Polymer Chemistry

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ARTICLE

pH-Responsive and Selective Protein Adsorption on an Amino Acid-Based Zwitterionic Polymer Surface

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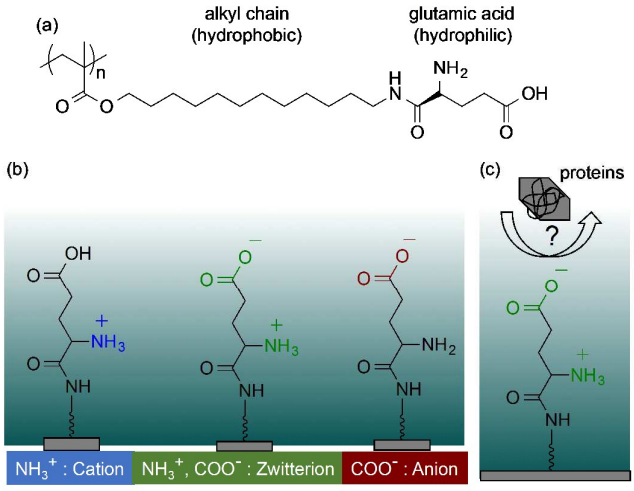
The synergistic interactions between the α-amine and the carboxylic acid in an amino acid have recently been studied as bio-based zwitterions. Here, we report a new amphiphilic polymer containing glutamic acid grafted to the end of a dodecyl polymer side chain, which contains the α-amine and the γ-carboxylic acid of the glutamic acid moiety. The polymer self-assembled into a multilayer structure in the thin film, and the glutamic acid moieties in the polymer side chains were exposed to the polymer film/water interface. Due to the presence of the glutamic acid moieties at the interface, the surface charge was controllable by pH in buffer solutions, resulting in zwitterionic character at neutral pH. It has been widely accepted that zwitterionic surfaces can exhibit non-fouling for proteins. Interestingly enough, the polymer film showed charge-selective protein adsorption since the synergistic interaction between the α-amine and the γ-carboxylic acid was weaker than conventional amino acid-based zwitterionic systems. This is due to the separated state of the functional groups by a three carbons spacer.

Introduction

Controlling surface properties of materials by functionalization with organic materials, such as polymers, makes it possible to expand the possibilities of applications. [1-3] In the field of surface modification, interest in design of biomaterial surfaces with biocompatible properties, especially non-fouling for proteins, has increased in both academia and industry. [4, 5] This has led to improvements in surface modification technology and produced many devices. For biomedical devices used in the human body (eg, catheters and stents) adsorption of proteins in biological fluids onto the device surfaces cannot be ignored due to the important performance problem stemming from protein adsorption. [6] To solve the problem, surface modification by immobilizing or coating bio-based molecules on the surfaces is often employed. It is well known that surface with betaine-based zwitterions, such as carboxybetaine, sulfobetaine and phosphobetaine, provide non-fouling properties. [7, 8] This is especially true of phosphobetaine whose structure is similar to the main components of a cell membrane, thus promoting the high biocompatibility. [9-11]

The zwitterionic property of amino acids has only just begun to be considered as a new bio-based surface modifier in recent years. Some groups demonstrated the biocompatibility of cysteine-anchored nanoparticles. [12, 13] The thiol group of cysteine was used as the reaction point to immobilize the amino acid onto the particle surfaces, producing nanoparticles covered with the α-amines and the carboxylic acids of the amino acid. The difference of their pK_a values makes it possible to control the surface charge by pH in buffer solutions. At acidic pH (pH < pK_a^{COOH}), the amines are protonated and ionized on the surface to positive, while a negative surface is provided at basic pH values (pH > pK_a^{NH3+}) due to the deprotonated carboxylic acids. Both of the groups are ionized at intermediate pH (pK_a^{COOH} < pH < pK_a^{NH3+}), providing non-fouling behavior resulting from the zwitterionic character. Other recent research on preparation of surfaces with amino acids has used polymer brushes. [14-17] Similar to the above example, the polymer brushes containing amino acids composed of the α-amine and the



Scheme 1. (a) Chemical structure of PGluDMA. (b) pH-responsiveness of the glutaimic acid in the polymer side chains exposing to the polymer film/water interface. (c) Unknown protein adsorption behavior of the zwitterionic state on the polymer film at neutral pH .

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carboxylic acid showed biocompatibility and pH responsiveness. In previous research on the surface functionalization with amino acid-based zwitterions, the synergistic interaction as zwitterions in amino acids was focused only on the combination of the α-amine and the α-carboxylic acid, whereas there has been no report considering the side chain of amino acids to design zwitterions. A synergistic interaction of a functional group in the side chain with the α-amine or the α-carboxylic acid in amino acids may provide new zwitterionic properties.

Herein, we designed and synthesized an amino acid-based zwitterionic polymer (PGluDMA) containing a glutamic acid grafted to the end of a dodecyl hydrocarbon chain as the side chain (Scheme 1a). It is worthwhile to note that the zwitterionic part of the glutamic acid moiety is composed of the synergistic interaction between the α-amine and the γ-carboxylic acid, which is different from the conventional amino acid-based zwitterions. Amphiphilic structures in polymer side chains, such seen in PGluDMA, lead the polymers to self-assemble into multilayer structures in the film on a substrate. [18] Additionally, the hydrophilic moieties in the amphiphilic structure are exposed to the polymer film/water interface, which presents the characteristic properties of the hydrophilic moieties on the surface. [19-21] Hence, as illustrated in Scheme 1, we predicted that if the glutamic acid moieties in PGluDMA are exposed to the polymer film/water interface, the surface charges should be controllable by changing the charge state of the functional groups by pH in buffer solutions. Furthermore, we expected that the synergistic interaction between the functional groups provides characteristic protein adsorption behavior.

Results and Discussion

Synthesis and characterization of PGluDMA films

PGluDMA was synthesized as outlined in Scheme S1. We first prepared a methacrylate monomer with a glutamic acid whose α-amine and γ-carboxylic acid were protected. The monomer was polymerized by reversible addition-fragmentation chain transfer (RAFT) polymerization, and then deprotected the Boc and t-Bu groups by TFA treatment to produce the final compound. We confirmed the chemical structures in each step by ^{1}H and ^{13}C NMR and FAB-MS (see supporting information). The weight average molecular weight was calculated from the molecular weight of the precursor polymer because the amphiphilic nature of the PGluDMA solution does not allow its introduction into a gel permeation chromatography column. The weight average molecular weight and the polydispersity of the precursor polymer were determined to be 4.3 $\times$ 10^4 g/mol and 1.10 by gel permeation chromatography coupled with multi-angle light scattering (SEC-MALS) measurements (Figure S1). From these values, the molecular weight of PGluDMA was calculated to be 3.9 $\times 10^4$ g/mol.

A thin film of PGluDMA was prepared on a silicon substrate by spin coating from 2,2,2-trifluoroethanol solution at a concentration of 1.0 wt% polymer. The film thickness was determined to be 50 nm by eilipsometry. The self-assembled structure in the film was investigated with grazing incident small angle X-ray scattering (GISAXS) measurements. Figure 1a-d shows representative 2D GISAXS patterns measured with incident angle, α_i, of 0.08° and 0.16° for the as-cast and the thermally annealed polymer films. The in-plane and out-of-plane scattering profiles extracted along the q_y direction at q_z = 0.094 nm^{-1} and the q_z direction at q_y = 0.35 nm^{-1}, respectively, are shown in Figure 1e-f. Isotropic scattering patterns whose relative scattering vector ratio is 1 : 2 indicated that lamellar structures are present in the as-cast film, whereas the patterns changed to two spots along the meridian after annealing. As can be seen in the scattering profiles described in Figure 1e-f, the peaks in the in-plane scattering profile from as-cast film disappeared after annealing as the out-of-plane peaks became sharper. These results indicate that the lamellar layers were oriented and ordered parallel to the substrate after thermal annealing. As displayed in Figure 1c, the scattering with α_i = 0.08° for the thermally annealed film also showed spot patterns, indicating that the oriented and ordered nanostructure was formed through to the outermost surface. The anisotropic scattering pattern at q = 12.6 nm^{-1} from the annealed film was also observed (Figure S4a). The *d*-spacing determined from the q value is 0.499 nm, being assignable to the reflection from interdistance between adjacent alkyl chains in the polymer side chains. The anisotropic scattering behavior indicates that the side chains were ordered parallel to the thickness direction in the lamellar structures. The lamellar layer thickness determined from the q value of the first peak position (q = 1.57 nm^{-1}) was 4.00 nm, which is shorter than twice the polymer side chain (4.80 nm) for the fully stretched alkyl chains. This difference can be explained by the FT-IR spectrum of the bulk polymer, which indicated that the alkyl chains in the polymer side chains are not all-trans (Figure S4b). In addition, the polymer side chains may be partially interdigitated between the lamellar layers because of the formation of a quadrupole from two primary amine and carboxylic acid pairs. With these considerations, we propose a model of multilayer structure in PGluDMA film, and it is shown in Figure S4c.

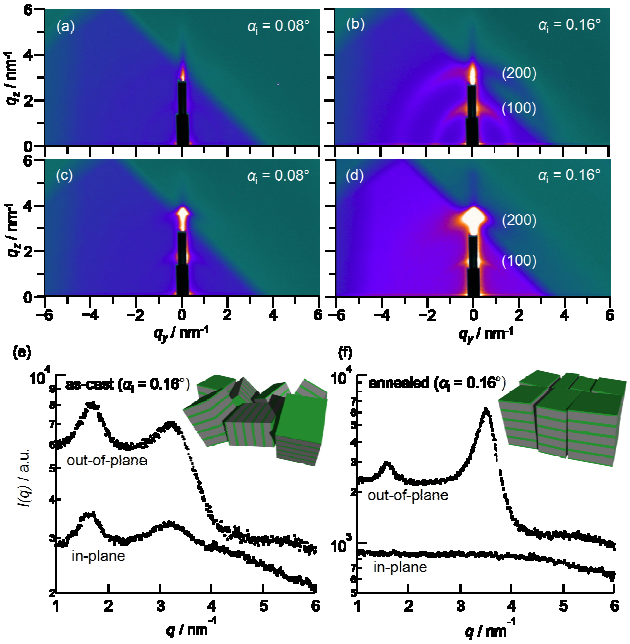

Figure 1. Representative 2D GISAXS patterns for PGluDMA films coated on silicon substrates, which were measured with α_i = 0.08° and 0.16°. Out-of-plane and in-plane scattering profiles extracted along the q_z direction at q_y = 0.35 nm^{-1} and the q_y direction at q_z = 0.094 nm^{-1}, respectively: as-cast film (a, b, e); thermally annealed film (c, d, f). The as-cast film was dried under vacuum at 25 ℃ for over 24 h after spin coating. The thermally annealed film was prepared by annealing at 100 ℃ for over 6 h under vacuum after spin coating, and drying at 25 ℃ for over 24 h.

Surface wettability and pH-responsiveness on PGluDMA films

The results of static contact angle measurements for water (ϑ) in air and for an air bubble in water (φ) for the polymer films are summarized in Table 1, and typical photographs of water and air bubble droplets on the surfaces are displayed in Figure 2. The definition of the static contact angles of ϑ and φ are shown in Figure S5. The static contact angles of water on the as-cast and the thermally annealed polymer film were 65° and 81°, respectively, demonstrating low hydrophilicity. However, the films repelled air bubbles in water with static contact angles (φ) indicating greater than 140°. For the as-cast film, air bubbles were not pinned to the surface in water, thus showing superhydrophilicity. The enhancement of hydrophilicity on the surfaces in water indicates that the surface chemical compositions were switched in response to their surrounding environment to minimize the interfacial energy as has been observed with an amphiphilic block copolymer. [22-25] This led us to believe that the glutamic acid moieties in the polymer side chains were laid into the bulk at air interface, whereas the moieties were exposed to the water interface when the film was immersed into water. If so, the more hydrophobic behavior of the thermally annealed film compared to that of the as-cast one can be assigned to the difference of the molecular motion of the side chains. This motion should be relatively restricted in the oriented and ordered nanostructure.

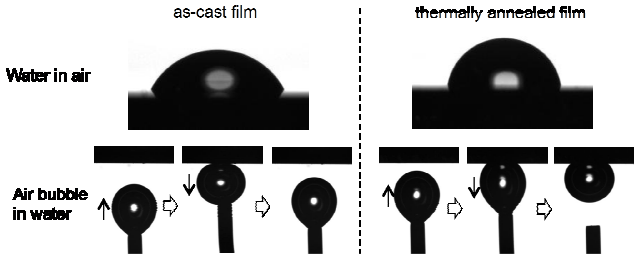

Figure 2. Photographs of water droplet in air and an air bubble droplet in water on the as-cast and the thermally annealed PGluDMA films.

Table 1. Contact Angles of Static of Water in Air (ϑ) and Air Bubble in Water (ψ), Advancing (ϑ_A) and Receding (ϑ_R) on As-cast and Thermally-annealed PGluDMA Films

sample	ϑ [a] (deg)	ψ [b] (deg)	ϑ_A /ϑ_R (deg)	$\vartheta_A - \vartheta_R$ (deg)
	Water in air	Air bubble in water		
as-cat	65	–	82/25	57
annealed	81	140	83/32	51

[a] Static contact angles were measured with a 2 µL droplet of water in air.
[b] Static contact angle of an air bubble (10 µL) were measured in water.
[c] Dynamic contact angles were measured by the controlled drop method with 30 µL of water.

To confirm the surface reorganization, the advancing (ϑ_A) and receding (ϑ_R) contact angles were measured by the controlled drop method. A large hysteresis in the advancing and the receding contact angle for the polymer films was observed (Table 1). The magnitude of $\Delta\vartheta$ ($\vartheta_A - \vartheta_R$) depends on the surface roughness and reorganization. [26] The root-mean-square surface roughness values of the polymer films in air and in water were less than 0.5 nm in a 5 × 5 µm^2 scanning area (Figure S6). Therefore, the large hysteresis was attributed to the surface reorganization, in agreement with the above observations. In addition, the smaller $\Delta\vartheta$ value for the thermally annealed film compared with the as-cast one also indicates the restricted motion of the polymer side chain due to the relatively oriented and ordered nanostructure.

Figure 3a shows the pH dependence of the surface zeta potential for the as-cast and the thermally annealed polymer films. Since the glutamic acid in the polymer side chain includes α-amine and γ-carboxylic acid, it can form three charged states; positive (pH < pK_a^{COOH}), neutral (pK_a^{COOH} < pH < pK_a^{NH3+}) and negative (pH > pK_a^{NH3+}). The surface charge observed to undergo these three states with response to pH. The switching points of the surface charge indicate the estimated pK_a values of the α-amine and γ-carboxylic acid in the polymer side chains. The pK_a values of the functional groups in this polymer are slightly different from that of L-glutamic acid (pK_a^{COOH} ~ 4.1, pK_a^{NH3+} ~ 9.5), which is explained by the polyelectrolyte effect: the crowded states of charges in polyelectrolytes induce a weaker pH response compared with that of the corresponding small molecules. [27] We also investigated the pH dependence of the static contact angle on the films. The static contact angle on the as-cast film obviously changed depending on pH in buffer solutions, whereas the thermally annealed film showed constant static contact angle throughout the pH region. In the positive and negative charged states, local surface organization should occur due to the electrostatic repulsion among the charged glutamic acid moieties, which made the surfaces more wettable than the neutral condition where the ionized functional groups compensated each other. The pH-independent wettability of the

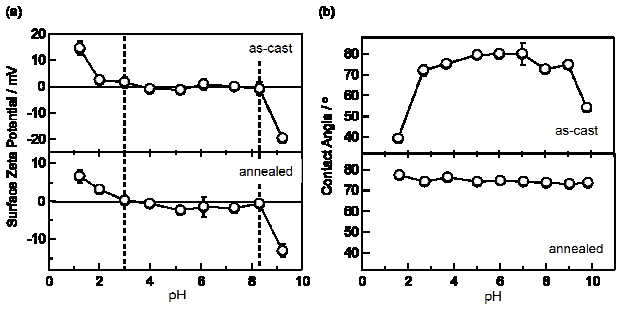

Figure 3. pH-dependence of surface zeta potential (a) and static contact angle (b) on the as-cast and the thermally annealed PGluDMA films. Upper and lower sides represent the as-cast and the thermally annealed films, respectively. Various buffer solutions were used to control the solution pH in these measurements.

thermally annealed film resulted from the more restricted side chain motion than the one of as-cast film.

Charge-selective protein adsorption on PGluDMA films

Protein adsorption on PGluDMA film was studied with quartz crystal microbalance (QCM) measurements. To investigate the charge effect of the proteins on the adsorption property, bovine serum albumin (BSA) and lysozyme (Lys), whose isoelectric points are pH 4.7 and pH 11, were used in this study. The proteins were dissolved in 100 mM Tris-HCl buffer (pH 7.5), in which BSA and Lys are charged negative and positive, respectively. Figure 4 shows typical frequency shifts for the adsorption of the proteins (1.0 g/L) on the as-cast and the thermally annealed polymer films in the buffer solution. There was no detectable frequency shifts for BSA, indicating that the non-fouling property was observed for BSA. On the other hand, immediate frequency decreases were observed

after the injection of Lys. The magnitudes of the frequency shifts for the polymer films were larger than those for a gold substrate (Figure S7). As mentioned in the above, the thermal annealing indicates a nanostructure in the polymer film that is oriented and ordered to the outmost surface, leading to restriction of the motion of the side chains, which resulted in differences in the amount of lysozyme adsorption on the as-cast and the thermally annealed films.

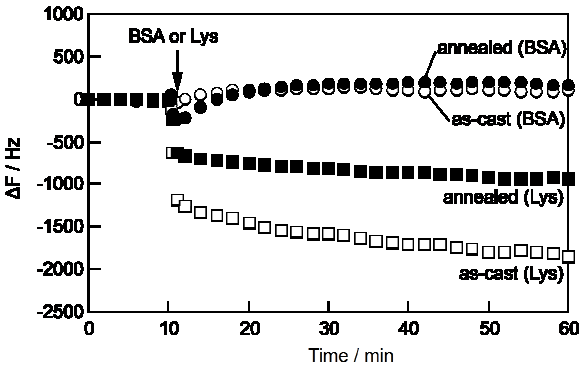

Figure 4. Typical frequency shifts for BSA and Lys adsorption on the as-cast and the thermally annealed PGluDMA films in 100 mM Tris-HCl buffer solution (pH 7.5). Circle and square represent BSA and Lys, respectively, and the filled symbols represent the thermally annealed films.

Kusumo and co-workers demonstrated charge-selective protein adsorption on a poly(2-(dimethylamino)ethylmethacrylate) (PDMAEMA) brush. [28] Negatively charged BSA adsorbed on the PDMAEMA brush surface, but no adsorption ability appeared in positively charged lysozyme. However, the antifouling for proteins with the same net charge as polyelectrolyte brushes is not always observed. Poly(styrenesulfonic acid) brushs and poly(acrylic acid) brushs exhibited considerable adsorption of negatively charged proteins including fibrinogen and BSA, indicating that protein adsorption may derive from local interactions with oppositely charged parts on the protein surface. [29, 30] In addition, the size of proteins, the charge distribution on protein surfaces, and the pH and ionic strength in buffer solutions also may reflect protein adsorption behavior. [31-35] It is generally assumed that surfaces covered with zwitterions show a non-fouling property for proteins irrespective of the charge. In other reported cases of amino acid-based zwitterions comprised of the α-amine and the carboxylic acid, [12-17] though those functional groups are weakly basic and acidic at neutral state, they can synergistically interact with each other owing to their adjacent placement, resulting in providing non-fouling properties. According to a report by Jiang et al., the number of carbons between an amine and a carboxylic acid in a side chain of a betaine-based polymer plays a significant role in the fouling behavior. [36] It is suggested that the longer spacer localizes the charge densities of the functional groups, resulting in protein adsorption onto the surfaces.

For the PGluDMA system, there are three carbons between the α-amine and the γ-carboxylic acid of the glutamic acid in the polymer side chain as the spacer, which separates the two functional groups resulting in localization of their charge densities (Figure S8). The charge localization in the glutamic acid moieties makes the synergistic interaction weaker than the one in the conventional amino acid-based zwitterions. Because amine and guanidine in the side chain of basic amino acids, including lysine and arginine, possess high affinity to carboxylic acids, the weak synergistic interaction pair of the α-amine and the γ-carboxylic acid in the glutamic acid may be ion-exchanged to the pair of the basic functional groups and the γ-carboxylic acid when the basic amino acid moieties on protein surfaces approach to the polymer film surface. Although the basic amino acids are also comprised in negatively charged BSA as well as positively charged lysozyme, lysozyme only adsorbed onto the surface of PGluDMA films. As mentioned above, the protein size, the charge distribution on protein surfaces, and the pH and ionic strength in buffer solutions are important factors in protein adsorption. These effects may also lead the charge-selective protein adsorption behavior on the surface of PGluDMA films.

Conclusions

In conclusion, we have synthesized a new amphiphilic polymer, PGluDMA, containing side chains of glutamic acid grafted to the end of a dodecyl hydrocarbon chain, and have found that the polymer spontaneously self-assembles into multilayer structures in the thin film on a silicon substrate. At the water interface, the surface charge was controllable by pH in buffer solutions owing to the presence of the α-amine and γ-carboxylic acid groups of the glutamic acid in the polymer side chains, resulting in a zwitterionic surface at neutral pH. The polymer films showed charge selective protein adsorption behavior, which is attributable to the relatively localized functional groups in the glutamic acid moieties by the long carbon spacer between the α-amine and the γ-carboxylic acid. The separated state of the functional groups in the glutamic acid make the synergistic interaction weak, which might make it possible to induce an ion-exchange reaction between the carboxylic acid on the outermost surface and the cationic groups on positively charged proteins. Such surface functionalization with a new zwitterion derived from the combination of a functional group attached to α-carbon with a side chain of amino acids may open up a new avenue to design smart materials.

Experimental

Materials

All chemical agents were purchased from Tokyo Chemical Industry Co., Sigma-Aldrich Co., or Watanabe Chemical Co., and were used without further purification.

Synthesis of PGluDMA

Synthesis of Boc-L-Glu(OtBu)-dodecanol (1): 12-amino-1-dodecanol (0.600g, 2.98 mmol), Boc-L-Glu(OtBu) (0.994 g, 3.28 mmol), and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) (0.910g, 3.28 mmol) were dissolved in dry methanol (20 mL) at RT. The mixture was stirred for 4 hr at RT. The reaction was quenched with water (10mL), and the mixture was washed with saturated NaCl solution. The organics was dried over $MgSO_4$ and concentrated in *vacuo*. The crude product was purified by column chromatography eluted with ethyl acetate/hexane (1:1), which afforded colorless oil (yield: 1.32 g, 91 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.34 (s, 1H), 5.30 (d, J = 7.76 Hz, 1H), 4.07 (br, 1H), 3.62 (t, J = 6.62 Hz, 2H), 3.22 (q, J = 6.67

Hz, 2H), 2.42 – 2.23 (m, 2H), 2.08 – 1.81 (m, 2H), 1.55 (m, 2H), 1.47 – 1.41 (m, 18H), 1.32 – 1.22 (m, 18H).

Synthesis of Boc-L-Glu(OtBu)-dodecyl methacrylate (2): Compound 1 (1.32 g, 2.71 mmol) dissolved in DCM (10 mL) was added in trietylamine (1.37 g, 0.136 mol) at RT. The solution was cooled to 0 °C and DCM solution (10 mL) of Methacryloyl chloride (0.425 g, 4.07 mmol) was added slowly. The reaction mixture was stirred for 1 hr at 0 °C, and then allowed to RT and stirred for 1 hr. The reaction was quenched with saturated $NaHCO_3$ solution (10mL), and the mixture was washed with saturated solution of sodium chloride. The organics was dried over $MgSO_4$ and concentrated in *vacuo*. The crude product was purified by column chromatography eluted with ethyl acetate/hexane (1:3), which afforded colorless oil (yield: 1.19 g, 79 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.49 (s, 1H), 6.04 (s, 1H), 5.49 (s, 1H), 5.41 (d, J = 7.96 Hz, 1H), 4.08 (t, J = 6.68 Hz, 2H), 4.10 – 4.00 (br, 1H), 3.18 (m, 2H), 2.35 – 2.22 (m, 2H), 2.05 – 1.79 (m, 2H), 1.89 (s, 3H), 1.62 (m, 2H), 1.39 (m, 18H), 1.21 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 172.7, 171.5, 167.5, 155.7, 136.5, 125.1, 80.7, 79.8, 64.8, 53.9, 39.5, 31.8, 29.5, 29.5, 29.4, 29.2, 29.2, 28.6, 28.3, 29.7, 26.8, 25.9, 18.3. FAB-MS (M+H): calc for 554.4, found 555.1.

Synthesis of Poly(Boc-L-Glu(OtBu)-dodecyl methacrylate) (3): Compound 2 (0.540g, 0.973 mmol), 4-cyanopentanoic acid dithiobenzoate (CPDB) (2.72 mg, 9.73 µmol), and 2,2'-Azobis(2-methylpropionitrile) (AIBN) (0.32 mg, 1.95 µmol) were dissolved in anisole (2.2 mL). After argon gas bubbling for 20 min at RT, the reaction mixture was stirred for 4 days at 60 °C. The reaction was quenched in liquid nitrogen. The polymer solution was precipitated into hexane and dried under *vacuo* (yield: 0.459 g, 85 %). The polymer and AIBN (120 mg) were dissolved in anisole (3 mL) at RT. The reaction solution was allowed to 80 °C and stirred for 2 hr. After cooling to RT, the polymer solution was precipitated into hexane and dried under *vacuo*. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.98 (br, 1H), 5.74 (br, 1H), 4.16 (br, 1H), 3.91 (br, 2H), 3.19 (br, 2H), 2.32 (br, 2H), 2.04 – 1.87 (br, 2H), 1.59 (br 2H), 1.42 (br, 18H), 1.26 (br, 18H), 1.00 – 0.80 (br, 3H).

Synthesis of Poly(Glu-dodecyl methacrylate) (PGluDMA): Polymer 3 (0.328 g) was dissolved in trifluoroacetic acid (TFA) (2 mL) at RT. The reaction solution was stirred for 2 hr. The polymer solution was precipitated into dichloromethane and dried under vacuo (yield: 0.267 g, 91 %). ^{1}H NMR (400 MHz, methanol-d_4): δ (ppm) = 4.00 (br, 1H), 3.96 (br, 2H), 3.24 (br, 2H), 2.50 (br, 2H), 2.16 (br, 2H), 1.69 (br, 2H), 1.57 (br, 2H), 1.36 (br 18H), 1.00 – 0.80 (br, 3H).

Characterizations

Sample preparation. Polymer thin films were prepared by spin coating at 2000 rpm for 60 s from 1.0 wt% polymer solution in 2,2,2-trifluoroethanol (TFE). The films were annealed at 100 °C for over 6 h under *vacuo* to prepare the annealed films.

SEC-MALS. Size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) measurement was carried out for polymer 3 solution using the polystylene columns (TOSOH TSK-GEL G3000HXL, 1 mL/min) at 40 °C with THF as the eluent. We prepared the polymer solution of 1.0 wt% in THF. The solution was optically purified with PTFE membrane with 0.2 µm pore and injected it into the column. The output from the column was then passed sequentially through a multi- angle light scattering (MALS) detector (Wyatt Technology, Dawn HeleosII, wavelength: λ = 658 nm) and an RI detector (Shimadzu, RID-10A). We determined the specific refractive index increments ($\partial n / \partial c$) of the polymer in THF solution with a differential refractometer (Otsuka Electronics DRM-1020, wavelength: λ = 633 nm).

GISAXS and SAXS measurements. GISAXS and SAXS measurements were carried out on the BL40B2 beamline at the SPring-8 facility, Hyogo Prefecture, Japan. We used a 3000 × 3000 pixel size of 100 × 100 µm^2 imaging plate (Rigaku R-AXIS VII) detector placed 64.2 or 59.0 cm from the sample. The wavelength of the X-ray beam was 0.10 nm. The magnitude of the scattering vector (q) defined as $q = 4\pi \sin\theta / \lambda$ with a scattering angle of 2ϑ.

For GISAXS measurements, the polymer thin films were prepared on disk-shaped silicon (111) wafers with 1-inch diameter and 3 mm thickness by spin coating. For α_i = 0.08° < 0.12 °, only the surface structure information of the polymer thin film is provided due to the limited penetration depth (~1 nm) of the X-ray. When incident angle is adjusted to α_i = 0.16° > 0.12 °, the X-ray penetrates the film down to the substrate, and the scattered X-ray includes the structure information of the overall film including bulk and surface structures. In this study, we used the X-ray with α_i = 0.08°, 0.12°, 0.16° to evaluate the structure of the polymer films.

Contact angle measurements. The static and dynamic contact angel measurements were performed with a Theta T-200 Auto 3 (Biolin Scientific Oy, Helsinki, Finland) equipped with a video camera. A 2 µL water droplet was used in static contact angle measurements. The contact angles of an air bubble in water were measured on the polymer film facing downward in a square transparent glass vessel filled with deionized water. The air bubble droplet (10 µL) was released from beneath the polymer film substrate using a microsyringe. The dynamic contact angle was measured by the controlled drop method with 30 µL water. In this method, ϑ_A and ϑ_R can be determined by increasing or decreasing the volume until the three-phase boundary moved over the solid surface. The pH dependence of the contact angle for the polymer film was measured with pH buffer solutions of 100 mM glycine-HCl buffers (pH 1 − 3), 100 mM citrate buffers (pH 4 − 6), 100 mM Tris-HCl buffers (pH 7 − 8) and 100 mM sodium bicarbonate buffers (pH 9 − 10).

Surface zeta potential measurements. Surface zeta potential measurements were performed at room temperature with an ELSZ-2 (Otsuka Electronics, Japan). We used polystyrene latex particles (500 nm in diameter) coated with hydroxypropyl cellulose (M_w = 30000 g/mol) dispersed in pH buffer solutions as a tracer. Before the measurements, the polymer films were immersed in the buffer solution for at least 10 min to reach equilibrium.

AFM observations. AFM observations were performed with a Cypher AFM system (Asylum Research, Santa Barbara, CA, USA) fitted with a standard silicon nitride cantilever (OMCL-TR800PSA (S)) and operated in contact mode at room temperature in air and water atmosphere. Topographic imaging was performed in air and in water. Before the observations in water, the polymer films were immersed in water for at least 10 min to reach equilibrium.

QCM. Interactions between proteins, such as BSA and Lysozyme, and PGluDMA films was evaluated with an Affinix QN using 27 MHz gold electrodes (initium Inc., Japan). Gold electrodes were cleaned by washing with piranha solution for 5 min three times. The polymer films were prepared on the gold electrodes by spin coating at 2000 rpm for 60 s from 1.0 wt% polymer solution in 2,2,2-

trifluoroethanol (TFE). The interaction of the proteins with the polymer films were evaluated at 25 ℃ in 100 mM Tris-HCl buffer solution (pH = 7.5) with the proteins (1.0 g/L).

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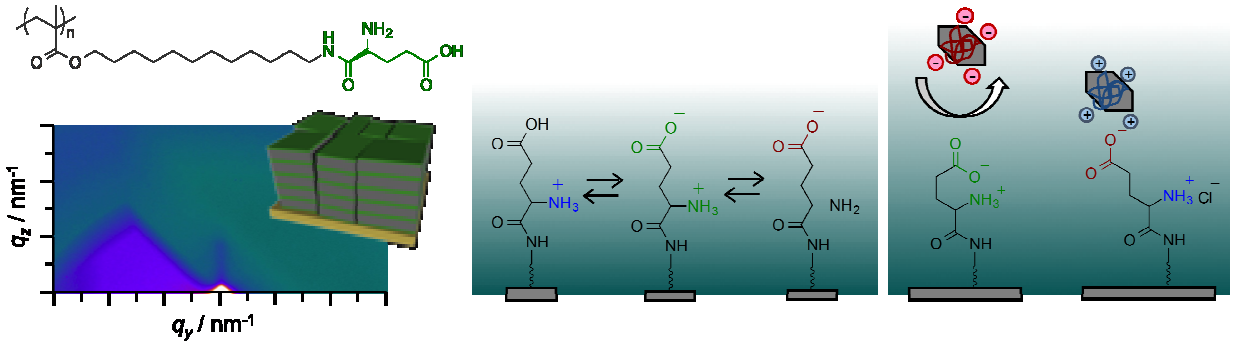

An amphipihlic polymer bearing glutamic acid in the polymer side chain was used as a surface modifier to produce an amino acid-based zwitterionic surface. The synergistic interaction between the α-amine and γ-carboxylic acid in the glutamic acid moiety provided a pH-responsive and selective protein adsorption surface.