

Oriented Antibody Immobilization and Immunoassay Based on Boronic Acid-containing Polymer Brush

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Electronic Supplementary Information

Abstract High sensitive immunoassay platforms have gained intense attention due to their vital roles in early-stage disease diagnosis and therapeutic information feedback. Although random covalent-binding of antibody has been widely adopted in immunoassays due to its simplicity and effectiveness, it readily loses its activity and fails to exhibit high antigen-binding capacity. In this work, copolymer of zwitterionic sulfobetaine methacrylate (SBMA) and glycidyl methacrylate (GMA) brushes were immobilized onto inert polypropylene (PP) *via* surface-initiated atom transfer radical polymerization (ATRP) based on biomimetic dopamine pretreatment. Subsequently, boronic acid (BA) groups were covalently bonded *via* active GMA units, followed by the introduction of oriented immobilization of antibody. Owing to the oriented immobilization of antibody facilitated by BA groups in polymer brush, the bioactivity of antibody is well preserved, which endows the surface with significantly enhanced antigen-binding capacity. Moreover, the existence of SBMA segments in polymer brushes renders the surface high resistance to nonspecific protein adsorption, significantly alleviating the signal interference of antigen recognition. This strategy could find potential applications in developing high sensitive immunoassay platforms based on the different substrates.

Keywords Biomimetic dopamine; Atom transfer radical polymerization (ATRP); Boronic acid (BA); Immunoassay; Biomimetic

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INTRODUCTION

Detection of biomarkers with high sensitivity has attracted great attention in past decades due to its significant roles in disease detection, environment/food monitoring and therapeutic information feedback^[1]. To date, enzyme-linked immunosorbent assay (ELISA) is still a popular and powerful immunoassay method in clinical biomarkers detection^[2, 3]. However, several issues associated with ELISA, *e.g.*, anti-bioadhesion performances, antibody bioactivity and immobilization strategies, are still challenges for sensitive recognition of targets.

In the immunoassay, antibodies are often immobilized on a solid surface to specifically recognize their corresponding antigens under a complicated condition. Hence, the accessibility of antigen-binding domain (Fab) of antibody and the stability of immobilized antibody are influenced significantly by the orientation of antibody on the surface. The results indicate that a proper antibody immobilization strategy is vital to enhance the interaction between antibody and its antigen to acquire high detection efficiency^[4, 5]. Currently, various immobilization strategies of antibody have

been developed. Chemical covalent immobilization, as one of the most commonly used methods, has been widely adopted due to its effectiveness, stability and repeatability. However, the random antibody immobilization readily causes the reduction in antibody bioactivity^[6]. Alternatively, the site-specific conjunction of antibody is more preferable since it facilitates a more homogeneous surface coverage and exhibited higher antigen-binding capacity^[7, 8]. Most of the non-covalent strategies generally rely on using antibody-binding proteins (protein A/G) to specific binding the fragment crystallizable region (Fc) of antibody^[9]. In spite of the efficiency in optimal exposure of Fab, the non-covalent immobilization lacks the control over the immobilization of protein A/G themselves. In comparison, the immobilization of antibody based on chemical/enzymic oxidations of carbohydrate moieties located at Fc region has shown to provide better accessibility of Fab sites, but easily causes the loss of antibody bioactivity^[10, 11]. Boronic acids (BAs) are well-known for their ability to form a stable but reversible cyclic ester structure with *cis*-1,2-diols and 1,3-diols of saccharide in aqueous media at room temperature^[12–14]. Benefited by the carbohydrate moiety at Fc domain and mild incubation conditions, the BA-based immobilization strategy showed great advantages in

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fabricating the uniform and optimal antibody-bonded surface in a modification-free manner^[15].

On the other side, owing to the abundant proteins existing in blood plasma or body fluid, it remains challenging to suppress nonspecific protein adsorption, which might cause reduction of interference signal as well as antigen recognition efficiency. To this end, modifying the antibody-bonded surface with various hydrophilic polymers, such as poly(ethylene oxide) (PEG)^[16], poly(*N*-vinyl pyrrolidone)^[17], zwitterionic polymers^[18–20], is considered as a highly effective way to resist nonspecific protein adsorption, primarily due to the formation of a stable hydrated layer. Among these, antifouling zwitterionic sulfobetaine (SB) polymers have received much attention due to their high stability in physiological conditions, low toxicity and favorable hemocompatibility^[21]. Therefore, the poly(SB)-based surfaces can exhibit excellent nonspecific protein adsorption resistance but avoid the risk of coagulation in blood plasma. As mentioned in our previous reports^[22], polypropylene (PP), as one of the promising medical materials, has many desirable properties such as good mechanical property, convenient processability and chemical stability, low cost, *etc.* However, the inert nature of PP limits its broad applications, particularly, for those in which the active groups are needed to conduct further surface modification. Recently, mussel-inspired polydopamine

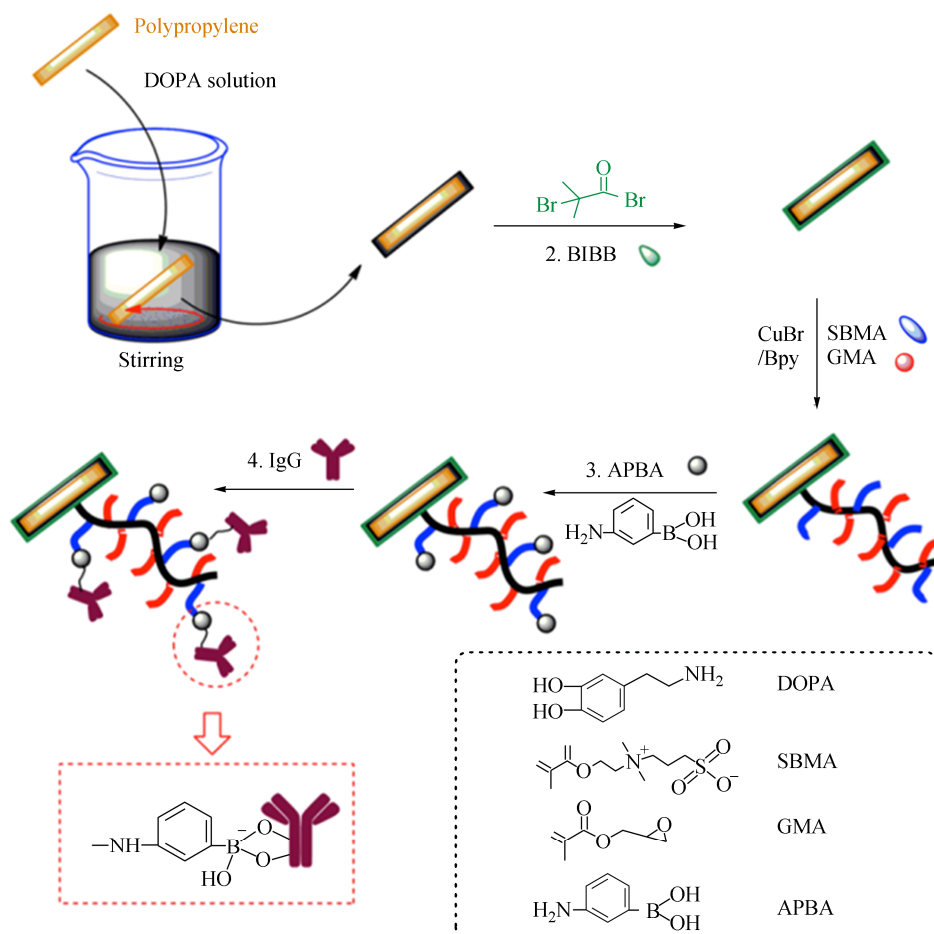
(PDA) has been used as a versatile and intriguing starting material for surface modification under mild conditions, and the adhesive mechanisms and unique properties of dopamine (DOPA) have been widely reported^[23, 24].

In this work, the DOPA was adopted to build an active layer on inert PP films to get support substrates. Copolymer brushes containing dual functional components, poly(sulfobetaine methacrylate) (pSBMA) and poly(glycidyl methacrylate) (pGMA) were co-grafted on the DOPA modified PP surface *via* surface-initiated atom transfer radical polymerization (SI-ATRP). After that, aminophenylboronic acid was introduced on the polymeric brushes *via* the reaction between its amine and the epoxy groups in pGMA, facilitating the surface specific oriented immobilization of antibody (Scheme 1). In the end, the nonspecific protein adsorption resistance, bioassay, and antibody dissociation on the prepared surfaces were systematically investigated.

EXPERIMENTAL

Materials

3-[Dimethyl-[2-(2-methylprop-2-enoyloxy) ethyl] azaniumyl] propane-1-sulfonate (SBMA), dopamine (DOPA), glycidyl methacrylate (GMA), 3-aminophenylboronic acid (APBA), copper(I) bromide (CuBr), 2-bromopropionyl-bromide (BIBB), and trihydroxymethyl aminomethane (Tris) were



Scheme 1 Fabrication scheme of nonspecific protein resistant surface with oriented immobilization of IgG property

purchased from Sigma-Aldrich. 2,2'-Bipyridine (Bpy) was obtained from Alfa-Aesar. Phosphate buffered saline (PBS, pH = 7.4) was supplied by Dingguo Biology. All proteins with or without fluorescence labeled (fluorescein isothiocyanate, FITC) including bovine serum albumin (BSA), goat to rabbit IgG (Go-to-Ra IgG), rabbit IgG, mouse to goat IgG (Mo-to-Go IgG) were provided by Bioss Biotechnology. GMA was passed through a basic alumina column to remove inhibitors. Solvents such as dichloromethane (DCM) and triethylamine (TEA) and toluene were immediately subjected to distillation in the presence of drying agents before use. Other reagents were AR grade and used as received.

Immobilization of SI-ATRP Initiator

The PP films (2.0 cm × 2.0 cm) were sonicated consecutively in acetone and ethanol for 30 min, followed by air blow-drying. The dried films were then activated by oxygen plasma at room temperature under a pressure of ~15 Pa. (DT-03 Plasma apparatus, Suzhou Omega Technology Co., Ltd.). The O₂ plasma pretreated films were immersed in 4 mL of Tris-HCl solution (pH = 8.5) containing DOPA (2 mg/mL) overnight. Then the films were rinsed with deionized water and dried in vacuum oven to get the PP-DOPA sample. For immobilization of ATRP initiators, the PP-DOPA samples were placed in 2 mL of anhydrous DCM solution containing TEA (10 vol%) in an ice/water bath for 30 min. When 1.2 mL of BIBB was added, the mixture was stirred for 4 h at room temperature, washed with ethanol to produce the PP-DOPA-BIBB samples.

Preparation of Polymeric Brush Based PP Film

Random polymer brushes on PP film were made as follows. Briefly, SBMA (7 mmol), Bpy (2 mmol) and 15 mL of DI water-methanol mixture (1/1, *V/V*) were added to a Schlenk flask containing PP-DOPA-BIBB. The mixture was degassed three times by freeze-pump-thaw cycles. Both CuBr (1 mmol) and CuBr₂ (0.2 mmol) were introduced into the container and the reaction was carried out under nitrogen atmosphere at 40 °C for 6 h. After being sequentially washed with methanol and ultrapure water, the resultant samples were obtained (referred to as PP-g-pSBMA). Similarly, different copolymer brushes-based samples with varied feed ratios were fabricated and denoted as PP-g-pGMA, PP-g-p(SBMA-*co*-GMA)_{1:1} and PP-g-p(SBMA-*co*-GMA)_{10:1}, respectively.

Oriented Immobilization of Primary Antibody

Firstly, APBA was introduced on the polymer brushes by the reaction between epoxy groups and amine groups. Briefly, the as-prepared samples were first immersed in 10 mL of DMF solution containing APBA (20 mg/mL), then the reaction was conducted at 50 °C for 24 h, followed by transferring the samples into ethanolamine aqueous solution (0.5 mol/L) for 2 h to completely eliminate the residual epoxy groups to get APBA-modified samples (PP-g-pGMA-APBA, PP-g-p(SBMA-*co*-GMA)_{1:1}-APBA, PP-g-p(SBMA-*co*-GMA)_{10:1}-APBA, respectively). To realize the orientation immobilization of primary antibody, the APBA-modified samples were incubated in Tris-HCl solution (pH = 8.5) for 2 h, and then transferred into Go-to-Ra IgG primary antibody

aqueous solutions (10, 20, 50, 100, 200 and 500 ng/mL) at 4 °C for 24 h. Finally, the resultant samples were obtained after being rinsed with Tris-HCl solution and ultrapure water to remove physically adsorbed antibodies, referring as PP-g-pGMA-APBA-IgG, PP-g-p(SBMA-*co*-GMA)_{1:1}-APBA-IgG and PP-g-p(SBMA-*co*-GMA)_{10:1}-APBA-IgG, respectively. To evaluate the antibody immobilization efficiency, the aforementioned IgG-immobilized samples were firstly blocked with BSA and dextran solution (1 mg/mL) at room temperature for 2 h. Then they were incubated with PBS buffer containing secondary antibody (FITC labeled mouse-to-goat IgG, 20 µg/mL) at 4 °C for 24 h, followed by rinsing, drying and evaluating by fluorescence intensity scanning.

Antigen Recognition

The specific IgG-immobilized surface was incubated with Go-to-Ra IgG solution (200 µg/mL), rinsed with Tris-HCl buffer and ultrapure water, and then incubated with FITC-labeled rabbit IgG antigen solutions with different concentrations (0.1 ng/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, 10 µg/mL and 100 µg/mL, PBS) at 4 °C for 24 h. After being washed by plenty of PBS solution and ultrapure water, the surface recognition capacities of the rabbit IgG antigen were examined by fluorescence intensity scanning.

Primary Antibody Dissociation

To prepare the IgG-immobilized surfaces, APBA modified samples were incubated with Rhodamine B isothiocyanate (RBITC)-labeled Go-to-Ra IgG solution (20 µg/mL), followed by washing with PBST solution (Tween-20, 0.05 g/mL). The samples were incubated with dissociation solution (citrate-phosphate buffer, pH = 3) for a certain period. After being thoroughly washed and dried, the samples were examined by confocal laser scanning microscopy (CLSM) to evaluate their antibody dissociation capacities.

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy

ATR-FTIR curves of the samples were obtained by Bruker Vertex 70 Fourier transform infrared spectroscopy equipped with an attenuated total reflection (ATR) unit (crystal 45°) at a resolution of 4 cm⁻¹ for 32 scans.

Nonspecific Protein Adsorption Evaluation

IgG-functionalized samples were pre-equilibrated in PBS solution for 2 h, then soaked in FITC-BSA-containing PBS solution (200 µg/mL) at 4 °C for 12 h. After that, the samples were rinsed with PBS buffer, dried by air flow and examined by CLSM.

Surface Chemical Composition

X-ray photoelectron spectrometer with Al/Kα (*hν* = 1486.6 eV) anode mono-X-ray source (XPS, VG Scientific ESCA MK II Thermo Advantage V 3.20 analyzer) was used to examine surface elemental compositions of all samples at the detection angle of 90°. All spectra over a range of 0–1200 eV, and high-resolution spectra of C1s, N1s, O1s, B1s, Br3d and S2p regions were collected. The relative atomic concentration of chemical elements was calculated from their related peak areas.

Surface Wettability

Static water contact angle was measured by the drop shape analysis instrument (DSA, KRÜSS GMBH, Hamburg 100) at room temperature. For each sample, 2 μL of water droplet was added and at least five contact angle measurements were made for calculating the average value.

Fluorescence Intensity Scanning and Data Analysis

Fluorescent images of the samples were collected by confocal laser scanning microscope (Zeiss, LSM 700). Target biomolecules labeled with FITC and RBITC were respectively excited by an argon ion laser at 488 and 555 nm. In order to obtain fluorescence intensity, original fluorescent images were analyzed using Image Pro software. Fluorescence intensity value was measured from six different positions of each fluorescence image.

RESULTS AND DISCUSSION

Surface Characteristics

ATR-FTIR spectra were employed to distinguish the functional groups of the samples (Fig. 1). Compared with virgin PP (Fig. 1a), the two new peaks at $\sim 3370\text{ cm}^{-1}$ (N—H/O—H stretching vibrations) and $\sim 1604\text{ cm}^{-1}$ (the overlap of C=C resonance vibrations in aromatic ring and N—H bending vibrations) appeared on the spectrum of PP-DOPA (Fig. 1b)^[25]. Since the linked DOPA layers in PP-DOPA can offer abundant bioactive groups to anchor the ATRP initiator BIBB, a new shoulder at $\sim 1758\text{ cm}^{-1}$ appeared, which was assigned to the carboxyl groups in BIBB (Fig. 1c).

After grafting of pGMA and pSBMA, the enhanced peak at 1727 cm^{-1} was apparently observed on a series of surfaces modified with GMA and SBMA in varied proportions (Figs. 1d–1g), attributed to the stretching of carboxyl both in GMA and SBMA. Meanwhile, the peaks at ~ 1035 and $\sim 1189\text{ cm}^{-1}$, belonging to the stretching of —S—O— and SO_3^- , evidently confirmed the existence of pSBMA on the surface when compared with that of the PP-g-pGMA film.

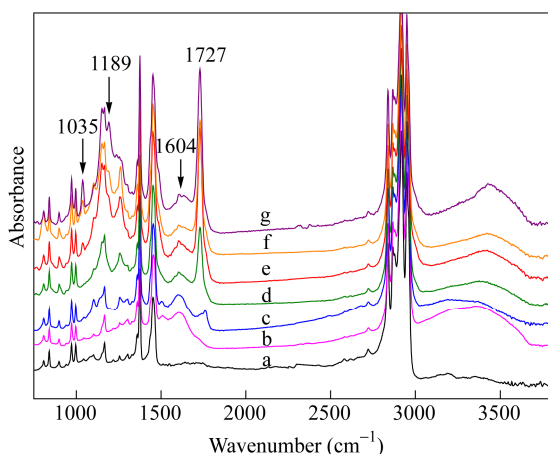


Fig. 1 ATR-FTIR spectra of PP film before and after modification: (a) virgin PP, (b) PP-DOPA, (c) PP-DOPA-BIBB, (d) PP-g-pGMA, (e) PP-g-p(SBMA-co-GMA)_{1:1}, (f) PP-g-p(SBMA-co-GMA)_{10:1} and (g) PP-g-pSBMA

Moreover, XPS was also used to confirm chemical compositions of the modified surfaces (Fig. 2 and Table S1, in electronic supplementary information, ESI). The strong N1s peak at 399.2 eV (11.33%) was observed on the sample of PP-DOPA. The successful immobilization of BIBB was confirmed by the appearance of Br peak at 66.3 eV (4.88%). After ATRP reaction, strong S2p peaks at 168.1 eV were present on the PP-g-p(SBMA-co-GMA)_{1:1}, PP-g-p(SBMA-co-GMA)_{10:1} and PP-g-pSBMA surfaces, which showed obvious difference from that of PP-g-pGMA. The S2p contents in XPS spectra were consistent with the monomer feed ratios of [SBMA]/[GMA]. Increasing SBMA monomer contents gradually increased the S element contents from 6.88% to 10.50% and 11.28% (Fig. S1, in ESI). All these results evidently confirmed the successful fabrication of polymeric brushes on substrate surfaces.

Water contact angle (WCA) measurement was used to assess surface wettability. The changes of surface wettability associated with corresponding surface modification were recorded. As shown in Fig. 3(A), the virgin PP surface showed a WCA of $\sim 108^\circ$ due to its hydrophobic nature. The sequential introduction of DOPA and BIBB caused apparent decreases in WCA ($\sim 67^\circ$ for PP-DOPA and $\sim 90^\circ$ for

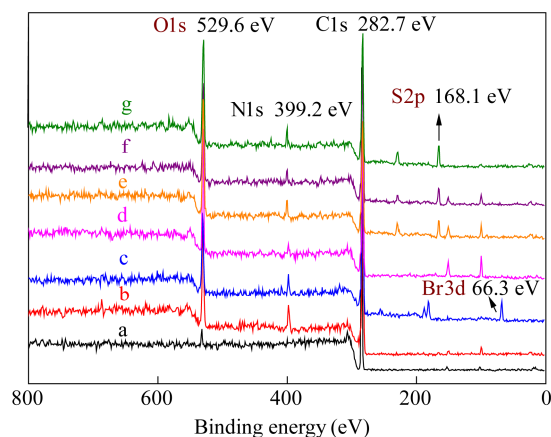


Fig. 2 XPS survey spectra of (a) virgin PP, (b) PP-DOPA, (c) PP-DOPA-BIBB, (d) PP-g-pGMA, (e) PP-g-p(SBMA-co-GMA)_{1:1}, (f) PP-g-p(SBMA-co-GMA)_{10:1} and (g) PP-g-pSBMA

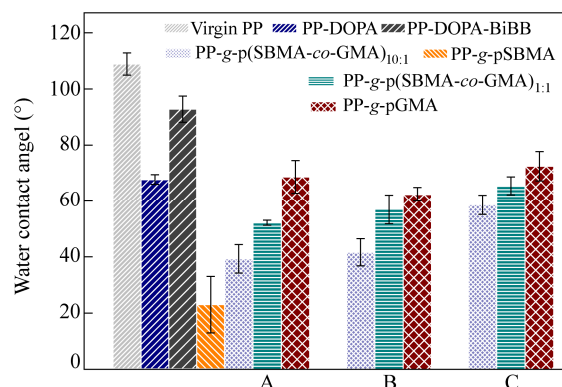


Fig. 3 Water contact angles of the samples after surface modifications of (A) initiator immobilization and SI-ATRP polymerization, (B) APBA binding, (C) IgG loading (The error bars: standard deviations, $n = 5$)

PP-DOPA-BIBB, respectively), consistent with their wettability characteristics. As for the surfaces modified with polymer brushes, the lowest WCA value ($\sim 22^\circ$) was found on pSBMA-modified surface. Further increasing GMA contents resulted in gradual increases of WCA values ($\sim 39^\circ$ for PP-g-p(SBMA-co-GMA) $_{10:1}$, $\sim 51^\circ$ for PP-g-p(SBMA-co-GMA) $_{1:1}$ and $\sim 68^\circ$ for PP-g-pGMA), which was attributed to the adding of less hydrophilic GMA units in polymer brushes. Some changes in WCA ($\sim 41^\circ$ for PP-g-p(SBMA-co-GMA) $_{10:1}$ -ABPA, $\sim 58^\circ$ for PP-g-p(SBMA-co-GMA) $_{1:1}$ -ABPA and $\sim 60^\circ$ for PP-g-pGMA-ABPA, respectively) were observed for the APBA-binding samples (Fig. 3B). In comparison, the subsequent immobilization of IgG antibody triggered evident increases in their WCA values, $\sim 59^\circ$ for PP-g-p(SBMA-co-GMA) $_{10:1}$ -ABPA-IgG, $\sim 66^\circ$ for PP-g-p(SBMA-co-GMA) $_{1:1}$ -ABPA-IgG and $\sim 71^\circ$ for PP-g-pGMA-ABPA-IgG, respectively (Fig. 3C).

Oriented Immobilization of Primary Antibody

Boronic acid (BA) can form a stable but reversible boronate with the 1,2-/1,3-*cis* diols structures to get a 5-/6-membered cyclic boronic ester in aqueous media at room temperature^[15,26]. Previous studies proved that BA-based surfaces could bind Fc region of an antibody to allow optimal exposure of the antigen-binding domain (Fab) on the surface, which is vital to maintain antibody high activity^[27]. To evaluate antibody-loading capacities of different samples, FITC-labeled second antibody was used to determine the amount of immobilized primary antibody. As shown in Fig. 4, significantly different fluorescence intensities with different concentrations of FITC-Ab were observed on the samples, directly reflecting the amount of immobilized antibody on the surfaces. Specifically, the stronger fluorescence signal was observed on the surface of PP-g-pGMA-APBA-IgG, mainly due to the largest amount of loaded boronic acid groups. As a result, the increased feed ratios of [GMA]/[SBMA] led to obvious increases on the amount of immobilized antibody on the samples of PP-g-p(SBMA-co-GMA) $_{1:1}$ -ABPA-IgG and PP-g-p(SBMA-co-GMA) $_{10:1}$ -ABPA-IgG.

On one side, PP-g-pGMA-APBA-IgG exhibited higher antigen detection capacity than PP-g-p(SBMA-co-GMA) $_{1:1}$ -ABPA-IgG and PP-g-p(SBMA-co-GMA) $_{10:1}$ -ABPA-IgG (Fig. 5), indicating that the antigen detection capacity of the

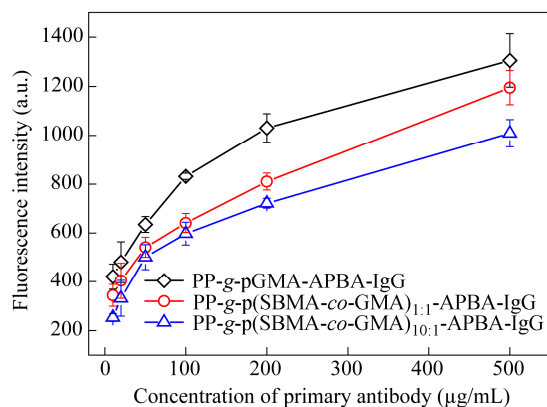


Fig. 4 Fluorescence intensity evaluation of antibody loading (The error bars: standard deviations, $n = 6$)

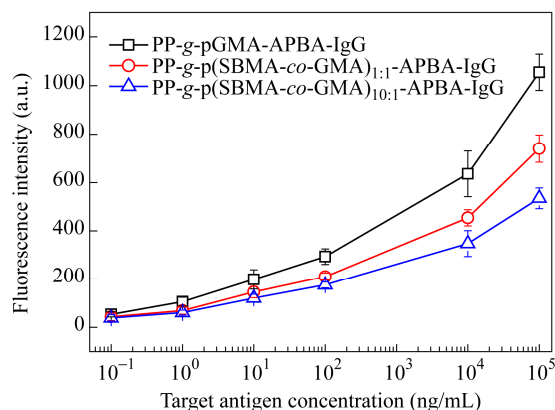


Fig. 5 Fluorescence intensity for the specific antigen recognition (The error bars: standard deviations, $n = 6$) (Antigen)

resultant samples gradually increased with increasing amount of immobilized antibody. On the other side, the differentiation tendency between PP-g-pGMA-APBA-IgG, PP-g-p(SBMA-co-GMA) $_{1:1}$ -ABPA-IgG and PP-g-p(SBMA-co-GMA) $_{10:1}$ -ABPA-IgG clearly decreased by reducing specific antigen concentration, which guaranteed the high antigen detection efficiency of PP-g-p(SBMA-co-GMA) $_{10:1}$ -ABPA-IgG at a low antigen concentration.

Signal-to-Noise (S/N) Evaluation

Practically, the detection of a specific disease marker is generally conducted in a complicated crude blood or body fluid media. As a result, the undesired nonspecific components may seriously hinder and interfere with the sensitive and accurate detection of the analytes. To evaluate the effect of nonspecific protein adsorption on antigen recognition, FITC-labeled BSA (FITC-BSA) was used as a model protein. All the samples with antibody-loaded were incubated with PBS buffer containing FITC-BSA for 2 h, subsequently examined by the CLSM (Fig. 6). PP-g-pGMA-APBA-IgG exhibited the highest fluorescence intensity (~ 2500 a.u.), which is nearly 2.5 times higher than that of virgin PP, (~ 980 a.u.). In contrast, PP-g-pSBMA showed considerably lower fluorescence intensity (~ 60 a.u.), primarily due to the hydrated layer formed by the electrostatic interaction between zwitterions of pSBMA brushes and water

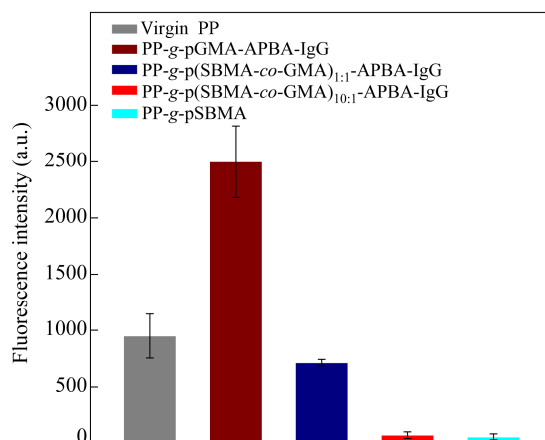


Fig. 6 Fluorescence intensities of FITC-BSA adsorption on the surfaces

molecules, significantly suppressing non-specific BSA adsorption. The introduction of pSBMA into pGMA brushes could remarkably inhibit nonspecific protein adsorption. The fluorescence intensities of PP-g-p(SBMA-co-GMA)_{1:1}-APBA-IgG and PP-g-p(SBMA-co-GMA)_{10:1}-APBA-IgG were ~714 and ~75 a.u., respectively, definitely confirming that pSBMA component can effectively suppress the interference signal.

To evaluate the influence of nonspecific protein adsorption on antigen recognition, the ratio of fluorescence intensity of specific antigen recognition to that of nonspecific protein adsorption was calculated to determine the signal-to-noise (S/N). As shown in Fig. 7, PP-g-p(SBMA-co-GMA)_{10:1}-APBA-IgG demonstrated the highest S/N values than their counterparts (PP-g-pGMA-APBA-IgG and PP-g-p(SBMA-co-GMA)_{1:1}-APBA-IgG). Owing to the optimal balance between the loading capacity of antibodies and their antifouling properties, PP-g-p(SBMA-co-GMA)_{10:1}-APBA-IgG possessed the remarkable antigen recognition capability with high S/N value.

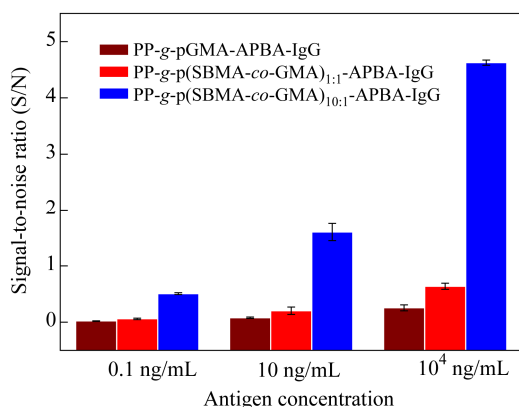


Fig. 7 Signal-to-noise values (S/N) of modified samples in different antigen concentrations (The error bars: standard deviations, $n = 6$)

Antibody Dissociation

The boronic acid (BA) can form robust covalent bonding with the *cis*-diol group under alkaline conditions, while this bond is reversibly cleavable under acidic conditions, revealing the potential applications in renewable biosensors^[19, 28]. In our work, antibody dissociation behaviors were examined in acid solution (pH = 3) for 6 h *via* ATR-FTIR. As illustrated in the normalized ATR-FTIR spectra (Fig. S2, in ESI), the obviously enhanced adsorption peaks at 1609 and 3359 cm⁻¹ were observed on the spectra of PP-g-pGMA, PP-g-p(SBMA-co-GMA)_{1:1} and PP-g-p(SBMA-co-GMA)_{10:1} after the site-specific capture of IgG. Due to different capacities of IgG immobilization, the strongest adsorption peak at 1609 cm⁻¹ was observed for PP-g-pGMA, followed in descending order by PP-g-p(SBMA-co-GMA)_{1:1} and PP-g-p(SBMA-co-GMA)_{10:1}. When the samples were treated with acid solutions, the apparent decrease in peak intensities of 1609 cm⁻¹ occurred, implying that most of the bonded IgG were dissociated from the surfaces.

Moreover, the dissociation ratios of various samples were quantitatively examined by CLSM. As shown in Fig. 8, the

fluorescence intensities of the samples before and after antibody dissociation followed the descending order of PP-g-pGMA-APBA, PP-g-p(SBMA-co-GMA)_{1:1}-APBA and PP-g-p(SBMA-co-GMA)_{10:1}-APBA due to their different capacities of antibody immobilization. Among them, the PP-g-p(SBMA-co-GMA)_{10:1}-APBA showed the highest dissociation ratio of ~75.8%, compared with those of PP-g-p(SBMA-co-GMA)_{1:1}-APBA (~64.8%) and PP-g-pGMA-APBA (~58.6%). This result can be explained by the fact that pure pGMA-modified surface readily triggers strong physisorption of antibody, whereas surface modified with pSBMA exhibits high antibody dissociation efficiency due to its significant antifouling characteristics.

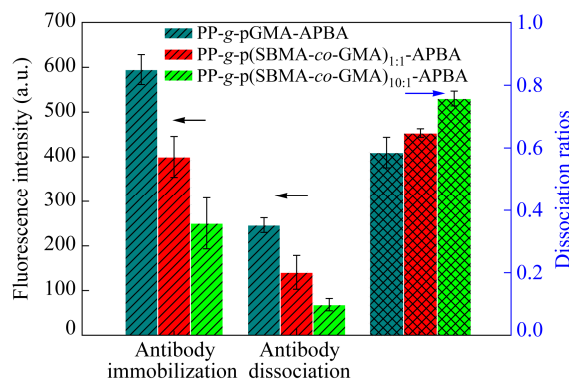


Fig. 8 Fluorescence intensity (black line) and dissociation ratios (blue line) after the antibody immobilization and dissociation of the samples

CONCLUSIONS

In summary, the immunoassay platform modified with BA groups and zwitterionic SB-containing polymer brushes has been developed, which simultaneously present a reversible antibody loading capacity and reduced background noise. It was demonstrated that the PP-g-p(SBMA-co-GMA)_{10:1}-APBA showed the optimal antigen detection efficiency due to its optimized balance between its resistance of non-specific proteins and appropriate loading capacity of antibodies. Additionally, this sample also displayed higher antibody dissociation ability due to the weakened interactions between the surface and antibody by hydrophilic pSBMA. Moreover, benefiting from DOPA pretreated adhesive layer, the immunoassay platform can be built upon various substrates regardless of their shape and dimensionality.

Electronic Supplementary Information

Electronic supplementary information (ESI) includes details of XPS atomic compositions and concentrations of the samples (Table S1); representative fluorescence images of FITC-BSA protein adsorption on the sample surfaces (Fig. S1); ATR spectra of the samples for site-specific capture of IgG and IgG dissociation in acid condition (Fig. S2), which is available free of charge in the online version of this article at <https://doi.org/10.1007/s10118-018-2031-0>.

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REFERENCES

- Wu, Y. M.; Cen, Y.; Huang, L.J.; Yu, R. Q.; Chu, X. Upconversion fluorescence resonance energy transfer biosensor for sensitive detection of human immunodeficiency virus antibodies in human serum. *Chem. Commun.* 2014, 50, 4759–4762.
- Bi, X.; Liu, Z. Facile preparation of glycoprotein-imprinted 96-well microplates for enzyme-linked immunosorbent assay by boronate affinity-based oriented surface imprinting. *Anal. Chem.* 2014, 86(1), 959–966.
- Gan, S. D.; Patel, K. R. Enzyme immunoassay and enzyme-linked immunosorbent assay. *J. Invest. Dermatol.* 2013, 133(9), 1–3.
- Bae, Y. M.; Oh, B. K.; Lee, W.; Lee, W. H.; Choi, J. W. Study on orientation of immunoglobulin G on protein G layer. *Biosens. Bioelectron.* 2005, 21(1), 103–110.
- Bhadra, P.; Shajahan, M. S.; Bhattacharya, E.; Chadha, A. Studies on varying *n*-alkanethiol chain lengths on a gold coated surface and their effect on antibody-antigen binding efficiency. *RSC Adv.* 2015, 5(98), 80480–80487.
- Jung, Y.; Jeong, J. Y.; Chung, B. H. Recent advances in immobilization methods of antibodies on solid supports. *Analyst* 2008, 133(6), 697–701.
- Soellner, M. B.; Dickson, K. A.; Nilsson, B. L.; Raines, R. T. Site-specific protein immobilization by staudinger ligation. *J. Am. Chem. Soc.* 2003, 125(39), 11790–11791.
- Trilling, A. K.; Beekwilder, J.; Zuilhof, H. Antibody orientation on biosensor surfaces: a minireview. *Analyst* 2013, 138(6), 1619–1627.
- Wang, Z.; Jin, G. J. Feasibility of protein A for the oriented immobilization of immunoglobulin on silicon surface for a biosensor with imaging ellipsometry. *Biochem. Biophys. Methods* 2003, 57(3), 203–211.
- Kim, J.; Cho, J.; Seidler, P. M.; Kurland, N. E.; Yadavalli, V. K. Investigations of chemical modifications of amino-terminated organic films on silicon substrates and controlled protein immobilization. *Langmuir*, 2010, 26(4), 2599–2608.
- de Juan-Franco, E.; Caruz, A.; Pedrajas, J. R.; Lechuga, L. M. Site-directed antibody immobilization using a protein A-gold binding domain fusion protein for enhanced SPR immunosensing. *Analyst* 2013, 138(7), 2023–2031.
- Wang, S.; Ye, J.; Li, X.; Liu, Z. Boronate affinity fluorescent nanoparticles for forster resonance energy transfer inhibition assay of *cis*-diol biomolecules. *Anal. Chem.* 2016, 88(10), 5088–5096.
- Peters, J. A. Interactions between boric acid derivatives and saccharides in aqueous media: structures and stabilities of resulting esters. *Coord. Chem. Rev.* 2014, 268(2), 1–22.
- Brooks, W. L.; Sumerlin, B. S. Synthesis and applications of boronic acid-containing polymers: from materials to medicine. *Chem. Rev.* 2016, 116(3), 1375–1397.
- Chen, M. L.; Adak, A. K.; Yeh, N. C. Fabrication of an oriented Fc-fused lectin microarray through boronate formation. *Angew. Chem.* 2008, 47(45), 8627–8630.
- Tan, L.; Xing, J.; Cao, F.; Chen, L.; Zhang, C.; Shi, R.; Wang, Y. Synthesis of double-hydrophilic double-grafted copolymers PMA-g-PEG/PDMA and their protein-resistant properties. *Chinese J. Polym. Sci.* 2013, 31(4), 691–701.
- Luan, S.; Zhao, J.; Yang, H.; Shi, H.; Jin, J.; Li, X.; Liu, J.; Wang, J.; Yin, J.; Stagnaro, P. Surface modification of poly(styrene-*b*-(ethylene-*co*-butylene)-*b*-styrene) elastomer via UV-induced graft polymerization of *N*-vinyl pyrrolidone. *J. Colloid Interf. Sci.* 2012, 93(1), 127–134.
- Huang, C. J.; Li, Y.; Krause, J. B.; Brault, N. D.; Jiang, S. Internal architecture of zwitterionic polymer brushes regulates nonfouling properties. *Macromol. Rapid Commun.* 2012, 33, 1003–1007.
- Chien, H.; Tsai, C.; Tsai, W.; Wang, M. J.; Kuo, W. H.; Wei, T. C.; Huang, S. T. Surface conjugation of zwitterionic polymers to inhibit cell adhesion and protein adsorption. *J. Colloid Interf. Sci.* 2013, 107(7), 152–159.
- Yu, Q.; Zhang, Y.; Wang, H.; Brash, J.; Chen, H. Anti-fouling bioactive surfaces. *Acta Biomater.* 2011, 7(4), 1550–1557.
- Zong, M.; Gong, Y. Fabrication and biocompatibility of cell outer membrane mimetic surfaces. *Chinese J. Polym. Sci.* 2011, 29(1), 53–64.
- Zhao, J.; Shi, Q.; Luan, S.; Song, L. Polypropylene non-woven fabric membrane via surface modification with biomimetic phosphorylcholine in Ce(IV)/HNO₃ redox system. *Mater. Sci. Eng. C* 2012, 32(7), 1785–1789.
- Li, Y.; Zhou, M.; Geng, C.; Chen, F.; Fu, Q. Simultaneous improvements of thermal stability and mechanical properties of poly(propylene carbonate) via incorporation of environmental-friendly polydopamine. *Chinese J. Polym. Sci.* 2014, 32(12), 1724–1736.
- Lyng, M. E.; van der Westen, R.; Postma, A.; Stadler, B. Polydopamine-a nature-inspired polymer coating for biomedical science. *Nanoscale* 2011, 3(12), 4916–4928.
- Jiang, J. H.; Zhu, L. P.; Li, X. L.; Xu, Y. Y.; Zhu, B. K. Surface modification of PE porous membranes based on the strong adhesion of polydopamine and covalent immobilization of heparin. *J. Membr. Sci.* 2010, 364(1–2), 194–202.
- Zhong, X.; Bai, H. J.; Xu, J. J.; Chen, H. Y.; Zhu, Y. H. A reusable interface constructed by 3-aminophenylboronic acid-functionalized multiwalled carbon nanotubes for cell capture, release, and cytosensing. *Adv. Funct. Mater.* 2010, 20(6), 992–999.
- Jung, Y.; Lee, J. M.; Jung, H.; Chung, B. H. Self-directed and self-oriented immobilization of antibody by protein G-DNA conjugate. *Anal. Chem.* 2007, 79(17), 6534–6541.
- Li, D.; Chen, Y.; Liu, Z. Boronate affinity materials for separation and molecular recognition: structure, properties and applications. *Chem. Soc. Rev.* 2015, 44(22), 8097–8123.