG incubation time on sensing system were investigated.

Influence of deposition time

The precise positioning and electrodeposition of conducting polymer PEDOT determines the optimal antifouling and sensing performance of the platform. Therefore, the deposition time of PEDOT, as it will control the thickness of the deposited PEDOT film, on the antifouling and sensing performance of the biosensor was investigated. As shown in Fig. S3A, when the deposition time was shorter than 40 s, only part of the negatively charged doping sequences were deposited by the PEDOT, and those exposed negatively charged doping sequences will allow protein adsorption because of the electrostatic interactions, leading to biofouling. When the deposition time was longer than 40 s, part of the linking or antifouling sequence may be covered by PEDOT, weakening the helix conformation or antifouling capability. While the deposition time at 40 s, the doping sequence were entirely covered by the PEDOT and kept the linking, antifouling and recognizing sequences exposed on the surface, corresponding to the best antifouling performance of the biosensor. At the same time, an ideal sensing signal was also obtained for the biosensor at the deposition time of 40 s (Fig. S3B). This is because that when the deposition time less than 40 s, the non-conductive peptides will weaken the electrode reactivity and cannot meet the sensing requirements, which inhibits the sensitivity of sensing interface. The deposition time longer than 40 s will partially or even completely cover the recognizing sequence, seriously affecting the binding of the target.

Influence of peptides concentration.

The functional branched peptides showed a significant effect on antifouling performance by incubation with 20% FBS, and reached maximum antifouling characteristic at 0.2 mg/mL (Fig. S3C).

Influence of IgG incubation time.

Incubation time of target molecular is an important parameter for the specific binding between

the peptide-aptamer and IgG. It can be clearly observed that the signal suppression increased with the increasing of the incubation time and reached a maximum at 75 min as shown in Fig. S3D, indicating that the binding achieved equilibrium. Therefore, the optimal incubation time of IgG was 75 min.

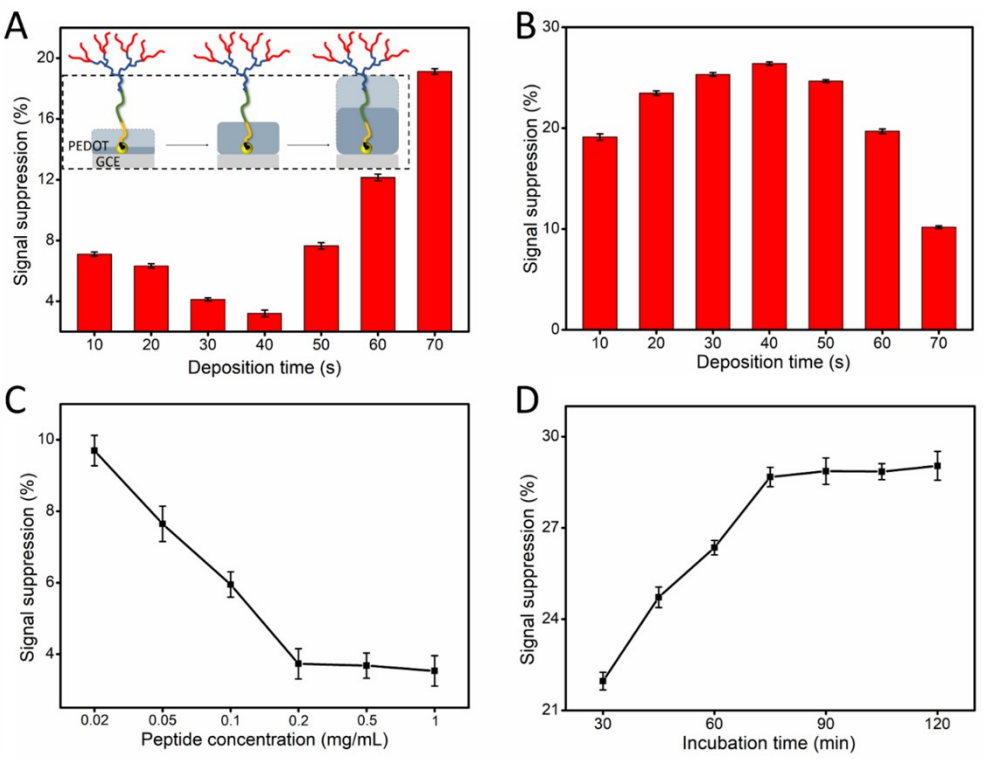


Fig. S3 Effects of PEDOT deposition time on the antifouling performance of the GPPE in 20% FBS (A) and sensing response of the biosensor to 1.0 μg/mL IgG (B). (C) Effects of the peptide concentration on the antifouling ability in 20% FBS. (D) Incubation time on the DPV responses of the electrochemical biosensor toward 1.0 μg/mL IgG.

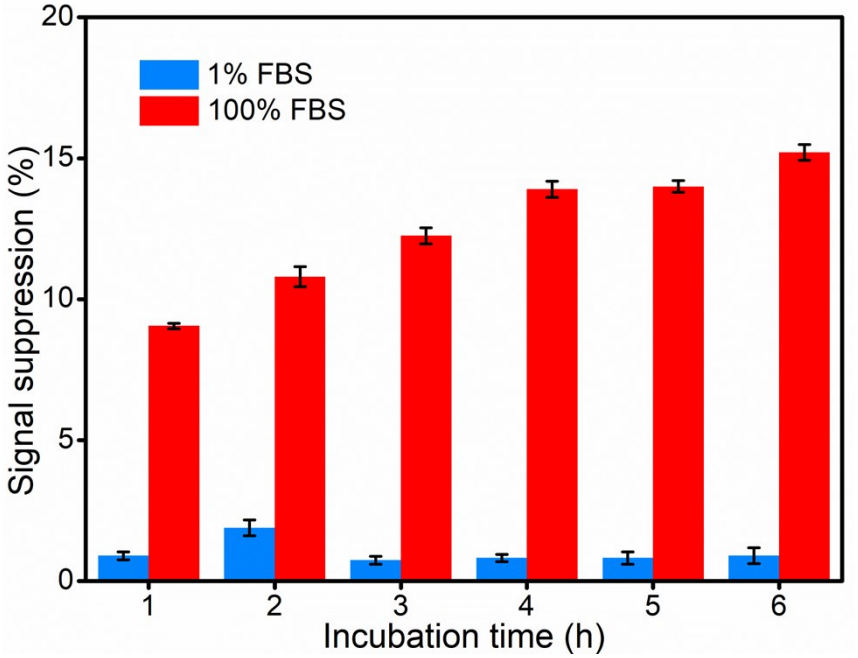


Fig. S4 Antifouling performances of the GPPE after incubation in 1% and 100% FBS samples for a considerably long time of 1-6 h.

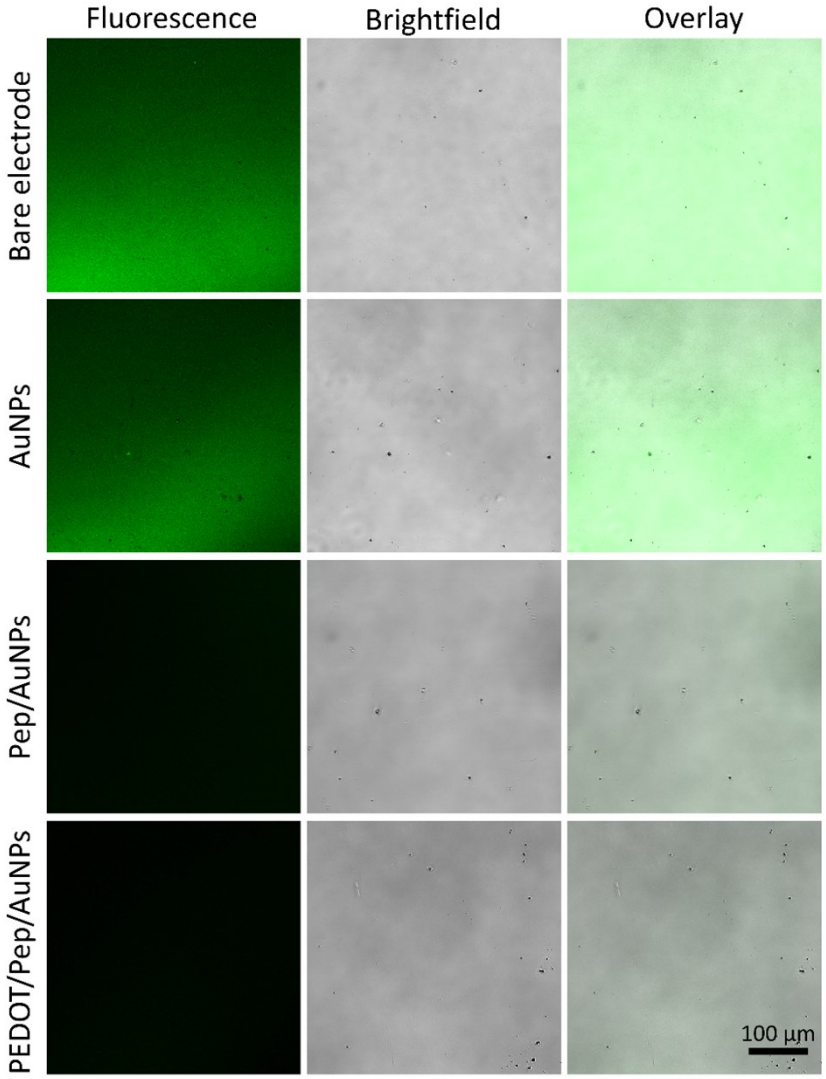


Fig. S5 Representative fluorescence microscopy images of bare electrode, AuNPs, Pep/AuNPs and PEDOT/Pep/AuNPs modified electrodes after incubation in 0.2 mg/mL FITC-BSA solution for 2 h.

Table S2. Comparison of sensing performance for the IgG detection.

Detection method	Material used	Linear range (ng/mL)	Detection limit (ng/mL)	Ref.
Self-powered biosensor	ZnO nanowire	100-10^6	6.9	1
Sandwich-type voltammetric immunosensor	Chemical reduced graphene and carbon nanotube	1.0-500	0.2	2
DPV	ZnO@ZIF-8/IL/GA/anti-IgG/BSA-CPE	0.1-10, 10-400	0.03	3
Self-powered biosensor	SiO_2/ZnO nanowire	10-10^6	5.7	4
CV	AuNPs/SPCE	50-200	9.4	5
DPV	Peptides-based PANI nanowire biosensor	1-10^4	0.26	6
SWV	Bipyramidal gold nanoparticles	20-4×10^4	20	7
Voltammetric immunosensor	COOH-multiwalled carbon nanotubes/Fe_3O_4	300-1000	25	8
DPV	PEDOT/Pep/AuNPs/GCE	0.1-10^4	0.045	This work

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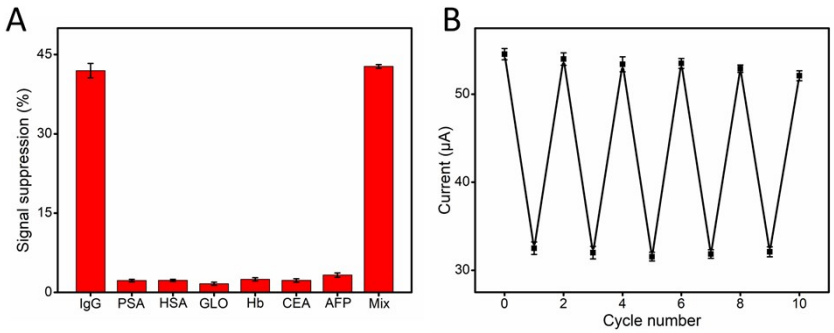

Fig. S6 (A) Signal suppression of the IgG biosensor to 1.0 μg m L^{-1} IgG, and 1.0 mg mL^{-1} of prostate specific antigen (PSA), HSA, globulin (GLO), hemoglobin (Hb), carcinoembryonic antigen (CEA), alpha fetoprotein (AFP) and their mixture (Mix), respectively. (B) The regeneration of the biosensor (responses to 1.0 μg m L^{-1} IgG).

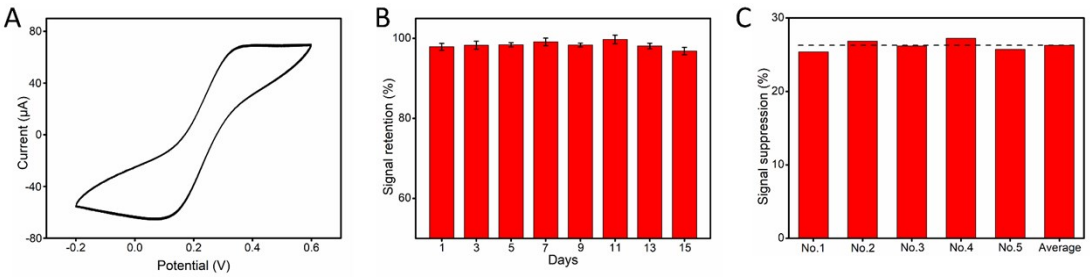

Fig. S7 (A) Representative assessment of interfacial stability with CV at a scan of 0.1 V/s in PBS (10.0 mM pH 7.4) containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. (B) Stability of the prepared biosensor stored at PBS (10 mM, pH 7.4). (C) The signal suppression of five different biosensors for the detection of 1.0 μg/mL IgG.