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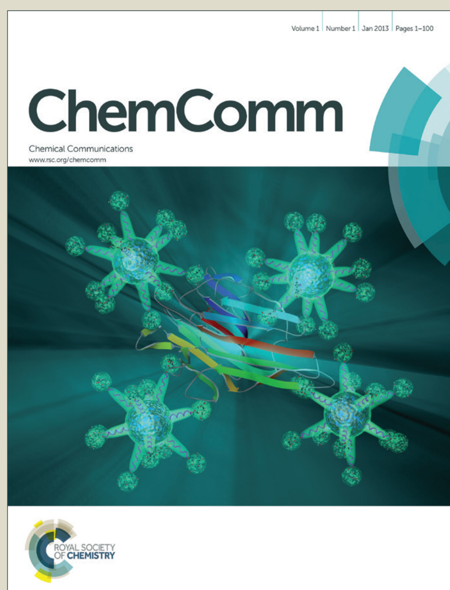

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ARTICLE TYPE

An anti-fouling aptasensor for detection of thrombin by dual polarization interferometry

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An anti-fouling surface was designed to effectively resist nonspecific protein adsorption using dual polarization interferometry, based on which the aptasensor for detection of thrombin was fabricated according to the specific interaction between thrombin and its 15-mer aptamer.

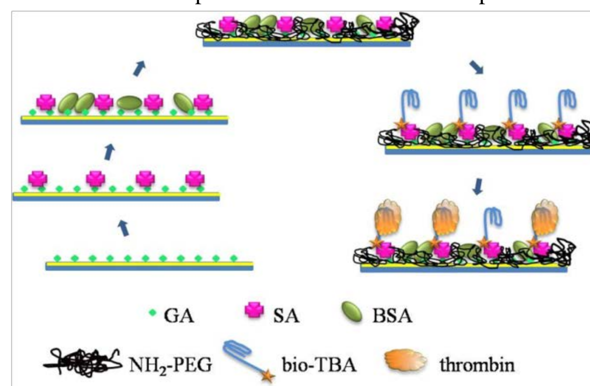
Biochips are substantially miniaturized laboratories that can perform biomolecular reactions. It has been reported that numerous proteins have been sensitively determined through specific interactions with molecules immobilized on the biochip surfaces[1]. Besides, biochips have also been widely used for drug screening, disease diagnosing and therapy[2]. However, when chips were applied for protein-related experiments, a most serious problem was nonspecific protein adsorption, which would increase the background noise, block active sites and decrease the detection efficiency and sensitivity. Moreover, nonspecific protein adsorption on surfaces of medical chips might induce the conformational changes of effective proteins, thus affected the chip functions[3]. Consequently, nonspecific protein adsorption must be minimized when chips were utilized for bioassays, biosensing or drug developments[4].

The nonspecific adsorption of proteins was mainly caused by hydrophobic interactions and electrostatic interactions, thus through inhibiting these two factors the nonspecific adsorption could be resisted effectively[5]. Polyethylene glycol (PEG) has been proved to be a good choice for reducing or eliminating nonspecific protein adsorption, which was mostly attributed to its hydrophilicity, electric neutrality, high mobility and flexibility[6]. In recent years, the surface modification with PEG to fabricate anti-fouling surfaces has attracted more and more attention[7]. Wolter prepared PEG surfaces for protein microarrays to gain high signal-to-noise ratios[7b]. Chu modified PEG on DNA chip surfaces to improve resistance to protein adsorption[7g]. Besides, incorporation of hexa (ethylene glycol) segments into DNA linkers, which were used to provide chemical spacers between surfaces and receptors, were also reported to reduce nonspecific adsorption[8].

Dual polarization interferometry (DPI) is an effective and sensitive analytical tool for real-time and quantitative analysis of adsorption of biomolecules on interfaces or to study biomolecular interactions[9]. Every slight change in the solution would result in the thickness, mass and density changes of the chip surface, thus, not only could DPI give out the signal response, but also it could reveal structural information. An explanation of this technique could be found in ESI. Chips of DPI were made of silicon oxynitride, which was regarded as a favorable material for producing DNA biochips owing to its thermodynamic stability and chemical stability[10]. Substances such as ethanolamine[11], tris (hydroxymethyl) aminomethane (Tris)[12] and PEG[13] have been reported to block silicon chips, among which the effect of PEG was the best[13]. However, ethanolamine[14], Tris[15], bovine serum albumin (BSA)[16] have ever been used in DPI experiments to block chip surfaces, whereas there was no report on employing PEG to reduce nonspecfic protein adsorption on DPI chips.

Aptamers are oligonucleotides that have high selectivity and affinity for various target molecules such as dyes, metal ions and proteins[17]. The protein recognition mode of aptamer is similar to that of antibody, thus, in recent years, more and more aptamers have been utilized to detect proteins instead of antibodies[18]. Thrombin is the key factor of blood coagulation, whose activity could be influenced obviously when bodies hurt and blood coagulation systems are disordered. Detecting the thrombin level will help to judge the coagulation ability of blood and prevent diseases such as thrombus. It is reported that 15-mer aptamer for thrombin (TBA, 5'-GGT TGG TGT GGT TGG-3') has been approved widely since it was selected[19]. Consequently, herein the interaction of TBA with thrombin was chosen as a model system to study the capability of resistance to nonspecific protein adsorption of the anti-fouling surface we designed and to fabricate thrombin aptasensor using DPI.

Scheme 1 displays the whole process of functional assembly and the mechanism of detecting thrombin. Streptavidin was first linked onto the chip surface which had been pretreated with



Scheme 1 Fabrication of aptasensing interface and detection of thrombin using DPI technique.

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glutaraldehyde. Then, BSA and PEG were used respectively to block surfaces for fabricating anti-fouling surfaces. After that, biotin-modified TBA was immobilized onto the surface through the specific interaction between biotin and streptavidin to form the probe layer. For comparison purpose, another 15-mer oligonucleotide with a random sequence (RAN, 5'-GGT GGT TGT TGT GGT-3') was chosen as a control DNA. The DNA sequences used in these experiments were listed in Table S1. Subsequently, DPI was utilized to measure the interaction of thrombin with TBA in real time and detect thrombin accordingly. The addition of thrombin to the TBA immobilized surface greatly interferes with the chip surface due to the thrombin-binding-induced TBA conformational change from random coil structure to G-quadruplex structure. Thus, the binding of thrombin onto the surface could be reflected by the changes of the surface layer thickness, density and mass given by DPI.

Fig. S1 exhibits the real-time measurements of the mass, thickness and density value changes during the whole immobilization process. The changes of the layer thickness and density values were plotted against the changes of layer mass values to more clearly display the extent of changes of each layer, which is shown in Fig.S2. As could be noted from Fig. S1 and Fig. S2, upon the injection of each material, the initial immobilization rate was very fast, during which the thickness and mass values increased sharply. Then the immobilization rate slowed down and reached a plateau, embodied by the stability of the mass, thichkness and density values of each layer, indicating that the immobilization of each material was successful and reached equilibrium. Table S2 presented the detailed parameters (mass, thickness and density) for each immobilized layer. The mass loading of SA was 0.610 ng/mm^2, corresponding to 5.5×10^9 molecule/mm^2. The mass loading of TBA and RAN were 0.055 ng/mm^2 and 0.052 ng/mm^2, respectively, corresponding to 5×10^9 molecule/mm^2 and 4.8×10^9 molecule/mm^2. The calculating data indicated that approximately one SA molecule bound with one DNA molecule, suggesting that TBA or RAN was sparsely immobilized on the surface, thus interacting with thrombin flexibly.

To study the capability of resistance to nonspecific protein adsorption of the anti-fouling surface we designed, thrombin was injected to layers blocked with both BSA and PEG. From Fig. 1, it could be observed that when the layer was modified with EG-T_5-TBA, the mass and thickness values increased quickly while the density value decreased accordingly at the beginning, then after slight fluctuation, these parameters reached stable values, indicating that quite a lot of thrombin was bound onto the layer. However, when the layer was not modified with EG-T_5-TBA, mass, thickness and density values only underwent little change initially and then backed to the baseline, indicating that almost no thrombin was captured onto the layer. We defined net amount as amount of thrombin bound on aptamer-modified layer minus that on corresponding layer without aptamer. Since the only difference between the two layers was the modification of EG-T_5-TBA, thus, the net amount denoted the specific binding part of thrombin with layers. It was proved that thrombin specifically interacted with its aptamer and there was no adsorption of thrombin on other portion of the layer blocked with both BSA and PEG, suggesting that this designed anti-fouling surface effectively resisted nonspecific protein adsorption.

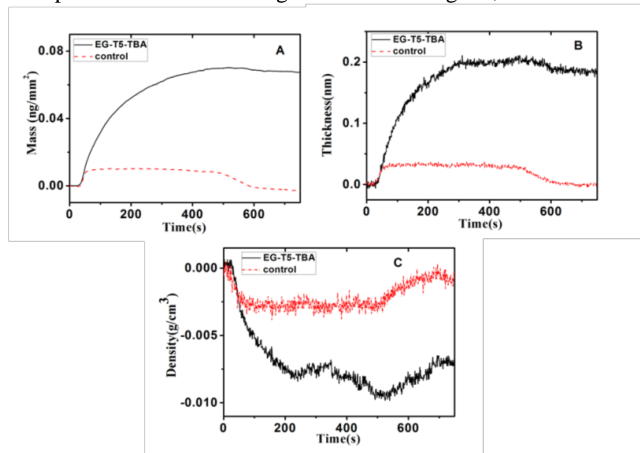

Fig. 1 Real-time changes of mass (A), thickness (B) and density (C) values upon 200 μL 100 nM thrombin injection into chip layers modified with (black line) and without (red line) EG-T_5-TBA.

Chip surfaces blocked with either BSA or PEG were also constructed and compared with the surfaces blocked with both BSA and PEG. As shown in Fig. S3, the nonspecific protein adsorption on surface blocked with PEG was still much and clearly observed, resulting in the amount of thrombin bound on surface blocked with PEG more than that on surface blocked with both BSA and PEG. However, the net amount on surface blocked with both BSA and PEG was more than that on surface blocked with PEG. From Fig. S4, it can be noted that the nonspecific protein adsorption on surface blocked with BSA was as little as that on surface blocked with both BSA and PEG, suggesting similar resistance to nonspecific adsorption. Whereas, the net amount on surface blocked with both BSA and PEG was more than that on surface blocked with BSA. Besides, the sensitivity of surface blocked with both was higher than those blocked with either. Consequently, it was concluded that the layer surface blocked with both BSA and PEG was superior to that blocked with either in resisting nonspecific protein adsorption.

EG-T_5-TBA incorporated two linkers of T_5 and hexa (ethylene oxide) units. As can be noticed in Fig. S5, compared to thrombin aptamer with or without either linker, EG-T_5-TBA provided the highest sensitivity. This might be because that T_5 and hexa (ethylene oxide) units increased the distance between aptamers and layer surfaces, which improved the flexibility and freedom of aptamers, effectively reduced the steric hindrance of thrombin binding to aptamers. Hence, EG-T_5-TBA was chosen to fabricate thrombin aptasensor on layer surfaces blocked with both BSA and PEG.

Thrombin with different concentrations ranging from 1 nM to 500 nM were injected independently onto the EG-T_5-TBA layer. The corresponding real-time mass, thickness and density value changes were shown in Fig. S6. As the concentration of thrombin increased, the changes of mass, thickness and density values increased accordingly. The changes of mass and thickness values at 750 s were selected to be plotted against the concentration of thrombin. As is observed in Fig. S7, the mass increase was linearly related to the concentration of thrombin ranging from 2.5 nM to 250 nM while the thickness increase was linearly related to the concentration of thrombin ranging from 2.5 nM to 100 nM.

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The linear equation could be fitted as Mass (ng/mm^2) = -0.0015 + 0.00063c (THR, nM) (R^2 = 0.991) and Thickness (nm) = 0.0033 + 0.00194c (THR, nM) (R^2 = 0.999), respectively. The limits of detection (3σ) were 0.5 nM for mass calibration and 0.1 nM for thickness calibration, which was much lower than many reported methods[20].

To further demonstrate the specific interaction between thrombin and its aptamer, control experiments were performed using different RANs instead of the aptamer. As can be noted from Fig. S8, RAN layers underwent little mass and thickness value changes after the addition of thrombin, indicating the high specific binding of thrombin towards its aptamer. Then, other proteins such as lysozyme, casein, BSA and their mixture with thrombin were investigated to study the selectivity of this aptasensor. As shown in Fig. S9, the mass and thickness value changes upon the addition of these proteins were much less than that of thrombin. Furthermore, the changes of mass and thickness values of their mixture with thrombin were in accord with that of thrombin alone, suggesting the high selectivity of this aptasensor towards thrombin.

Conclusions

In summary, BSA and PEG were employed cooperatively to block the surface of DPI chip made of silicon, which has not been reported ever and successfully eliminated the nonspecific protein adsorption. Meanwhile, thrombin aptasensor was fabricated based on the anti-fouling surface using DPI. This aptasensor had a high specificity and selectivity towards thrombin, besides, the detection limit was rather low due to the high sensitivity of this method. It is expected that the strategy for fabricating anti-fouling surfaces can be employed in biochips and the aptasensor strategy can be expanded to detect other target molecules by using their corresponding aptamers.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Explanation on DPI instrument and principle, experimental details, Fig. S1–S9, Table S1 and Table S2]. See DOI: 10.1039/b000000x/

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