Analytica Chimica Acta 1208 (2022) 339841

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Designed multifunctional peptides with two recognizing branches specific for one target to achieve highly sensitive and low fouling electrochemical protein assay in human serum

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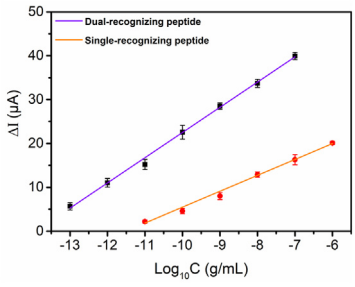
HIGHLIGHTS

- A highly sensitive and low fouling electrochemical biosensor for IgG was prepared.
- Antifouling peptide with two recognizing branches specific for one target was designed.
- The biosensor based on the designed peptide exhibited a wide linear range and low LOD.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 March 2022
Received in revised form
1 April 2022
Accepted 13 April 2022
Available online 16 April 2022

Keywords:
Dual-recognizing peptide
Antifouling
Electrochemical biosensor
Immunoglobulin G

ABSTRACT

Herein, an antifouling electrochemical biosensor based on designed multifunctional peptides with two recognizing branches specific for one target was proposed to improve the target recognition efficiency and sensitivity. The designed multifunctional peptide contains two different recognizing branches (with sequences FYWHCLDE and FYCHTIDE) for immunoglobulin G (IgG), an antifouling sequence (EKEKEK) and an anchoring sequence (CPPPP), which can be immobilized onto the gold nanoparticles (AuNPs) and poly(3,4-ethylenedioxythiophene) (PEDOT) modified electrode surface. Owing to the synergistic effect of the two recognizing branches, the dual-recognizing peptide-based biosensor exhibited significantly enhanced sensitivity. Under the optimal experimental conditions, the biosensor for IgG exhibited a linear response range of 0.1 pg/mL to 0.1 μg/mL, with a limit of detection of 0.031 pg/mL (about 2 orders of magnitude lower than that of the normal biosensor). Moreover, the biosensor was also capable of assaying IgG in real biological samples such as human serum without suffering from significant biofouling. This strategy for biosensor construction not only ensures the ultra-sensitivity for target detection, but also effectively avoids biofouling on sensing interfaces in complex biological media.

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https://doi.org/10.1016/j.aca.2022.339841
0003-2670/

1. Introduction

Early diagnose and treatment could effectively avoid and reduce the incidence rate of human disease and help to save and prolong life. In general, the occurrence of diseases is accompanied by the abnormal concentration and distribution of some biomolecules in human body, such as nucleic acid, proteins, small metabolites and enzymes, which are known as disease markers [1,2]. Thus, effective and sensitive analysis of disease markers is vital for timely diagnosis and treatment of the diseases.

Enzyme-linked immunosorbent assay [3–5], fluorescence [6–9], surface plasmon resonance [10,11] and electrochemical methods [12–14] are commonly methods used for quantitative detection of disease markers. Due to simple operation, high sensitivity and low-cost [15–17], the electrochemical method has been favored by researchers. However, the disease biomarkers often exist in complex biological media, such as blood, saliva and urine, and the concentration is extremely low. The detection process is susceptible to the interference of coexistence of biological molecules, such as some protein or small organisms, seriously affecting the reliability of the detection methods. Therefore, the construction of low fouling biosensors that are capable of resisting nonspecific interferents in the complex biological media remains a serious challenge that needs to be solved. Among most of the peptide-based biosensors, peptide with one recognizing sequence, which can only identify a single binding site of the targets, is commonly used for the detection of target through specific binding [18,19]. Nowadays, a range of peptide-based sensing platforms with two recognizing branches coexisting in one probe or sensing interfaces, where two recognition sites can play a synergistic role with each other, have been reported [20,21]. In the process of specific affinity adsorption, two recognizing branches can bind to different sites of the target. Research shows that the synergistic effect between two recognizing branches and the related two specific reactions are both helpful to enhance the capability of biosensor for assaying of target [22], thereby improving the detection sensitivity. Inspired by these, the sensor sensitivity can be improved by introducing two recognizing branches into one peptide through reasonable design.

In addition, biofouling has always been a severe challenge in the clinical application of electrochemical biosensors [23,24], since biofouling affect not only the sensing sensitivity but also the analytical accuracy of the biosensor. And constructing antifouling layers on the sensing interface is considered as an effective treatment [25]. To date, Polyethylene glycol (PEG) and zwitterionic polymers are two kinds of popular antifouling materials. However, owing to the shortcomings of oxidation and poor stability, they are limited in practical use [26–28]. Due to the ease of synthesis, stable in nature and multi-functionality [29,30], peptide was selected as an effective antifouling material to resist biofouling [31–33].

In this work, we innovatively designed multifunctional peptides with a hydrophilic antifouling main chain and two recognizing branches specific for one target and constructed an antifouling electrochemical biosensor based on designed peptides for human immunoglobulin G (IgG) assaying. These two recognizing branches can specifically bind the different sites of immunoglobulin G in the Fc region [22,34], and the synergistic binding effect of these two branches could obviously enhance the binding affinity for IgG. These are also conducive to improve the sensitivity and accuracy of the developed biosensor. As illustrated in Scheme 1, the electrode was firstly coated with poly(3,4-ethylenedioxythiophene) (EDOT) and AuNPs by two-step electrodeposition, where the PEDOT was used to increase not only the specific surface area but also the conductivity of the modified electrode, and AuNPs were used to immobilize the peptide. Furthermore, along with the immobilization of the designed multifunctional peptides, an ultrasensitive and antifouling biosensor was obtained for IgG assaying in complex media. As expected, the developed biosensor not only exhibits a superior sensitivity but also can achieve the direct detection in actual samples, such as human serum, suggesting a significant application prospect in future clinical medicine.

2. Experimental section

2.1. Fabrication of the ultrasensitive and antifouling biosensor

The electrodes were carefully polished with 0.3 μm and 0.05 μm aluminum powder before modification, and then they were ultrasonically washed by Milll-Q water, anhydrous ethanol and water for 2 min, respectively. Afterward, 0.2 M EDOT and 0.0434 g of sodium citrate, which acted as dopants, were added to 5.0 mL water forming the conductive polymer mixture solution, and electrochemical deposition was performed at the constant potential of 1.1 V for 200 s. Then, the PEDOT modified electrode was soaked in 0.5 mM chloroauric acid ($HAuCl_4$) solution containing 0.5 M KNO_3 for AuNPs electrodeposition with the potential range from −1.5 V to 0.5 V using CV technique for four scanning segments, which was consistent with the previous report. Finally, the AuNPs/PEDOT modified electrode was incubated in 0.25 mg/mL peptide solution overnight. The disulfide bond formed between sulfhydryl groups in peptides shall be reduced with 50 mM TCEP solution before the peptide was used, so that peptides were self-assembled onto the electrode through the Au−S bond. At last, the prepared biosensor was stored at room temperature for later use.

2.2. Characterization of the dual-recognizing peptide and biosensor

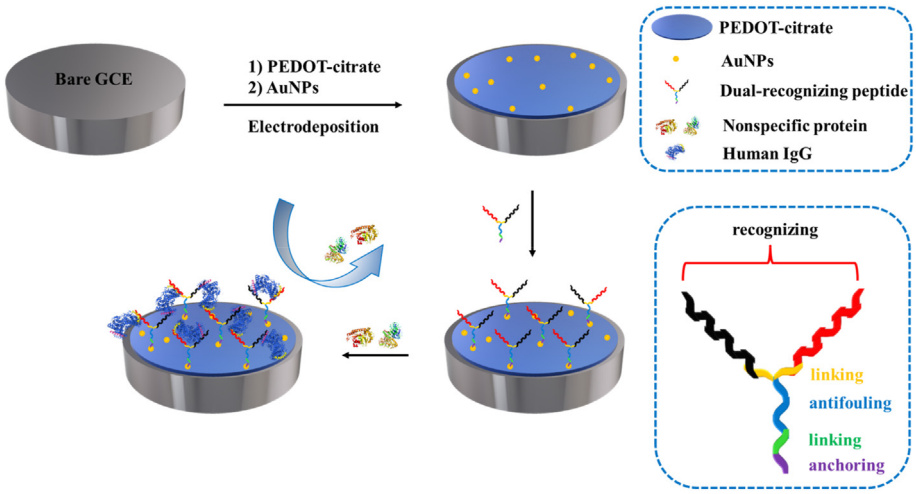
The circular dichroism (CD) spectra were applied to elucidate the secondary structure of the dual-recognizing peptide, it was recorded on 0.25 mg/mL peptide solution with a wavelength range from 190 to 280 nm and a step resolution of 0.5 nm using Jasco J-1100 CD Spectrophotometer (Jasco Inc, Japan). The final measured data was the buffer spectrum subtracted from the spectrum of the peptide sample. Hydrophilicity of various assembled interfaces of the constructed biosensor was monitored by the water contact angle meter (JC 2000D1, Zhongchen Instrument, China). X-ray photoelectron spectroscopy (XPS) spectra were recorded with an ESCALAB 250Xi spectrometry (Thermo Fisher Scientific, U.K.) instrument with a monochromatic Al Kα X-ray source ($h\nu = 15$ kV), characterizing the successful modification of each material on the electrode surface.

2.3. Assessment of the antifouling interface

In order to evaluate and compare the antifouling performances of different interfaces, each modified interface was soaked in different concentrations of human serum for 30 min, and the DPV signal responses were recorded before and after soaked in human serum. Before the antifouling test, the prepared biosensor was saturated with 1.0 mg/mL target protein IgG to eliminate the interference of target IgG presented in human serum. Meanwhile, the fluorescence images of modified interfaces were monitored using the Leica TCS-SP5 microscope, after incubated in 0.25 mg/mL FITC-BSA for 30 min.

2.4. Detection of the target protein IgG

In order to compare the detection sensitivity of the biosensors based on designed dual-recognizing peptides and single-recognizing peptides, the prepared different biosensors were incubated in IgG solutions with different concentrations for 90 min.



Scheme 1. Fabrication process of the antifouling electrochemical biosensor based on the designed multifunctional peptide with two branches recognizing two different sites of the target IgG.

And the DPV signal responses before and after incubation were recorded.

2.5. Analysis of actual samples

IgG is the main component of immunoglobulin in real human serum, and the normal content of IgG in human serum is 7.0–17.0 mg/mL, accounting for roughly 75% of the total content of immunoglobulin in serum. For actual clinical samples analysis, the real blood provided by five patients were centrifuged at 10000 rpm for 5 min, and then the supernatants were collected and measured with the prepared biosensor. The obtained results were compared with those hospital detection results.

3. Results and discussions

3.1. Investigation of the designed dual-recognizing peptides and antifouling biosensor

The morphology of the modified interfaces was characterized by scanning electron microscopy (SEM). As shown in Fig. 1A and B, sodium citrate doped PEDOT film showed a relatively rough surface. After the electrodeposition of AuNPs, a uniform layer of small particles can be directly visualized on PEDOT surface, demonstrating the successful modification of AuNPs. CD spectra was used to characterize the secondary structure of the designed multifunctional peptide, as displayed in Fig. 1C. A strong negative peak at 200 nm and a weak positive peak at 220 nm demonstrated the α-

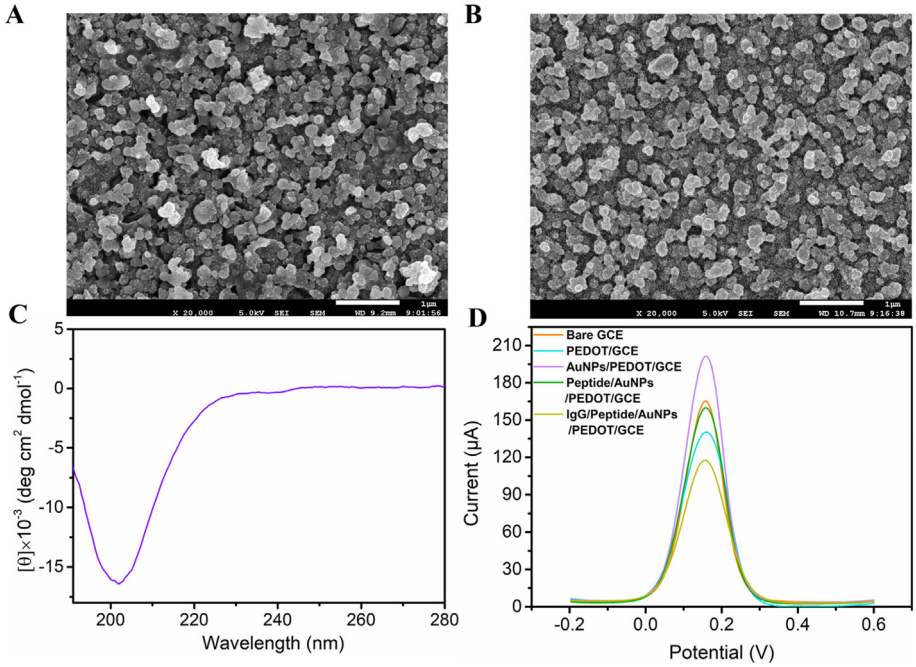


Fig. 1. SEM characterization of electrodes modified with the PEDOT (A) and the AuNPs/PEDOT (B). (C) CD spectra of 0.25 mg/mL designed multifunctional peptide in PBS. (D) DPV curves of different modified electrodes recorded in $[Fe(CN)_6]^{3-/4-}$ solution.

helix structure of the designed peptides, and this is due to the rigid construction of proline residues. XPS characterizations of PEDOT, AuNPs/PEDOT, and Peptide/AuNPs/PEDOT modified surfaces were revealed in Fig. S2. The PEDOT modified electrode mainly contained carbon (C), oxygen (O) and sulfur (S) elements. After the electrodeposition of AuNPs and the immobilization of peptides, two new elements (Au and N) appeared and N element was originated from the peptides. The XPS results effectively showed the modification of PEDOT and AuNPs, the immobilization of two-recognizing peptides, and the successful construction of the biosensor. The hydrophilicity of different modified electrodes was characterized by water contact angle test, as shown in Fig. S3. The contact angles of bare GCE, AuNPs/PEDOT/GCE and branched peptide/AuNPs/PEDOT/GCE were 67.4°, 57.4°, 34.8°, respectively. It can be clearly observed that the water contact angle decreased obviously after the modification of the designed peptides, indicating the superior hydrophilicity of the peptide modified interfaces.

In order to prove the basic feasibility of the experiment, the current signals of different modified interfaces were characterized by DPV. As can be seen from Fig. 1D, it was observed that bare electrode exhibited an obvious DPV peak, and after the electrodeposition of conductive PEDOT, the current value decreased due to the electrostatic repulsive force between the citrate and the $[Fe(CN)_6]^{3-/4-}$ redox probe. And the subsequent electrodeposition of

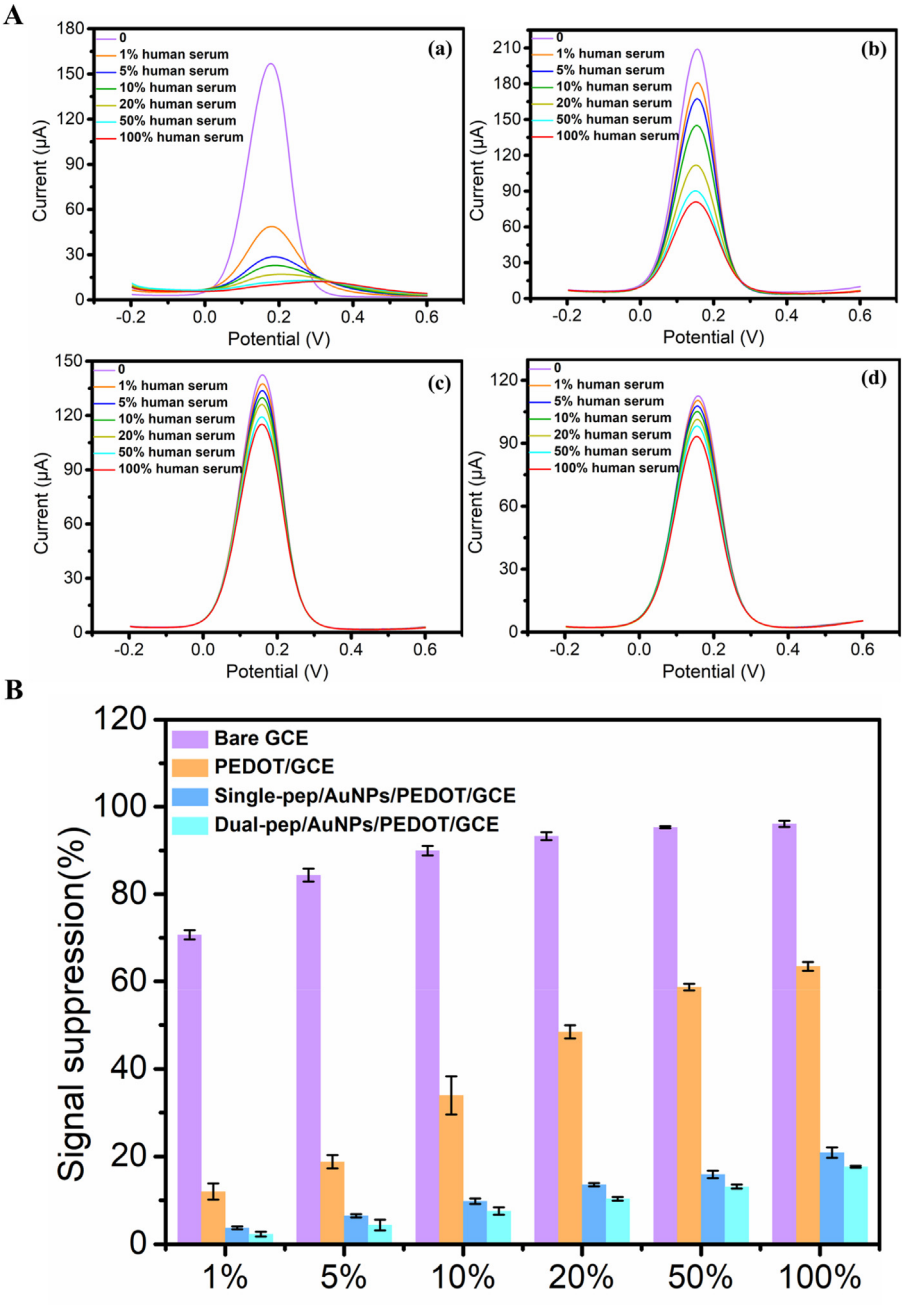


Fig. 2. (A) DPV responses of (a) bare GCE, (b) AuNPs/PEDOT/GCE, (c) single-pep/AuNPs/PEDOT/GCE and (d) dual-pep/AuNPs/PEDOT/GCE after soaked in human serum with different concentrations (V/V) for 30 min. (B) The corresponding signal suppression of different modified electrode interfaces. The error bar shows the standard deviations of three repeated measurements.

AuNPs, the current signal was remarkably increased because of the large surface area and excellent conductivity of AuNPs. When the dual-recognizing peptides were introduced to the modified electrodes, the current decreased significantly, ascribed to the insulating property of the peptides. After the further binding with target IgG, the DPV current continuously decreases. It is speculated that IgG protein could hinder the electron transfer between the solution and the electrode interface The gradual change of the current signal indicated the successful construction of the antifouling biosensor, which also provided a strong proof for the specific recognition and detection of targets.

3.2. Optimization of experimental conditions

In order to reach the optimal assay performance of the constructed biosensor, the effects of peptide concentration and incubation time of the target IgG were investigated. It can be observed in Fig. S4 A, the DPV current change increased with the increasing of peptide concentration and reached a platform when peptide concentration is 0.25 mg/mL. Thus, 0.25 mg/mL was chosen as the optimal peptide concentration. Fig. S4 B also demonstrated that the difference of DPV responses gradually increased with the increasing of the incubation time from 30 min to 90 min, and then became stable. Therefore, 90 min was employed as the optimum incubation time.

3.3. Antifouling performance of different modified interfaces

In order to explore the antifouling performance of different modified interfaces, the DPV responses of bare GCE, AuNPs/PEDOT/GCE, dual-pep/AuNPs/PEDOT/GCE and single-pep/AuNPs/PEDOT/GCE were recorded before and after incubation in different concentrations of human serum (Fig. 2A). As shown in Fig. 2B, after incubation in a series of concentrations of human serum, the signal suppression of the dual-pep/AuNPs/PEDOT/GCE was still less than 20% even in 100% human serum, suggesting a larger application perspective in practical complex samples. After that, the long-term antifouling performance was also investigated. As shown in Fig. S5, the signal suppression of dual-recognizing peptide modified interface was less than 20% within 6 h in 5% human serum. The antifouling abilities of different interfaces were further confirmed by fluorescence microscopy. As shown in Fig. S6, the interfaces of bare GCE and AuNPs/PEDOT/GCE had obvious green fluorescence, while the dual-recognizing peptide modified interface almost had no significant green fluorescence.

3.4. Detection performance of the biosensor

The prepared antifouling biosensor based on designed multifunctional peptides with two recognizing branches was used for the detection of different concentrations of target IgG, and linear peptide modified electrode was taken as a control group. Under the optimal conditions, the sensitivity of the constructed biosensor was investigated, as shown in Fig. 3A and Fig. 3B. With the increase of the IgG concentration from 0.1 pg/mL - 0.1 μg/mL, the current signal decreased gradually. And a linear relationship between the current responses of the developed biosensor and the logarithm of the target IgG concentration was obtained. The corresponding linear equation was $I_0 - I = 5.75 \log_{10} C + 79.95$ ($R^2 = 0.9977$), the limit of detection (LOD) was 0.031 pg/mL (S/N = 3). While the linear detection range of the control group was 10 pg/mL to 1.0 μg/mL, and the linear equation was $I_0 - I = 3.62 \log_{10} C + 41.70$ ($R^2 = 0.9950$), with the LOD of 3.8 pg/mL (S/N = 3). It can be seen from Fig. 3C and D that the LOD of the biosensor modified by the dual-recognizing peptide was 2 orders of magnitude lower than that of the biosensor modified by the single-recognizing peptide. What's more, due to the synergistic binding effect of the two recognizing branches, the developed biosensor also exhibited a higher detection sensitivity and a wider detection range.

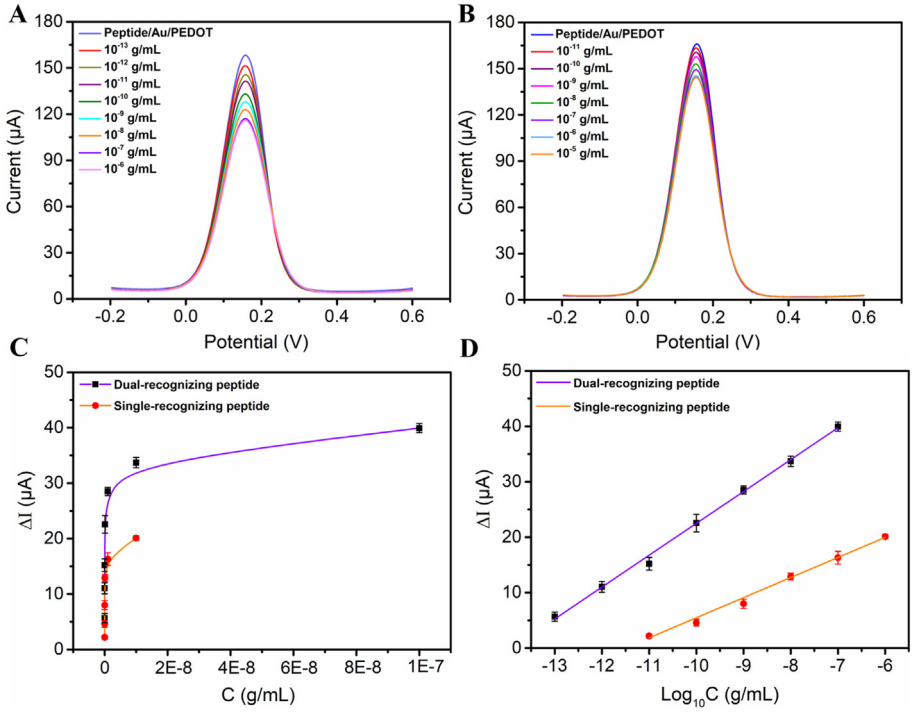


Fig. 3. DPV responses of the biosensors based on dual-recognizing peptide (A) and single-recognizing peptide (B) after incubation in different concentrations (V/V) of human IgG. (C) Corresponding response signal changes of the dual-recognizing and single-recognizing peptide-based biosensor. (D) Linear fitting curves of these two biosensors.

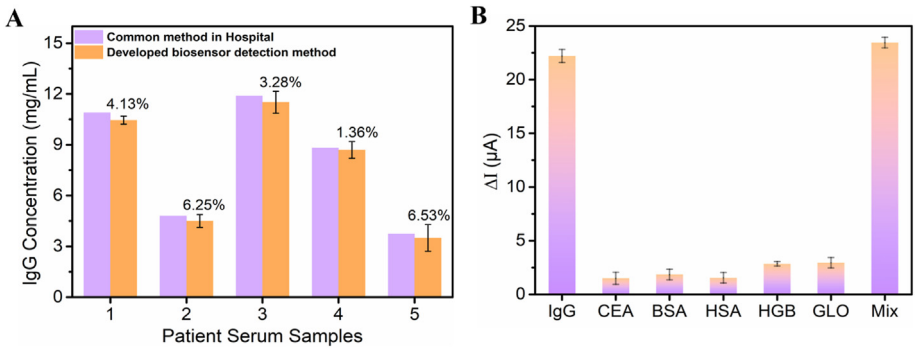


Fig. 4. (A) Results of testing clinical serum samples using our method (orange) and hospital turbidimetric method (lilac). (B) Current change (I_0—I) of the dual-recognizing peptide based-biosensor to IgG (0.1 ng/mL), and CEA (100 ng/mL), BSA (100 ng/mL), HSA (100 ng/mL), HGB (100 ng/mL), GLO (100 ng/mL) and their mixture (Mix), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.5. Real sample analysis with the biosensor

In order to verify the accuracy and applicability of the dual-recognizing peptide based-biosensor, five real human serum samples were tested, and the tested results were compared with those analyzed with the immunoturbidimetry method in the hospital. As can be seen from Fig. 4A, it is clear that the results obtained through our method were generally consistent with those measured by the immunoturbidimetry method commonly used in the hospital, which suggested that the dual-recognizing peptide-based biosensor was potentially useful for actual clinical monitoring.

3.6. Selectivity and stability of the biosensor

The selectivity of the dual-recognizing peptide based-biosensor was studied by using various interfering proteins such as CEA, BSA, HSA, HGB, GLO and their mixtures. The concentration of target IgG was 10^{-10} g/mL, and the concentrations of other interfering proteins were 10^{-7} g/mL. As shown in Fig. 4B, the obvious response signal was easily detected in the presence of the IgG, whereas low response signal was recorded in other interfering proteins, indicating the dual-recognizing peptide based-biosensor has outstanding selectivity for IgG detection.

In order to explore its stability, the prepared biosensor was immersed in the PBS containing $[Fe(CN)_6]^{3-/4-}$ for continuous CV test. As shown in Fig. S7, the signal was basically unchanged after 200 successive cycles, indicating good stability of the dual-recognizing peptide based-biosensor.

4. Conclusion

In summary, we have constructed an ultrasensitive and antifouling electrochemical biosensor based on designed multifunctional peptides with two recognizing branches specific for one target. The designed multifunctional peptides can resist the adsorption of nonspecific proteins, and greatly improve the sensitivity of the biosensor due to the synergistic binding effect of the two recognizing branches. Compared with the normal biosensor based on the single-recognizing peptide, the biosensor based on the peptide with dual-recognizing branches exhibited much wider linear range and lower LOD. Moreover, owing to the antifouling property of the designed peptide, the peptide-based biosensor was able to detect target IgG in complex human serum with high sensitivity and acceptable accuracy compared with the clinical method adopted from the hospital. The strategy for peptide design and biosensor construction provides a new thought for highly sensitive analysis of proteins in real complex biological samples.

CRediT authorship contribution statement

Yanxin Li: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization, Data curation. **Zhen Song:** Methodology, Writing – review & editing, Visualization. **Min Chen:** Conceptualization, Data curation, Writing – review & editing. **Zhenying Xu:** Resources, Data curation, Writing – review & editing. **Shuju Zhao:** Resources, Data curation, Writing – review & editing. **Yaqun Xu:** Writing – review & editing. **Xiliang Luo:** Conceptualization, Methodology, Investigation, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (21974075, 22174082), and the Taishan Scholar Program of Shandong Province of China (ts20110829).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2022.339841.

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