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# Chiral Design for Polymeric Biointerface: The Influence of Surface Chirality on Protein Adsorption

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## 1. Introduction

The biointerface is crucial for in vivo or in vitro biomedical applications.[1–4] When artificial materials come in contact with biological systems, they interact with each other through their interfaces, and protein adsorption on the biointerface is usually the first event that occurs. Therefore, it is especially important in the study of biomaterials. This process strongly influences biological events such as cell adhesion, platelet adhesion and activation,[5] etc., and ultimately determines the tissue responses toward these foreign materials.[6–8] Extensive efforts have been made to adjust and control protein adsorption on materials surface by various chemical or physical methods,[9–12] e.g., the use poly(ethylene glycol) hydrogel-based surfaces to resist protein adsorption, or nanostructural strategies to control protein adsorption and desorption dynamics, etc.[13–15] Recently, smart surfaces were also reported to modulate the protein adsorption by external stimuli.[16,17] However, biological systems are complex and thus new approaches are still desirable due to the demands of practical applications.

As one of the significant biochemical signatures of life, biomolecules in nature are usually chiral molecules and show high chiral preference for one specific enantiomer.[18–20] Hence, we were inspired to introduce the chiral effect in the study of protein/materials interactions. In this contribution, we show that surface chirality strongly influences the protein adsorption on the polymer surface and the interactions between them. Using a chiral brush polymer films based on L(D)-amino acid units as a model system, we report that the L-film has much stronger affinity with proteins than the corresponding D-film, although their other chemical and physical properties are identical. The thermodynamics analysis indicates that this effect is dominated by the stereoselective hydrophobic interactions between the polymers and the proteins. This reveals a novel biomimetic strategy for the design of biointerface materials, which is obviously different from the conventional ones that are based on the control of wettability, or construction of surface topographic features, etc. We anticipate that this work may lead to important applications including protein separation, biochips, and chiral biodevices.

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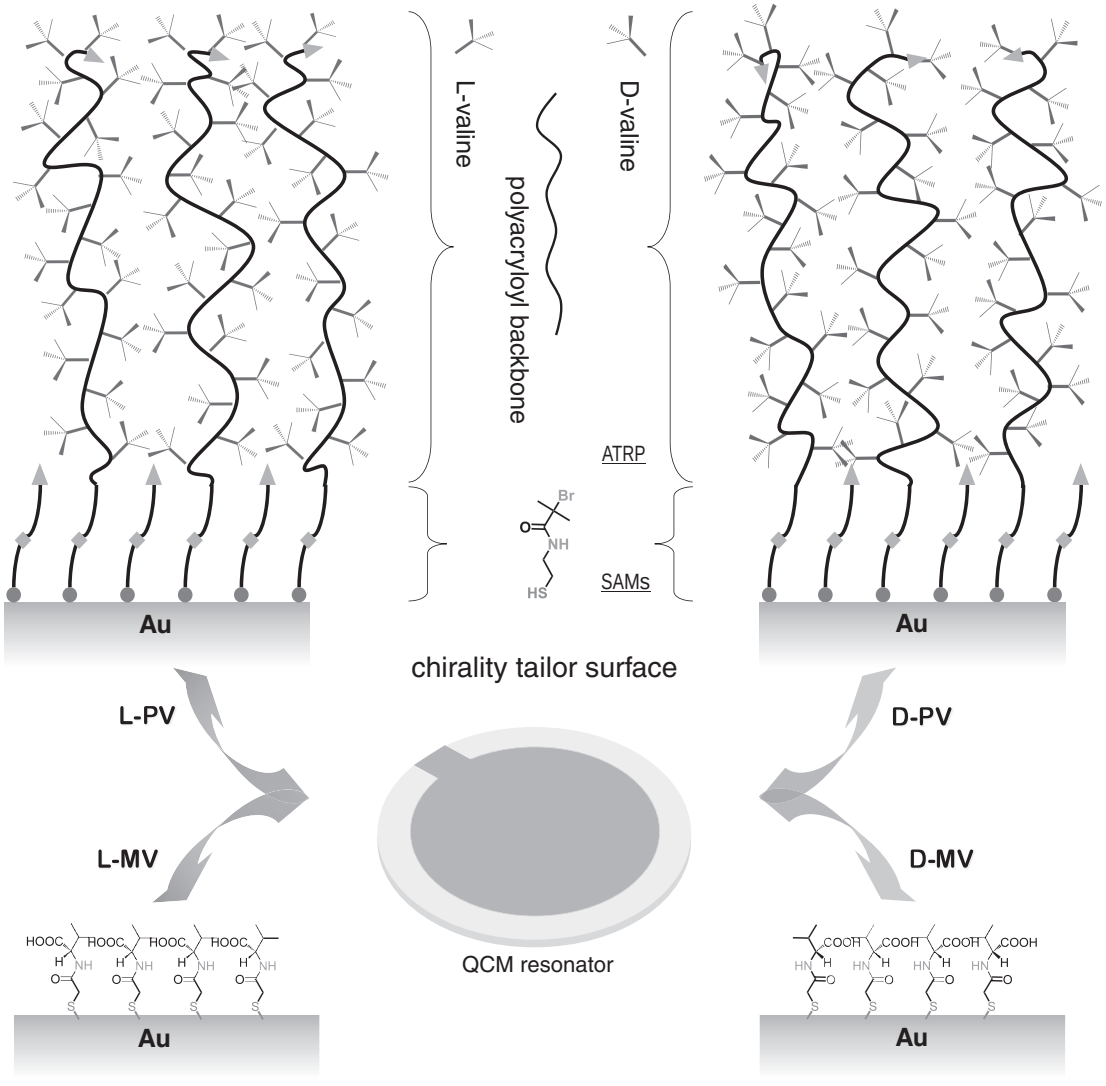
## 2. Results and Discussions

### 2.1. Synthesis and Characterization of the Chiral Polymer Films

Due to the obvious advantages of easy tailorability of chemical compositions and functions and precisely controlled surface properties,[21,22] we adopted a polymer brush framework for the chiral surface design. One of the important fundamental materials in life, most amino acids exist as the L-isomers in nature. The chirality of amino acids affects the steric configurations and higher-order conformations of proteins and other biomacromolecules, which strongly influence their bioactivities and the relevant biological processes.[23–25] On the other hand, it has been shown that the nature of the terminal group and the length of the chain have a significant influence on protein adsorption and cellular adhesion.[26–28] Based on these considerations, we selected the L- and D-amino acids as chiral centers. They were grafted onto the achiral polyacryloyl backbone as side groups in a comb-teeth style. The chiral comblike brush films were prepared on solid substrates via the surface initiated atom transfer radical polymerization (ATRP) method,[21] starting from monomers of L(D)-acryloylated amino acids. Our study shows that the optical rotation has been largely amplified after the polymerization process compared to the original monomer state (see the Supporting Information (SI)). The reason is that the chain structure of the linear polymer assembles the chiral units in a regular way, and thus greatly enhances the overall chirality characteristic of the system. Valine is one of the eight essential amino acids of human body, which plays crucial roles in a wide variety of physiological processes, and it has an isopropyl side group connected to the chiral center, which provides an ideal model to study the influence of hydrophobic side groups on the possible chiral effect. Therefore, although there are more than 20 natural amino acid species in nature, L(D)-valine grafted polymer (denoted as L-PV and D-PV, respectively) brush films are employed as a representative in the following study.

It is worthwhile to note that polymer brushes combined with comb-teeth functionalities have obvious advantages, including high coverage and no overlapping of chains as the polymer extends away from the interface, a high density of functional groups dispersed on the surface, etc.[29–32] In order to address




**Scheme 1.** Preparation scheme for the chiral polymer brush films (L- and D-PV, upper part) and chiral self-assembly monolayers (L- and D-MV, lower part) based on valine units on the gold surface of the QCM resonators.

this point, we also prepared the sufaces modified via the self-assembly process with monolyers of L(D)-valine monomers (denoted as L-MV and D-MV, respectively) to enable a comparison of the results. The protein adsorption on chiral surfaces was evaluated by using a quartz crystal microbalance (QCM) instrument,[33] with which the adsorbed mass of biomolecules could be analyzed *in situ* through the frequency shift ($\Delta f$) of a quartz crystal resonator which is linear to the change of adsorption quantity. For this purpose, all the L(D)-PV and L(D)-MV films were grafted onto the Au coated quartz crystal resonators, as schematically shown in **Scheme 1**.

As a pair of enantiomorphous surfaces, L-PV and D-PV brush films exhibit the same chemical compositions, as indicated by the X-ray photoelectron spectroscopic analysis (see SI). Water contact angle measurement shows that the wettability of L- and D-PV films is also the same (see SI). **Figure 1**a–c show atomic force microscopy (AFM) images of the gold surface of QCM crystals, L-PV and D-PV films, respectively, which indicate that the roughness of the QCM crystal surface increases for some extent, but remains identical for the enantiomorphous polymer films. A standard scratching method was used to measure the thickness of L- and D-PV films, as shown in Figure 1d–f. Both films exhibit a thickness of about 11.7 nm. According to the above data, and the analysis of the ATRP process, it is reasonable to conclude that the chain lengths of polymer brushes and the packing densities, as well as the surface charges, etc., should also be identical for L- and D-PV films.

### 2.2. Protein Adsorption on the Chiral Polymer Films

**Figure 2**a shows the time-dependent curves of $\Delta f$ on L- and D-PV tethered resonators using a flow of bovine serum albumin (BSA) solution (0.5 mg·mL$^{-1}$, PBS buffer, pH = 7.4). Interestingly, significant difference of BSA adsorption on L- and D-PV tethered resonators was observed. For the

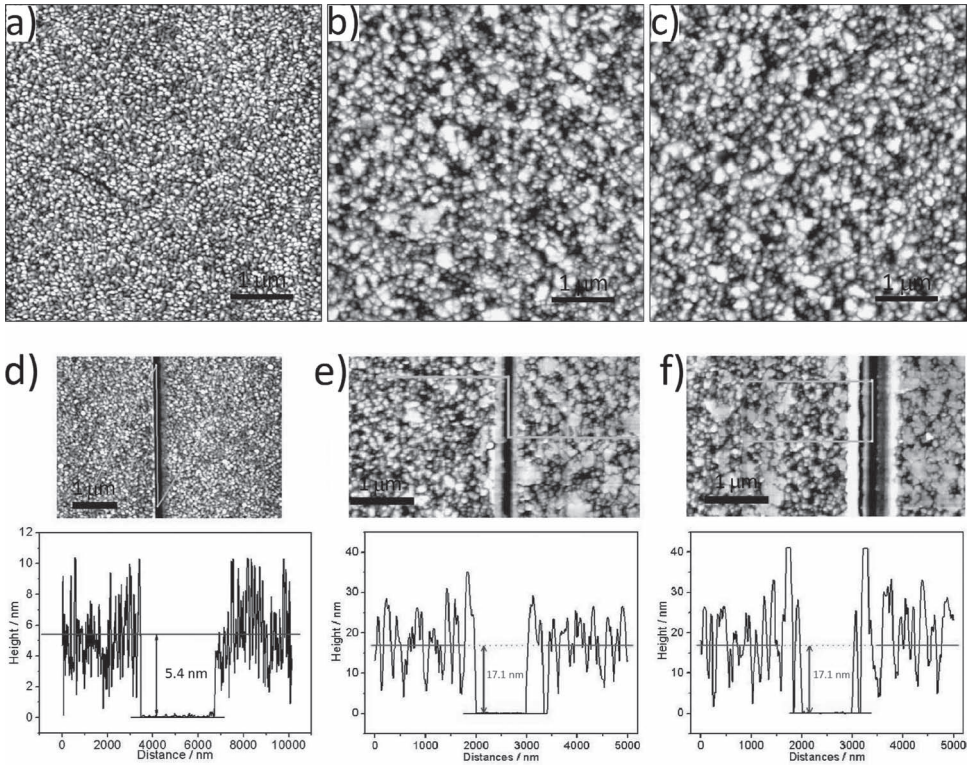



**Figure 1.** AFM studies of a) Au-coated QCM resonator and the tethered b) L- and c) D-PV films, and d) the scratching experiment results to study the film thickness of the Au coating, e) L-PV film tethered Au coating and f) D-PV film tethered Au coating. These results indicate that L- and D-PV films have identical surface roughness (10.49 nm for L-PV film and 10.66 nm for D-PV film) and film thickness (both are about 11.7 nm).

L-surface, the adsorption terrace appears at about 670 s, and the statistic average value for frequency shift at the terrace ($\Delta f_{t\text{-}L}$) is about −40.1 ± 1.8 Hz; whereas for the D-surface, the adsorption terrace appears at about 460 s with a $\Delta f_{t\text{-}D}$ value of about −27.0 ± 2.0 Hz, which corresponds to a difference in adsorption quantity for about 230.6 ng·cm$^{-2}$, according to the relationship between the adsorbed mass and $\Delta f$ (see SI). It clearly shows that the surface chirality strongly influences the protein adsorption dynamics, and proteins exhibit a stronger adsorption on the L-surface than that on the D-surface.

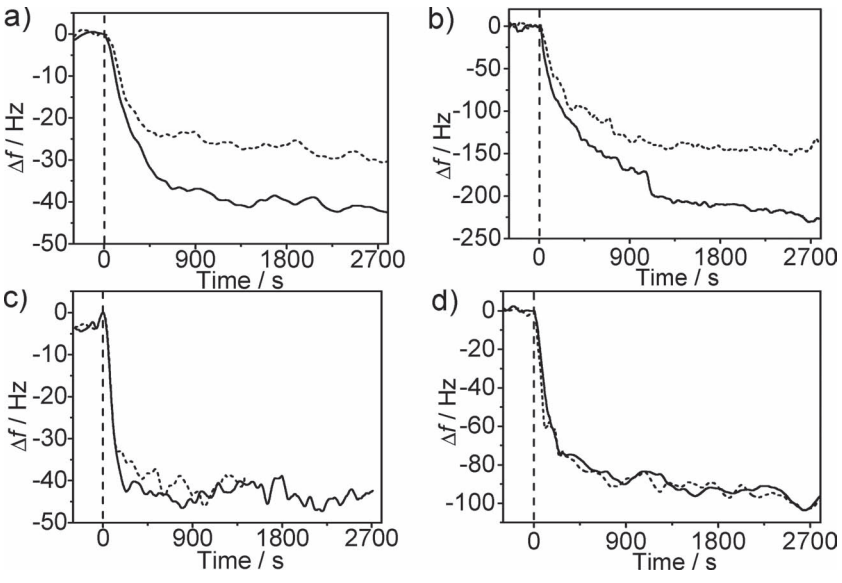



**Figure 2.** Time-dependent curves of frequency change ($\Delta f$) in QCM experiments. a,c) BSA adsorption and b,d) gelatin adsorption on a,b) chiral polymer (L-PV and D-PV) brush films and c,d) monolayer (L-MV and D-MV) surfaces. Solid lines: L-surfaces; dash lines: D-surfaces.

In order to investigate whether this effect is a special phenomenon dependent on specific protein or not, we repeated the experiment using other proteins, gelatin for example, under the same testing conditions. The curves were shown in Figure 1b, in which different adsorption behaviors of gelatin was also observed on the L- and D-PV films. Compared with BSA, the adsorption quantities of gelatin are much higher on both kinds of chiral polymer surfaces, and the difference between $\Delta f_{t\text{-}L}$ and $\Delta f_{t\text{-}D}$ reaches nearly 80 Hz (corresponds to an adsorption quantity difference of about 1408 ng·cm$^{-2}$). The corresponding AFM images of the films after BSA and gelatin adsorption are shown in **Figure 3**. Although the difference between AFM images of BSA adsorption on L- and D-PV films is insignificant (Figure 3a and

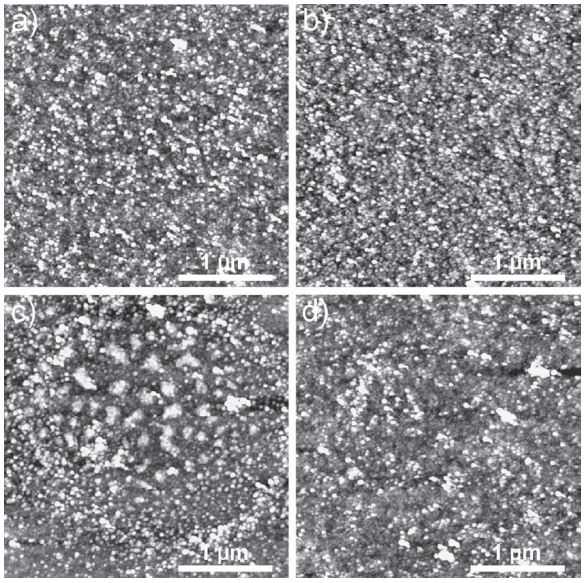



**Figure 3.** AFM images for a,c) L-PV and b,d) D-PV films after a,b) BSA and c,d) gelatin adsorption.

b), the gelatin adsorption on L-PV film (Figure 3c) exhibits obviously larger aggregates than that on the D-PV film (Figure 3d). This result is in good consistence with the QCM data shown above. Furthermore, it can be noticed that the adsorption quantity level of gelatin on both the L- and D-films is much higher than BSA. This can be explained by electrostatic interaction between proteins and the substrate, because both the L- and D-PV films are negatively charged, whereas BSA and gelatin are negatively and positively charged, respectively. This also indicates that the stereoselective interaction between proteins and chiral surfaces is so strong that it can even overwhelm the electrostatic interactions. Therefore, since that BSA and gelatin are two kinds of typical proteins with very different physicochemical properties, it can be inferred that the chiral effect for protein adsorption may be a general effect that is applicable for different protein species.

However, in the same experiment using the L(D)-MV coated resonators, the difference in protein adsorption is not significant. As shown in Figure 1c and d, the $\Delta f \sim t$ curves almost coincide with each other on L-MV and D-MV surfaces for the same protein (BSA or gelatin), although slight differences might still exist at the adsorption terrace. We suppose that this may be related to relatively weaker chiral characteristic of the self-assembly monolayers due to the ultrathin thickness and the consequently much lower surface density of the chiral terminal groups,[12] which also illustrates the advantages of chiral polymer brush films.

### 2.3. Fluorescent Titration and Fluorescent Microscopic Results for BSA/Polymer Interaction

The above measurements reveal that BSA and other proteins may have a stronger attraction to the L-PV film than the D-PV film. In order to clarify this point and the chemical nature of it, we used a fluorescent titration experiment to study the affinity between protein and chiral polymers.[34] In this experiment, a fluorescence labeled protein, BSA fluorescein isothiocyanate conjugate (FITC), was employed, which has a strong fluorescence emission with a peak at 521 nm upon excitation at 494 nm. Especially, this fluorescein has a property of proximity-dependent fluorescence self-quenching,[35] which means that guest binding to BSA-FITC can quench the fluorescent emission (fluorescence intensity decreases linearly with the increase of the interaction with guest molecules).

**Figure 4**a shows the relative fluorescence emission intensity of BSA-FITC at 521 nm in the presence of chiral polymers at room temperature (295 K). In this experiment, 5 μL L- or D-PV polymer solution probes (2 $mg \cdot mL^{-1}$) was titrated consecutively into 2 mL BSA-FITC mother liquors (20 $\mu g \cdot mL^{-1}$) every 6 min and the fluorescent emission was recorded in situ. The abscissa represents the number of titration times for the polymer probes. A significant reduction of fluorescent intensity appeared after the second time the polymer probe was added. Large differences were observed for L- and D-PV polymers, in which the declination speed for the L-PV polymer is apparently

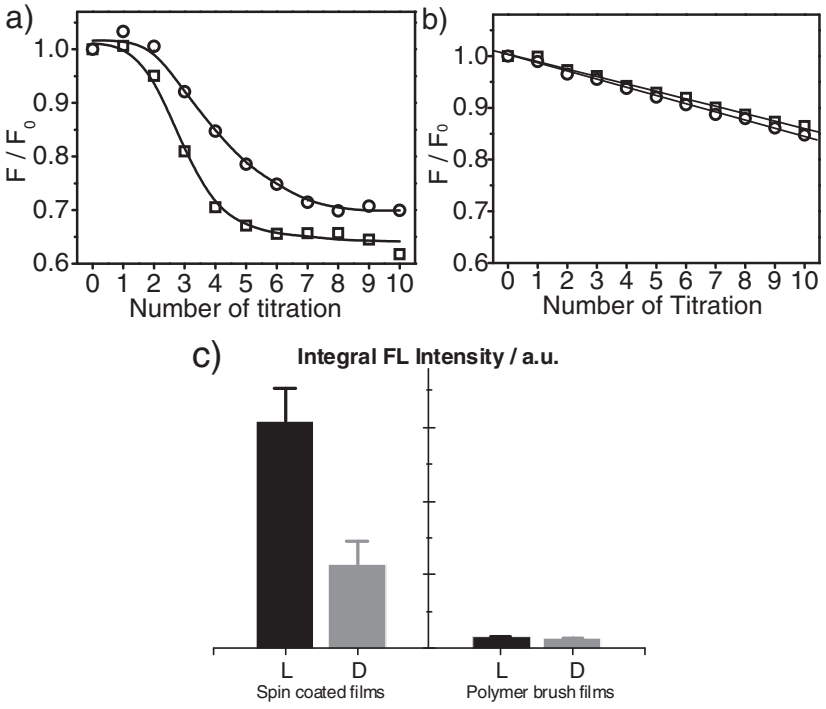



**Figure 4.** a,b) Fluorescent titration experiment results, in which the relative fluorescent intensity ($F/F_0$) at 521 nm was recorded in situ with the consecutive titration of 5 μL chiral probes into the BSA-FITC mother liquor. The abscissa is the number of times for the probe addition. a) Chiral polymer probes. Squares: L-PV; Circles: D-PV. b) Chiral monomer probes. Squares: L-MV; circles: D-MV. c) Integral fluorescent intensity of the adsorbed BSA-FITC on spin coated films and polymer brush films for L(D)-PV after deducting the background. The data were calculated by ImageJ software according to the FM images. Dark: L-PV film; grey: D-PV film.

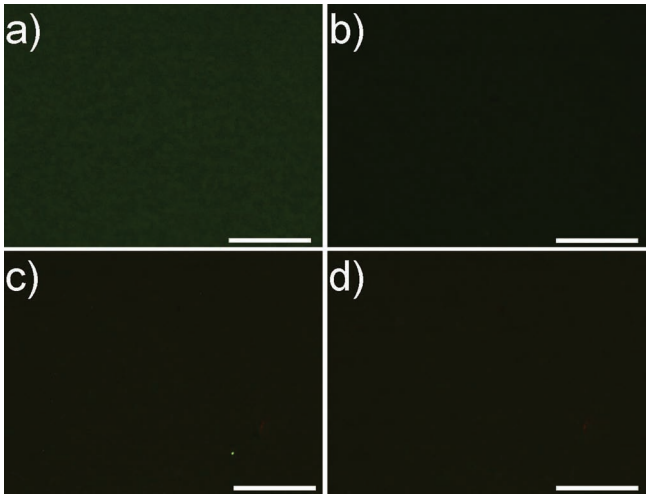



**Figure 5.** Fluorescence measurements for BSA-FITC adsorbed on chiral polymer films. a,b) spin coated films and c,d) polymer brush films. a,c) L-PV; b,d) D-PV. Scale bar: 100 µm. It can be noticed the BSA exhibit much larger adsorption quantities on the spin coated films, on which a distinctly higher fluorescent intensity was observed on the L-PV film than on the D-PV film. On the polymer brush films, since the adsorption level is low, the difference between the fluorescent intensities of L-PV and D-PV films is correspondingly very weak.

much faster than that for the D-PV, and shows an apparently higher quenching level at the equivalent state. Since the self-quenching of BSA-FITC is dependent on the proximity between proteins and the guest molecules, it can be inferred that in the polymer/BSA-FITC complexes, the L-PV is more proximate to the protein than the D-PV. Moreover, the association constants ($K_a$) between BSA-FITC and the chiral polymers can be calculated from the titration experiments according to literature (for calculation details, see SI).[36,37] The results of the calculation indicate that the $K_a$ values for L-PV are about one order of magnitude higher than those for D-PV, which represents a remarkably stronger affinity for L-PV towards BSA. This was further confirmed by a fluorescent microscopic (FM) study (Figure 4c, see **Figure 5** for the FM images) for the BSA-FITC adsorption on both of the spin-coated chiral polymer films and for the corresponding polymer brush films. It was a higher fluorescent intensity for the BSA-FITC adsorption on both two kinds of L-PV films than on the corresponding D-PV films. Figure 4b shows another fluorescent titration results (295 K) for the monomers of L(D)-acryloylated valine (L-MV and D-MV), in which no evidential difference could be observed. This indicates that, as small molecules, the monomer enantiomers exhibit approximate interactions with BSA-FITC. All these data are in good consistence with the QCM experiments.

Protein-guest interaction is driven by several distinct but interdependent mechanisms, e.g., hydrogen bonding (H-bonding), electrostatic, and hydrophobic interactions, etc.[38,39] In order to clarify this point, the thermodynamic parameters, including the enthalpy change ($\Delta H$) and the entropy change ($\Delta S$),[40] of the polymer/BSA-FITC binding process were studied by measuring the association constants at different temperatures (295 K and 301 K). The thermodynamic parameters were determined according to the van't Hoff equation, as summarized in SI. It shows that the formation of polymer/BSA-FITC complex is an exothermal reaction, being accompanied by positive $\Delta S$ values for both L- and D-PV, which are usually considered empirically as an evidence for a hydrophobic interaction dominated process. However, for the L- and D-MV monomers, it reveals an H-bonding dominated process for the monomer/BSA-FITC complex formation (endothermic reaction accompanied with negative $\Delta S$ values), which does not present an evidential stereoselectivity for L- and D-MV enantiomers. On the other hand, since the valine residues and BSA (pI = 4.7) are all negatively charged due to ionization in PBS, electrostatic interactions cannot be expected for the binding process and the stereoselective interaction between the chiral polymers and BSA in this study. Thus, it can be concluded that the main driving force for the differential adsorption of BSA and binding behaviors of the L- and D-PV polymer films is the stereoselective hydrophobic interaction. The steroselective hydrophobic interaction may be related to conformational matching (or lack thereof) between the terminal chiral moieties of the polymers and the hydrophobic domains of proteins. According to literature, hydrophobic interactions are the major driving forces governing protein adsorption on a substrate; therefore, the manipulation of hydrophobic interactions in protein adsorption is always an important topic in developing new protein-oriented biomaterials.[41] The stereoselective hydrophobic interaction between proteins and chiral polymer films provides a novel approach for such research. On the other hand, this also explains why L-PV and D-PV films exhibit differential adsorption quantities for different kinds of proteins, e.g., BSA and gelatin, although they have opposite electrostatic interactions with the negatively charged polymer films, which induces markedly different adsorption quantity ranges.

## 3. Conclusions

The influence of surface chirality of polymer films on protein adsorption shows that the chiral effect can act as a novel strategy for designing biointerface materials from a biomimetic point of view. Hydrophobic interactions are an important part of this effect for the L(D)-amino acids based chiral polymer films, suggesting that a successful matching (or lack thereof) between the stereo-configurations of the nonfunctional hydrophobic side groups (e.g. alkyl or aromatic groups, etc.) of the chiral polymers and the hydrophobic domains of proteins is an important factor governing the protein/polymer interaction. Further investigation of this effect may also give an interesting insight to the understanding of high chiral preference in natural biological systems. Furthermore, the design of side-chain chirality for the polymer brushes brings the possibility to conveniently combine other functionalities for developing novel functional bio-devices.[42–45]

## 4. Experimental Section

*Grafting Chiral Polymer Brushes on QCM Resonators*: A cleaned QCM resonator was immersed in a solution of ethanol (10.0 mL) containing 2-mercaptoethylamin hydrochlorid (22.7 mg) and triethylamine

(22.3 mg) for overnight to produce surface–$NH_2$ groups on gold. After that, it was rinsed with ethanol and dichloromethane sufficiently, dried by nitrogen flow, and then immersed in a solution of dichloromethane with 2% (v/v) pyridine. Bromoisobutyryl bromide (0.5 mL) was added dropwise into above solution at 0 °C, and the mixture was left for 1 h at this temperature, and then at room temperature for 12 h. The polymerization was conducted by immersing the cleaned and dried resonator in a degassed solution of *N*-acryloyl-L(D)-valine monomer (4.0 mmol) in a 1:1 (v/v) mixture of $H_2O$ and MeOH (8.0 mL) containing Cu(I)Br (23.0 mg) and PMDETA (0.1 mL) for 3 h at 60 °C. After the polymerization process, the resonator was immersed in and rinsed with methanol, ethanol, and de-ionized water, respectively, to remove all the possible impurities. Other experimental details, e.g., the monomer synthesis, characterizations of the monomers and polymer brushes can be found in the SI.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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