Specific Binding of Immunoglobulin G with Bioactive Short Peptides Supported on Antifouling Copolymer Layers for Detection in Quartz Crystal Microgravimetry and Surface Plasmon Resonance

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Supporting Information

ABSTRACT: A new peptide-based system supported on copolymer brushes grafted from gold sensors and with resistance to nonspecific adsorption is reported for selective binding of human immunoglobulin G (IgG). A random copolymer rich in primary amines, poly(2-aminoethyl methacrylate hydrochloride-co-2-hydroxyethyl methacrylate) (poly(AMA-co-HEMA)) was first grafted from initiator-coated gold substrates via activators regenerated by electron transfer radical polymerization (ARGET-ATRP), followed by immobilization of acetylated-HWRGWV peptide, which has specific binding affinity with IgG. The peptide ligands covalently linked to the soft copolymer layer were characterized by X-ray photoelectron spectroscopy (XPS), water contact angle, ellipsometry, and atomic force microscopy (AFM). The extent of binding, binding affinity, and selectivity for target IgG molecules as well as the capability to minimize nonspecific interactions with other proteins were examined by fluorescence imaging, surface plasmon resonance (SPR), and quartz crystal microgravimetry (QCM). The effect of copolymer molecular composition and analyze concentration was elucidated in order to design systems based on immobilized peptides for high signal-to-noise response and detection limits that meet the requirements for IgG biosensing in fluid matrixes.

Immunoglobulin G (IgG) has been widely used in biotechnology and immuno-biosensing due to its extremely high selectivity and sensitivity in the recognition of antigens.1–3 To date numerous techniques have been developed for IgG separation and purification. Affinity chromatography is one of the widely used methods due to its efficiency in purification and high IgG recovery during separation.4 Essential to affinity purification is the use of Staphylococcus aureus protein A and streptococcus protein G, which are the most common IgG ligands. In fact, these protein ligands can specifically bind the Fc region of IgG and show high affinity, up to 109–1010 M−1.5,6 Nevertheless, the major drawback of protein A/G ligands includes the expensive downstream processing of IgG, which can account for 50–80% of the total production costs.7 In addition, in order to recover IgG from respective affinity adsorption columns, it is necessary to use harsh elution conditions (mostly low pH elution buffer), which in turn results in the aggregation of IgG and reduces subsequent binding capacity.8,9,10 These effects could be countered if the binding affinity between IgG and the substrate was adjusted better. Overall, there is a strong motivation to design ligands for IgG affinity separation and purification that are selective, cost-effective, and with controlled binding affinity. A number of synthetic substrates have been proposed for this objective, including engineered proteins11 and histidine,12 thiophilic,13 and synthetic peptides.14 Among them, small synthetic ligands have received considerable attention due to their lower cost and higher stability compared to protein-based ligands. Unfortunately, the lack of selectivity for IgG by small ligands has prevented a more widespread use.

From combinatorial solid-phase hexamer peptide libraries, Carbonell et al. have identified HWRGWV (Supporting Information, Scheme S1a) as a new hexamer peptide that selectively binds to the Fc region of IgG (Supporting Information, Scheme S1b).10,15–17 It was found that chromatography resins based on acetylated-HWRGWV can purify IgG from complete mammalian cell culture medium (cMEM) containing 10% fetal calf serum and 5% tryptose phosphate broth, reaching purity and recovery as high as 96 and 95.5%, respectively.9 Additionally, HWRGWV ligand-based chromatographic resins were able to purify a humanized monoclonal antibody of IgG4 subclass from commercial Chinese hamster ovary (CHO) cell culture supernatant with...
a purity and recovery higher than 90 and 85%, respectively. Therefore, the purity that can be reached by using this peptide ligand is comparable with that obtained from protein A/G while the cost is reduced significantly. Moreover, since the binding affinity of the short peptide with IgG is ~10^4 M^{-1}, lower than that for protein A, the recovery of IgG can be performed under milder elution conditions, which averts the losses of activity and protein aggregation typical in low pH elution buffers. These advantages make HWRGWV hexamer peptide ligand for IgG detection and separation a viable alternative to conventional systems based on protein A/G.

Given these proven features of short peptide ligands, there exists a need to develop biosensors for deployment in affinity separation operations. For a biosensor to have good performance and high signal-to-noise ratio, the sensing component needs not only to bind selectively to target molecules but also requires elimination of biofouling background, which interferes by way of nonspecific interactions. A widely used strategy is to modify the biosensor surface with antifouling and hydrophilic polymers in matrices or spacers that carry the affinity ligand and resist nonspecific protein adsorption and unwanted interactions. In addition, antifouling chemistries provide a hydrophilic microenvironment that is advantageous in maintaining ligand activity. We propose a new hybrid system consisting of a combination of a grafted hydrophilic, antifouling copolymer as support for short peptides for IgG selective binding. To this end, QCM and SPR sensors based on gold chips were employed after modification with self-assembled monolayers. The copolymer used consisted of poly(2-carboxyethyl methacrylate)-bipyridyl, copper(II) chloride, and resist nonspecific protein adsorption and unwanted interactions. In addition, antifouling chemistries provide a hydrophilic microenvironment that is advantageous in maintaining ligand activity. We propose a new hybrid system consisting of a combination of a grafted hydrophilic, antifouling copolymer as support for short peptides for IgG selective binding. To this end, QCM and SPR sensors based on gold chips were employed after modification with self-assembled monolayers.

The copolymer used consisted of poly(2-aminoethyl methacrylate)-co-(2-hydroxyethyl methacrylate) (poly(AMA-co-HEMA)), which was prepared via activators regenerated by electron transfer atom transfer radical polymerization (ARGET-ATRP). The AMA segments in the copolymer endow the system with peptide immobilizing capability (via amine coupling) while the HEMA segments provide nonspecific protein resistance.

### MATERIALS AND METHODS

**Materials.** The initiator thiol (ω-mercaptoundecyl bromoisobutyrate) was purchased from Prochimia (Sopot, Poland). QCM and SPR gold chips were purchased from Q-Sense (Göteborg, Sweden) and BioNavis (Tampere, Finland), respectively. Acetylated-HWRGWV (95.1%) was purchased from GenScript (Piscataway, NJ). Human IgG (>97%) was obtained as a lyophilized powder from Equitech-Bio (Kerrville, TX). 2-Hydroxyethyl methacrylate (HEMA), 2-aminoethyl methacrylate hydrochloride (AMA), 2,2'-bipyridyl, copper(II) bromide, N,N-dimethylformamide (DMF, anhydrous), N,N-diisopropylethylamine (DIPEA), O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethylethlenediamine hexafluorophosphate (HATU) coupling agent, fluorescein isothiocyanate-labeled IgG from human serum (FITC-IgG), and albumin from bovine serum (BSA) were all purchased from Sigma-Aldrich (Milwaukee, WI) and used as received.

**Immobilization of Initiator Monolayer on Gold.** All SPR and QCM chips were immersed in “piranha” solution (H₂SO₄/H₂O₂ = 7:3 (v/v)) (Caution: piranha solution reacts violently with organic materials and should be handled carefully) at room temperature for 5 min to remove the organic residues on the surfaces. Then the surfaces were rinsed with copious amounts of Milli-Q water and dried under a flow of nitrogen. Finally, the surfaces were exposed to UV irradiation for 30 min just before use.

Self-assembled monolayers (SAMs) of initiator ω-mercaptoundecyl bromoisobutyrate were prepared by immersing the QCM and SPR gold chips into a 1 mmol initiator thiol in anhydrous ethanol solution for 15 h at room temperature, yielding Au—initiator surfaces (Au—Br). After reaction, these surfaces were thoroughly rinsed with ethanol to remove physiosorbed thiols and then dried under nitrogen flow.

**Grafting Hydrophilic, Antifouling Poly(AMA-co-HEMA) Peptide Support.** The poly(AMA-co-HEMA) modified surfaces were prepared via surface initiated ARGET-ATRP of AMA and HEMA randomly grafted from the Au—Br surface. The reaction solution was prepared by dissolving AMA (0.36 g, 2.2 mmol), HEMA (1.15 g, 8.8 mmol), bipyridine (7.7 mg, 49 μmol), CuBr₂ (1.6 mg, 7 μmol), and ascorbic acid (17.3 mg, 98 μmol) into a mixture of methanol (7 mL) and water (7 mL). The Au—Br surfaces were immersed into the reaction solution for given times (from 1 to 30 min) at room temperature. Finally, the surfaces were removed from the solution, rinsed with abundant Milli-Q water and anhydrous ethanol to remove unreacted monomers and physically adsorbed polymers, and then dried under a nitrogen flow to achieve poly(AMA-co-HEMA) surfaces.

**Poly(AMA-co-HEMA)-Short Peptide Sensor.** Acetylated-HWRGWV (Ac-HWRGWV) peptides were immobilized in the poly(AMA-co-HEMA) support layer via covalent amide bonds by the reaction of the peptide’s carboxylate groups with the free amines of AMA segments. Briefly, poly(AMA-co-HEMA) grafted surfaces were immersed in an anhydrous DMF solution containing Ac-HWRGWV (5 mg mL⁻¹) and HATU (3.8 mg mL⁻¹), followed by addition of DIPEA (2.58 μL/mL). The reaction continued for 12 h at room temperature under a nitrogen atmosphere. Finally, the surfaces were rinsed with DMF and Milli-Q water thoroughly to remove the impurities and dried under a nitrogen flow to achieve the poly(AMA-co-HEMA)-peptide surfaces.

**Surface Characterization.** Ellipsometry. The thickness of grafted copolymer layer was measured by spectroscopic ellipsometry model M-2000 V (J. A. Woollam Co., Inc.) at a 70° angle and wavelengths from 400 to 800 nm. Ellipsometric data were fitted using a Cauchy layer model with fixed (A₀, B₀) values of (1.46, 0.01). The ellipsometric thickness for each sample was independently measured at three different locations and was reported as the average.

**Attenuated Total Reflection Fourier Transform-Infrared (ATR-FT-IR).** ATR-FT-IR adsorption spectra were obtained with a Thermo Nicolet Nexus 670 FT-IR ESP spectrometer (Thermo Nicolet) equipped with a Smart OMNI sampler, and results were determined by using the OMNIC software (Nicolet Instrument Corp.).

**Atomic Force Microscopy.** Tapping-mode topographical images of poly(AMA-co-HEMA) and poly(AMA-co-HEMA)-peptide surfaces were obtained in air with a Digital Instruments D3000 atomic force microscope (AFM). The root-mean-square (RMS) surfaces roughness values were obtained for an averages of 5 × 5 μm areas.

**Contact Angle Measurement.** Static water contact angles (WCA) on the surfaces were measured using a contact angle goniometer (SEO Phoenix 300, Korea) via the sessile drop method at room temperature. Milli-Q water (18.2 MΩ cm⁻¹) with a drop volume of 20 μL was used; the water drops were allowed to equilibrate in contact with the surfaces for 15 s before reading the WCA. Each value was averaged by six independent measurements.
**RESULTS AND DISCUSSION**

The primary amine group containing AMA are widely used in systems for drug delivery,24 biomedical devices,25 etc. Armes et al. introduced the possibility to direct polymerization of AMA in its hydrochloride salt form by living radical polymerization.26 In our efforts, the random copolymer of HEMA and AMA (in its hydrochloride salt form) was prepared by ARGET-ATRP. The concentration of amine groups was varied in a series of poly(AMA-co-HEMA) supports that were prepared as illustrated in Scheme 1. First, a homogeneous and dense monolayer of initiator was immobilized on a gold surface. Then poly(AMA-co-HEMA) copolymers were grafted from initiator-immobilized substrates. The layer thickness of grafted poly(AMA-co-HEMA) was controlled by varying the polymerization time. It is worth noting that compared to conventional ATRP, ARGET-ATRP can be carried out in the presence of limited amounts of air27 and also provides a controlled polymerization with small amounts of Cu-based catalysts (a few ppm), due to the regeneration of the Cu(I) activator species by reducing agents.28 Immobilization of Ac-HWRGWWA via amide bonds between its carboxylate groups and amines from AMA was conducted by immersing the poly(AMA-co-HEMA) surface into a peptide reaction mixture at room temperature, yielding poly(AMA-co-HEMA)-peptide (Scheme 1). Overall, the amine-containing poly(AMA) side-chains were used for peptide bioconjugation24,29 while the poly(HEMA) was introduced to act as an antifouling component with expected good resistance to protein, bacteria, and cell adhesion10,30 in the biosensors.31,32

**Fluorescence Imaging.** An Olympus BX-61 optical microscope operated under transmitted- and fluorescence-mode was used to qualitatively access the extent of protein binding. The images were recorded using an Olympus DP-70 digital CCD camera. The respective modifed surface was equilibrated in PBSS for 12 h prior to incubation in 0.2 mg/mL FITC-IgG in PBSS for 2 h at room temperature. Before imaging, the surfaces were rinsed (5 min each) three times in PBSS and three times in Milli-Q water and then dried under a gentle flow of nitrogen.

**Surface Plasmon Resonance (SPR).** The adsorption of IgG on poly(AMA-co-HEMA)-peptide surfaces was measured with a SPR-Navis (KSV, Helsinki, Finland) instrument. SPR was operated in a flow-through mode with a flow rate of 10 \( \mu \text{L} \text{min}^{-1} \) at 25 °C. The PBSS running buffer was pumped to the poly(AMA-co-HEMA)-peptide surface until reaching a stable baseline and then a set of IgG solutions with different concentrations (0.01–5 mg/mL) were injected separately into the system. Finally, PBSS was passed through the surface to establish a new baseline. The changes of SPR signals were used to quantitatively determine the extent of protein adsorption. To investigate the fouling resistance of poly(AMA-co-HEMA)-peptide surfaces, 1 mg/mL BSA was introduced following the same protocol used for IgG.

**X-ray Photoelectron Spectroscopy (XPS).** X-ray photoelectron spectroscopy (XPS) was used to examine the surface chemical composition of the polymeric layers. A XPS (SPECS, Germany) with a monochromatic Mg Ka X-ray source (1253.6 eV) was employed and operated under 10\(^{-8}\)–10\(^{-9}\) Torr pressure. Survey as well as high-resolution C1s and N1s spectra were collected. Measurements were made at takeoff angles of 90° relative to the sample’s surface.

**Surface Characterization of poly(AMA-co-HEMA) Support Layer.** Analyses via ATR-FT-IR, WCA, and layer thickness (ellipsometry) were determined on the poly(AMA-co-HEMA) surfaces after each of the reaction steps (Scheme 1), and the results are shown in Figure 1. The Au surface was very hydrophilic (WCA < 10°) after treatment with “piranha” solution and UV irradiation but, as expected, it became less hydrophilic (WCA = 67°) after exposure to air for 12 h (Figure 1a). The immobilization of initiator SAM produced a layer of \( \sim 1.6 \text{ nm} \) thickness with a WCA of 77°; these values are consistent with the formation of a dense monolayer.33 Polymerization of AMA and HEMA from the initiator SAM during 25 min produced a copolymer layer of \( \sim 26 \text{ nm} \) thickness and reduced the WCA to 48°. This contact angle was very close to that reported for poly(AMA) and poly(HEMA) homopolymers (Supporting Information, Figure S1). ATR-FT-IR spectra of the poly(AMA-co-HEMA) surfaces (inset in Figure 1b) indicates the appearance of a peak at 1726 cm\(^{-1}\), which is ascribed to the carboxyl stretching vibration of the ester group from poly(AMA-co-HEMA) copolymer. The chemical composition of poly(AMA-co-HEMA) and its difference with poly(AMA) and poly(HEMA) were also obtained from XPS analyses (Supporting Information, Table S1).
The growth evolution of poly(AMA-co-HEMA) chains as a function of polymerization time was followed by measurements of the ellipsometric thickness (Figure 1b). An approximately linear increase in thickness of the grafted layer with polymerization time is observed up to 20 min, suggesting that the ARGET-ATRP grafting of AMA and HEMA from the Au−Br surface is a "controlled" process. As expected, the polymerization rate decreased slightly after long polymerization times, beyond ∼20 min, which can be ascribed to the consumption of the active chain ends via bimolecular reactions.34,35

Surface Characterization of Poly(AMA-co-HEMA)-peptide. HATU was used to couple the Ac-HWRGWVA directly to the poly(AMA-co-HEMA) copolymer layer. HATU is known as an effective peptide coupling additive and is widely used in immobilization of biomolecules.36 HATU reacted with the deprotonated carboxylic group of Ac-HWRGWVA first and formed an active intermediate, which then reacted with the amine groups of poly(AMA-co-HEMA) polymer to form amide bonds in poly(AMA-co-HEMA)-peptide.

Changes in the chemical composition of the surfaces were determined by XPS. The percentage of nitrogen in the poly(AMA-co-HEMA) surface was measured to be ∼1.7%, but it increased to 8.2% after peptide immobilization (Figure 2a and the Supporting Information, Table S1 and Figure S2). Thus, the success of peptide immobilization in the copolymer layer is confirmed. Moreover, after peptide immobilization, the WCA increased slightly, from 48 to 53° (Figure 2b) and the
polymer thickness increased from 23.8 to 30.7 nm (Supporting Information, Figure S3).

The surface morphology of poly(AMA-co-HEMA) before and after peptide immobilization was observed by AFM. The poly(AMA-co-HEMA) grafted surface had a RMS roughness of ∼1.39 nm and it decreased to ∼0.85 nm after peptide conjugation. Distinctive changes in the topographical features on the surface are evident after peptide immobilization (Figure 2c,d).

As is the case for protein A/G, HWRGWV peptide binds selectively to the Fc region of IgG, which can be confirmed by testing nonspecific interactions, for example, by challenging the system with a reference protein such as BSA. As such, the amounts of IgG and BSA adsorbed on poly(AMA-co-HEMA) surfaces before and after peptide immobilization were compared by using SPR measurements. From Figure 3a, only 0.05 and 0.01 mg/m² of IgG and BSA were adsorbed on the poly(AMA-co-HEMA) surface, respectively. The low adsorption levels for both proteins indicate that poly(AMA-co-HEMA) surface possess protein-resistant properties due to the presence of HEMA chains. This is in contrast with results obtained from experiments with the same supports after peptide immobilization: a significant increase in IgG adsorption, up to 4.2 mg/m² (nearly 84 times higher) was measured, while the surface displayed nonspecific protein resistance as indicated by the limited BSA adsorption. In fact, BSA binding was even lower than that in the absence of peptide. This result indicates that the poly(AMA-co-HEMA)-peptide surface used as a biosensing element exhibits both high capability for selectively binding IgG and nonspecific protein resistance. The specific IgG binding ability of poly(AMA-co-HEMA)-peptide surface was further confirmed by fluorescence microscopy. Figure 3b,c shows typical images of FITC-labeled IgG adsorbed on a poly(AMA-co-HEMA) support layer before and after immobilization of peptide. Strong fluorescence was detected only on the poly(AMA-co-HEMA)-peptide surface, indicating the selective binding of IgG, which is consistent with the SPR results.

**Effect of Molecular Composition of Poly(AMA-co-HEMA) Layers.** AMA and HEMA segments in the copolymer were used to immobilize the peptide and reduce or eliminate contributions from biofouling, respectively. The feed ratio of AMA and HEMA during polymerization is expected to be a key factor in ensuring support with good immobilization capacity and nonfouling properties. Therefore, copolymers of five different compositions were obtained by tuning the molar ratio of AMA and HEMA monomers in the reaction solutions (M_{AMA}/M_{HEMA} = 100:0, 50:50, 20:80, 10:90, and 0:100). Note: the total molar amount of monomer is constant (11 mmol) in the reaction solution, and $M_{AMA}/M_{HEMA} = 100:0$ and $0:100$ give homopolymers of poly(AMA) and poly(HEMA), respectively. As discussed before, the poly(AMA-co-HEMA) layers were functionalized with short peptides, yielding poly(AMA-co-HEMA)-peptide.

The adsorption of IgG and BSA on poly(AMA-co-HEMA) and poly(AMA-co-HEMA)-peptide surfaces with different molecular composition was measured by SPR and the main results are included in Figure 4. It is apparent that as the HEMA component increases in the copolymer, adsorption of BSA decreases while that of IgG increases. The copolymer with the AMA/HEMA ratio of 20:80 binds IgG to the largest extent while allowing the lowest level of BSA adsorption; thus, this copolymer composition (20:80) was used in further investigations. In addition, it was found that more IgG adsorption occurred on the poly(AMA-co-HEMA)-peptide surface compared with that on homopoly(AMA)-peptide surface. This observation can be explained by the incorporation of relative flexible HEMA segments facilitating greater access of IgG to peptide binding sites.

**Effect of IgG Concentration.** SPR and QCM were used to study the effect of IgG concentration on peptide-facilitated

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**Figure 3.** (a) BSA and IgG areal masses adsorbed on poly(AMA-co-HEMA) and poly(AMA-co-HEMA)-peptide are used as biosensing layers in SPR. Also included are representative fluorescence images of FITC-IgG adsorbed on (b) poly(AMA-co-HEMA) and (c) poly(AMA-co-HEMA)-peptide surfaces. The scale bar in the images is equivalent to 50 μm.

**Figure 4.** Protein (IgG and BSA) areal masses bound onto poly(AMA-co-HEMA)-peptide sensors as a function of the chemical compositions of the layer as indicated by the AMA/HEMA molar ratio.
binding. The adsorption of protein on the layers used as sensing elements leads to changes in the refractive index (SPR) of the interface in the vicinity of the sensor and in the QCM resonator frequency. SPR signals can be directly related to the amount of protein molecules present in the adsorbed layer (dry mass), while the QCM frequency shifts are related to the total mass including hydration or coupling water associated with the adsorbed proteins (wet mass). Therefore, the water content of the adsorbed protein layer can be determined by comparing results from SPR and QCM experiments. Both QCM and SPR were used to detect the dynamics and specificity of IgG binding on poly(AMA-co-HEMA)-peptide sensing layers.

SPR results for IgG adsorption as a function of IgG solution concentration (thereafter abbreviated as [IgG]), ranging from 0.01 to 5 mg/mL, are included in Figure 5a,b. With increasing [IgG], a dramatic but monotonic increase in IgG adsorption is observed, reaching a limiting adsorbed mass of 4.2 mg/m$^2$ (at 1 mg/mL IgG solution concentration). The adsorption isotherm was fitted to a simple exponential relationship ($R^2 = 0.987$), eq 1:

$$Y_{SPR} = 4.52(1 - e^{-1.75X})$$

where $Y_{SPR}$ is the amount of IgG adsorbed on the surface (mg/m$^2$) and $X$ is [IgG] (mg/mL).

QCM detection of protein binding is illustrated in Figure 5c,d. For the poly(AMA-co-HEMA)-peptide functionalized QCM chip, the raw QCM frequency profile was recorded as a function of time for different [IgG], ranging from 0.01 to 5 mg/mL. In this study, all QCM measurements were conducted at the third overtone number and the frequency changes reported as $\Delta f$. In Figure 5c, five representative QCM profiles are plotted for comparison. The frequency measured for QCM resonators in running buffer was taken as the baseline, and the frequency change with respect to the baseline was ascribed to protein adsorption on the surface. It is found that for high [IgG] the binding rate increased and $\Delta f$ decreased with increasing [IgG], suggesting a faster and greater extent of IgG adsorption on the surface. For [IgG] = 1 mg/mL, $\Delta f$ reaches a minimum of $-89$ Hz. In a control experiment with BSA (1 mg/mL), $\Delta f$ was only $-3$ Hz, suggesting that the peptide-modified surface binds IgG specifically while it displays excellent nonspecific protein resistance. QCM signals were found to be especially sensitive to IgG binding: large and noticeable frequency shifts, equivalent to $-28$ Hz, were recorded for [IgG] as low as 0.05 mg/mL.

The “solidified liquid layer” model was used to decouple the effect of viscoelasticity and allow quantification of the actual changes in mass from QCM data upon molecular adoption on the surfaces from the solution medium. On the basis of this model, the QCM data was analyzed and the IgG adsorption values were plotted as a function of [IgG] (Figure 5d). With increased [IgG], a dramatic yet monotonic increase of IgG adsorption was observed reaching a maximum of 16.1 mg/m$^2$ at

Figure 5. Time-resolved SPR and QCM isotherms for IgG adsorption from different solution concentrations on poly(AMA-co-HEMA)-peptide surfaces. (a) Representative SPR sensograms are shown for IgG recognition at concentrations of 0.01, 0.1, 0.25, 0.5, and 1 mg/mL. (b) The resulting SPR isotherms were fitted to $Y_{SPR} = 4.52(1 - e^{-1.75X})$, $R^2 = 0.987$. (c) Typical QCM IgG adsorption profiles for IgG concentrations of 0.01, 0.05, 0.25, 0.5, and 1 mg/mL. The frequency shifts from part c were used to calculate the adsorbed mass by using the “solid liquid model”, and the results were fitted to $Y_{QCM} = 15.9(1 - e^{-5.33X})$, $R^2 = 0.998$ (see isotherm in part d). Here, $Y_{SPR}$ and $Y_{QCM}$ represent the extent of IgG adsorption measured by SPR and QCM (mg/m$^2$), respectively, and $X$ is the IgG solution concentration (mg/mL).
resulting in $k_H$ and $k_W$. The immobilized peptides and IgG is determined to have association and dissociation constants were accurately obtained at concentrations as low as 0.05 mg/mL was achieved via ser at concentrations as low as 0.05 mg/mL was achieved via biological elution conditions compared with protein A, preventing the loss of activity and protein aggregation typically produced in low pH elution buffers. These observations can also be taken as beneficial in cyclic operations and in sensor regeneration. Finally, we note that the binding constants obtained from SPR results in Figure 5b ($k_a = 6.6 \times 10^{-3}$ mg$^{-1}$ mL s$^{-1}$, $k_d = 1.1 \times 10^{-3}$ s$^{-1}$, and $K_A = 9 \times 10^5$ M$^{-1}$) are slightly different than those obtained from QCM experiments presented before. This can be explained by a number of contributions such as mass transport and hydrodynamics. The peptide-IgG binding energy at 25 °C calculated from $K_A$ values obtained from QCM ($4.9 \times 10^5$ M$^{-1}$) and SPR ($9 \times 10^5$ M$^{-1}$) isotherms are −32.3 and −33.8 kJ mol$^{-1}$, respectively which are typical of affinity constants of proteins (Supporting Information).43

Overall, the short peptides ligands immobilized in soft copolymer layers can result in low cost and robust devices for the detection of IgG. These systems can be deployed in reactors or built-in downstream systems for IgG capture and to facilitate process monitoring and control. It also provides a potential as an indicator of many autoimmune diseases related to the concentration of IgG.44-46 We are currently conducting detailed studies to explore applications of related matrices in fields of sensor development in Chinese Hamster Ovary (CHO) cell culture fluids and its regeneration ability. The use of photo-cross-linking to enhance the performance of the matrix by incorporating a benzophenone in the peptide ligands is also a subject under consideration. In addition, the binding affinity with antigens can be of interest since the peptide only binds the Fc fragment of IgG, and thus the presented peptide-modified surface may be an effective way to orient IgG onto the surface through its Fc, leaving free Fa and Fb segments that can bind antigen specifically.

**CONCLUSIONS**

Novel biosensors based on random poly(AMA-co-HEMA) copolymers carrying immobilized Ac-HWRGWVA peptides were developed. The biosensors were found to have specific binding affinity with IgG while maintaining excellent nonspecific protein resistance. Several factors influence IgG binding including layer molecular composition and IgG concentration. Layers carrying short peptides with an AMA/HEMA molar ratio of 20:80 displayed excellent IgG specificity binding while very low nonspecific BSA adsorption. Detection of IgG in PBSS buffer at concentrations as low as 0.05 mg/mL was achieved via QCM. The affinity constants for the binding of Ac-
HWRGWVA peptides to IgG were calculated to be 4.9 × 10^{−5} M−1 and 9 × 10^{−6} M−1 for QCM and SPR, respectively. Overall, sensors based on short peptide ligands immobilized in soft copolymer layers can result in a new paradigm for low cost, robust, and potentially regenerable devices for the production and detection of IgG.

**ASSOCIATED CONTENT**

◆ Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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