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Mixed Self-Assembled Aptamer and Newly Designed Zwitterionic Peptide as Antifouling Biosensing Interface for Electrochemical Detection of alpha-Fetoprotein

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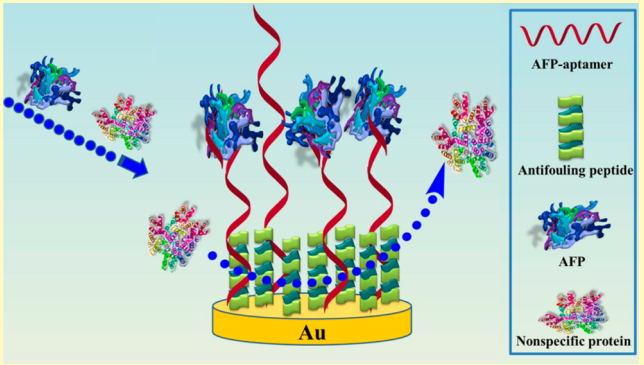
Supporting Information

ABSTRACT: A sensitive and low-fouling aptasensor for alpha-fetoprotein (AFP) was developed based on mixed self-assembled aptamers and newly designed zwitterionic peptides, where densely immobilized peptides formed an antifouling layer to resist nonspecific protein adsorption, and sparsely attached aptamers acted as the recognizing layer to achieve target binding. The obtained biosensing interface responded to the target AFP with a strikingly selective and sensitive manner, exhibited excellent protein-resistant performance even in complex human serum solution, and showed promising feasibility for the quantitative analysis of AFP in real human serum samples.



KEYWORDS: *mixed self-assembly, antifouling, zwitterionic peptide, aptamer, alpha-fetoprotein, electrochemical biosensor*

Aptamers, short and single-stranded oligonucleotides, are artificially synthesized and selected by SELEX (systematic evolution of ligands by exponential enrichment).[1] Aptamers can specifically bind with their preselected targets from small-sized nucleic acids to large-sized proteins and even whole cells with strong affinity.[2] Due to their considerable advantages of fast production, low cost, easy labeling, inherent selectivity, high stability, and reversible denaturation, as biorecognition elements, aptamers provide a feasible alternative in designing numerous types of biosensing platforms in a variety of fields such as analytical chemistry, biochemistry, and detection science.[3,4] Huang's group[5] screened the specific aptamers for alpha-fetoprotein (AFP), a biomarker of liver cancer, through an integrated microfluidic system, and confirmed their strong specific binding ability with AFP. Based on the screened AFP-specific aptamers, it is predictable that a great deal of biosensing platforms[5,6] can be directly developed for the quantitative analysis of AFP, which plays an important role in early diagnosis of cancer and assessment of treatment efficacy. Of the choice of available assays, electroanalytical techniques, with the characteristics of being simple, portable, cost-effective, disposable, selective, and sensitive, offer exceptional potential in reporting protein biomarker binding at as-prepared suitable surfaces.[7−9]

It is noteworthy that, although these biosensing platforms, directly grafting biorecognition elements such as AFP-aptamers to conventional substrates, have shown great achievements in analytical detection, the practical clinical application for these assays remains an extremely severe challenge, as they may suffer from highly nonspecific protein adsorption, which could produce a false positive response, decrease specificity and sensitivity of detection, and preclude measurement of a true kinetic rate,[10] especially in complex biological media, such as human serum solution and cell lysate.[11] So presently, to overcome this issue, many efforts have been carried out for the development of an antifouling surface based on antifouling materials[12−15] like poly(ethylene glycol) (PEG) polymer, zwitterionic polymer, and peptide. Among these antifouling materials, peptide has become a prospective candidate, because peptide is composed of natural amino acids, which are zwitterionic molecules in biological systems and possess inherently outstanding biocompatibility.[16] Besides superior biocompatibility and coordination ability, the remarkable antifouling property of some designed peptides, benefiting from their predominant hydrophilicity and charge neutrality,[15,17,18] conveniently extend their applications in the biomedical field. Nowadays, many antifouling peptides have

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been reported. For example, the sequence of SGKGSSGSST, in which glycine (G) was not hydrophilic but important for the formation of helical structure, was proven to be of excellent capability in preventing nonspecific protein adsorption by Chelmowski's group.[17] White and co-workers[19,20] have reported the capability of peptides with random lysine (K) and glutamic acid (E) or alternating poly EK in resisting nonspecific adsorptions. In compliance with the main design rules of hydrophilicity and uncharged,[17] a related peptide sequence of EESKSESKSGGGGC has been designed and its protein-resistance property has been explored through the development of a sensing interface in this work.

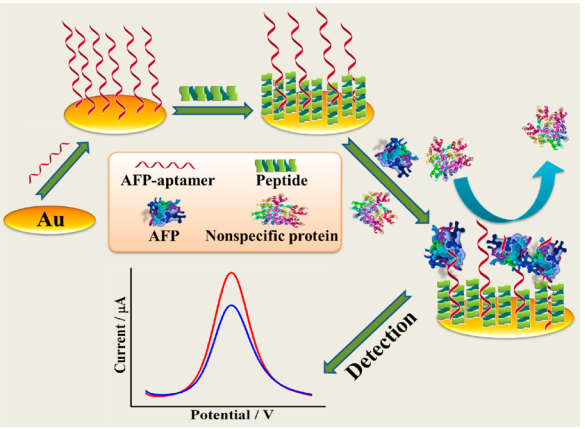
As stated earlier, the development of an antifouling biosensing interface has become the most desirable goal in clinical application, because it is effective in both reducing background interference and maintaining enough target binding ability.[21,22] Generally, one current manner of ordinary biosensing platforms, integrating both the recognition elements and the antifouling materials at the same layer, suffers the drawback of relatively low target loading, which further suppresses the sensing response, owing to the limited penetration of biomolecules in the highly packed brush film.[23] The other popular pattern is to graft the recognition probes to the antifouling materials directly, which can bring high target binding, but nevertheless can also cause large nonspecific adsorption, due to the very loose polymeric structure.[24] Recently, Jiang's group reported a two-layer architectural platform for low protein fouling and high target loading,[23] and Yu et al. proposed a generic concept of mixed self-assembled monolayers consisting of antifouling polymer and fructose probe.[25] Therefore, it can be desired that the mixed self-assembly with sparse recognition probes and dense antifouling materials may improve the sensitivity of analytical detection. Herein, we described the fabrication of a uniquely electrochemical aptasensor, based on mixed self-assembled AFP-specific aptamers and newly designed zwitterionic peptides (sequence of EESKSESKSGGGGC), for the sensitive and selective detection of AFP.

In brief, the long-chained AFP-specific aptamers which acted as the recognizing layer to achieve selective target binding and the newly designed short-chained zwitterionic peptides which formed an antifouling layer to resist nonspecific protein adsorption were immobilized successively onto the gold (Au) electrode surface as shown in Scheme 1. As the recognition elements, AFP-specific aptamers can bind with their target AFP with strong specificity and affinity, resulting in conformational changes of bound aptamers, which would play the key role in measurable electrochemical signals. Electroanalytical techniques with remarkable sensitivity were employed to investigate the sensing performance of developed aptasensor.

The newly designed zwitterionic peptide with the sequence of EESKSESKSGGGGC shows good hydrophilicity, and is nearly electrically neutral (Figure S-1), which endows the desirable antifouling ability. The detailed information about the peptide was provided in Supporting Information.

The surface modification process of the developed sensing strategy was characterized with electrochemical techniques of differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). The stepwise DPV response behaviors of the aptasensor were recorded (Figure S-2A). The DPV of the bare gold electrode with a sharp peak represents its typical curve in the presence of a redox mediator. Followed by the attachments of AFP-aptamers and peptides, a dramatic

Scheme 1. Schematic Illustration of the AFP Aptasensor Fabrication Protocol through the Stepwise Immobilizations of Long-Chained AFP-Specific Aptamers and Short-Chained Peptides onto Gold Electrode Surface via Self-Assembly



DPV drop occurred as the proof of peptides and aptamers covering the bare gold electrode surface. As a result of surface covering, the high charge-transfer capacity was hindered by the peptide and aptamer package step. Finally, along with the binding of AFP analytes to their specific aptamers, the DPV signal accordingly decreased. This occurrence manifested the strong interactions of target proteins and their specific aptamers that induced conformational changes[2,5] of the bound aptamers on the electrode surface. Conformational change based on the target-binding mechanism plays a key role in measurable electrochemical signals.[5] In this AFP-binding system, the conformational changes of aptamers led to enhanced resistance of charge-transfer, manifesting as the declining DPV signal response. Among the available electrochemical detection techniques, EIS is a dynamic tool for analyzing the information about the modified electrodes.[26] The Nyquist impedance plot creates a semicircle in diameter which is defined as the resistance of electron transfer (R_{ct}) of the electrode surface. The sensor fabrication process was also monitored using EIS (Figure S-2B). Undoubtedly, in contrast to that of bare gold electrode, an apparent augment of resistance (R_{ct}) to electron transfer of the AFP-aptamer/Peptide/Au was observed, indicating that the electrode modified with peptides and aptamers significantly prevented the electron transfer between the redox probe and the electrode surface. Subsequently, the R_{ct} further increased after the binding of AFP with AFP-aptamer/Peptide/Au, demonstrating lower charge transfer resulting from the conformation changes of bound aptamers. All EIS signals are consistent with DPV responses, which illustrated the successful development of AFP aptasensor with sensitive electrochemical signal changes.

To further characterize the modification of peptides and AFP-aptamers onto gold electrode (Au substrate) surfaces, X-ray photoelectron spectroscopy (XPS) was used to investigate the changes in chemistry of modified substrate surface (Figure S-2C and D). Figure S-2C showed the XPS spectra of a bare Au substrate, from which obvious Au 4f, C 1s, and O 1s peaks were observed. As expected, Figure S-2D certified the successful modification of peptides and aptamers, because of the appearances of P 2p peak (from aptamers), and S 2p and N 1s peaks (from both peptides and aptamers), accompanied by the lower Au 4f peak and higher C 1s and O 1s peaks resulting from the covering of aptamers and peptides onto Au surface.

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Consequently, peptides and AFP-aptamers had been densely assembled on the Au surface, providing capable protein resistance and target recognition elements for the constructed biosensor.

The hydrophilic ability of the newly designed peptide modified biosensing interfaces was evaluated with the static water contact angle assay[27] (Figure S-3 and Table S-1). The optimal aptasensor fabrication protocol was investigated through two different immobilization strategies (Figure S-4). According to the electrochemical signal change, the fabrication procedure concerning the stepwise immobilization of long-chained aptamers and short-chained peptides onto the gold electrode surface via self-assembly as shown in Scheme 1 was preferred.

The DPV technique was applied to monitor the binding of target AFP at the biosensing interface, initially in PBS (0.2 M, pH 7.4) solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. Specific target binding led to a DPV response decrease with AFP concentration increasing (Figure S-5). A more detailed analysis of the biosensor response to target AFP was shown in Figure 1A, which revealed that the DPV response

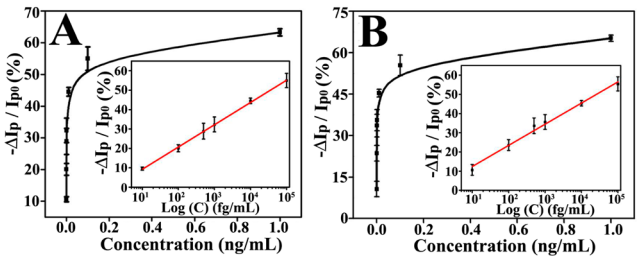

Figure 1. Dynamic response range and calibration curve (inset) of the aptasensor against AFP concentration in the absence (A) and presence (B) of 1.0 mg/mL HSA.

change ($-\Delta I_p/I_{p0}$) behaved as a good linear correlation (R^2 = 0.998) with the logarithmic value of AFP concentration, and a linear range from 10.0 fg/mL to 100.0 pg/mL. The regression equation was $-\Delta I_p/I_{p0}$ (%) = 11.52 log C (fg/mL) − 2.26, and the detection limit was calculated to be 3.1 fg/mL, which was lower than that of other antibody−antigen reports.[28,29] Evidently, such a biosensing interface exhibited superior target binding efficiency. The related analyses comfortably fulfilled the requirement of detection across the clinical application range, which was mainly ascribed to the incorporation of a biocompatible and antifouling supporting surface based on peptides, and a sensitive electrochemical DPV-based assessment of target binding.

Additionally, the target-binding performance of the fabricated biosensor was also measured in the presence of 1.0 mg/mL HSA. As is evident in Figure 1B, in this analysis solution, the response range toward AFP of the aptasensor (10.0 fg/mL to 100.0 pg/mL) is markedly consistent with that (Figure 1A) observed from previously mentioned PBS solution without HSA addition. The very close slope of the two analysis patterns (with and without the addition of HSA in measured solutions) indicated the excellent protein-resistance property of the peptide-based interface. The good protein-resistance capability provided powerful support for the practical clinical application of this biosensing system.

To further assess the performance in resisting nonspecific protein adsorption of these peptide-based biosensor interfaces, the DPV signal decrease (I_p decrease) of fabricated interfaces (AFP-aptamer/Peptide/Au) with different concentrations of HSA was analyzed, and observed to be strikingly negligible even to 5.0 mg/mL HSA (Figure 2B and Figure S-6), in contrast

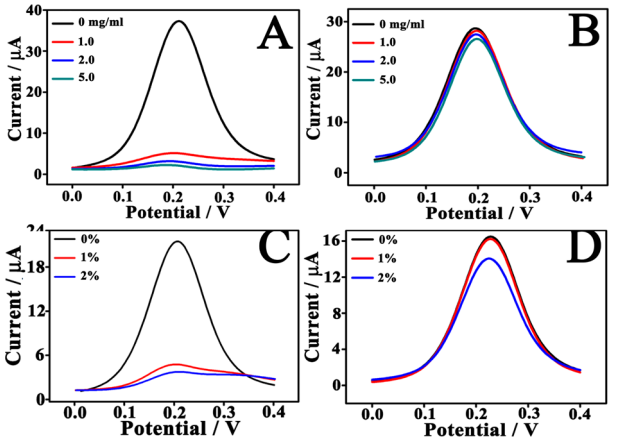

Figure 2. DPV responses of electrodes modified without (A, C) and with (B, D) peptides after immersions in different concentrations of HSA (0, 1.0, 2.0, 5.0 mg/mL) and human serum (0%, 1%, 2%) solutions.

with that of the sensing interfaces without peptide introductions of AFP-aptamer/Au (Figure 2A and Figure S-6). Moreover, in order to study the antifouling level of such fabricated interfaces within complex human serum samples (in 10 mM PBS, pH 7.4), which contain many positively and negatively charged proteins, the interfaces of AFP-aptamer/Peptide/Au (Figure 2C) (and interfaces without peptide introductions of AFP-aptamer/Au for control assays, Figure 2D), after AFP quantification in PBS, were presaturated prior to analysis. As described in Figure S-6, after serum immersions (30 min), the decreases of DPV response of presaturated AFP-aptamer/Peptide/Au surfaces were significantly less than those of presaturated AFP-aptamer/Au surfaces, evidencing excellent antifouling capability of the peptide-based interface in serum samples (although the biosensor only shows satisfying antifouling ability in 1−2% serum, which is not as high as other reports,[30] it is still valuable as it can be used for real serum assay after simple dilution). Such low fouling capability, ascribed to the prominent hydrophilicity and remarkable charge neutrality of the newly designed zwitterionic peptides, would be a great improvement for the clinical applications of various biosensors.

The direct and selective detection of disease biomarkers with the presence of a large excess of nonspecific ingredients (proteins and nucleic acids) is exceedingly in demand for various nonamplified biosensing systems. To test the interfacial specificity, the prepared aptasensors were suspended in solutions containing different concentrations of human serum albumin (HSA), γ-globulin from bovine blood (GLO), hemoglobin (HGB), single-strand DNA, and target AFP, separately. The sensor responses were summarized in Figure 3A, which displayed the advanced specificity of the constructed aptasensor, because such interfacial responses toward other ingredients, even in higher concentration, were negligible. Hence, the aptasensors possess excellent selectivity toward target AFP, attributed to the effective ability in preventing nonspecific protein adsorption and the satisfying performance

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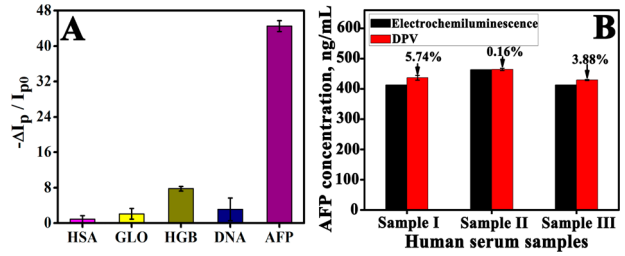

Figure 3. (A) Responses of the AFP aptasensor to 1.0 μg/mL HSA, GLO, and HGB; 1.0 nM DNA; and 10.0 pg/mL AFP. (B) Representative comparative real human serum sample assays with the developed aptasensor and a clinically approved electrochemiluminescence assay.

in the enhanced target binding of the constructed biosensing system.

The practical application of this constructed aptasensor for the quantification analysis of AFP in a series of clinic serum samples from pregnant women (AFP concentrations were high and known, and the serums were diluted with PBS before DPV measurements) was summarized in Figure 3B. Considering the high AFP concentrations of the serum samples, the antifouling property (Figure 2), and the analytical performance toward AFP of the constructed aptasensor (Figure 1), the obtained human serum samples were initially diluted to 1/10 000 (V/V) using PBS (10 mM, pH 7.4). The results indicated that the aptasensors for AFP detection were well behaved, and matched well with the quantification of the same serum samples through a clinical standard Roche electrochemiluminescence assay (differences are within 6.0%). The results revealed the potential practical applicability of such constructed aptasensors.

In conclusion, we present herein a uniquely selective and sensitive electrochemical aptasensor, with a sensing interface based on a dense antifouling layer of newly designed short-chained peptides to resist nonspecific protein adsorption, and a sparse recognition layer of long-chained AFP-specific aptamers to achieve selective target binding. Combining the effective antifouling characteristic of the newly designed peptides, the outstanding specificity of the AFP aptamers, and the high sensitivity of electroanalytical technique, the constructed biosensor was capable of detecting the tumor biomarker AFP across its clinically relevant range with high sensitivity and low fouling. Moreover, the fabricated aptasensor exhibited promising feasibility for the quantification analysis of AFP in real human serum samples. It is believed that the remarkable specificity incorporated with high antifouling property during the development of the analytical assay will improve convenience for early diagnosis and clinical therapy of human disease.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.7b00103.

Experimental section, information on the newly designed peptide, aptasensor fabrication protocol, and additional data (PDF)

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Notes

The authors declare no competing financial interest.

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