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Stimuli-responsive command polymer surface for generation of protein gradients

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Mixed polyelectrolyte brushes with a composition gradient were used as a platform for fabrication of stimuli-responsive command surfaces to control the generation of concentration gradients of adsorbed protein molecules. Switching between homogeneously adsorbed protein layers and adsorbed layers with protein concentration gradients was achieved by changing the *pH* of protein aqueous solutions. Protein adsorption and the direction of the adsorption gradient were tuned and also turned off and on or reversed by tuning the proton concentration in the *pH* range 4.0–8.6.

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I. INTRODUCTION

Investigation and control of protein adsorption are of great importance for developing proteomic and diagnostic tools, drug delivery systems, food technology, medical equipment and implants, and biosensors.^{1–4} The adsorption amount and conformation of adsorbed proteins are the key factors in controlling the interactions between the surface and biological environment.^{5–7} Numerous publications aimed to clarify the mechanism of the interaction between proteins and surfaces.^{8–10} Recently, surfaces with gradually changing properties have been successfully introduced for this purpose.^{11–16} Compared to conventional “one-by-one” experiments, the combinatorial approach based on gradient surfaces allows dramatically accelerated measurements and reduced variance due to environmental conditions.^{17,18} The gradient surfaces have been used to tune the protein adsorption and, consequently, cell adhesion¹³ to study both the effect of ligand/receptor density on biological recognition^{19,20} and polyvalency.²¹ A number of approaches for the gradient of surface density of proteins have been proposed, such as varying the dose of light during the protein photoimmobilization,²² using nanoparticles as protein carriers and optical tags,²³ adsorption on the gradient self-assembled monolayers (SAMs) or surface immobilized poly-

mer layers,¹⁴ ink-jet method,²⁴ and the depletion effect in the fluid.²⁵ The methods of gradient fabrication have gained considerable attention in recent reviews.^{26,27}

Here, we suggest a novel approach for fabricating gradients of surface density of proteins using adsorption on surfaces capable of reversible switching of lateral gradient of electrical charges. The main advantage of this method is that the character and direction of gradient of the protein surface density can be controlled by *pH*.

Stimuli-responsive materials and surfaces have attracted interest due to their unique ability to tune, regulate, and turn on and off a range of properties, in particular, interactions with the ingredients in surrounding environment upon external signal.^{28,29} Stimuli-responsive surfaces can be fabricated by grafting responsive molecules, typically polymer molecules,³⁰ polymer brushes,³¹ block-copolymer brushes,³² mixed polymer brushes,³³ and specially designed polymers of a complex structure,³⁴ or by engineering the nanostructured coatings consisting of organic and inorganic materials.³⁵

Recently, we have found that the charge, thickness, and wettability of grafted polymer layers consisting of two oppositely charged polyelectrolytes can be switched by changing *pH*.^{36,37} For example, a mixed polymer brush composed of two complementary polyelectrolytes, polyacrylic acid and poly(2-vinyl pyridine) (PAA-mix-P2VP) is charged positively at low *pH* and negatively at high *pH*. Being a Brønsted base, monomer units of P2VP are protonated at low *pH*, charged positively, and the polymer chain is swollen.^{38,39}

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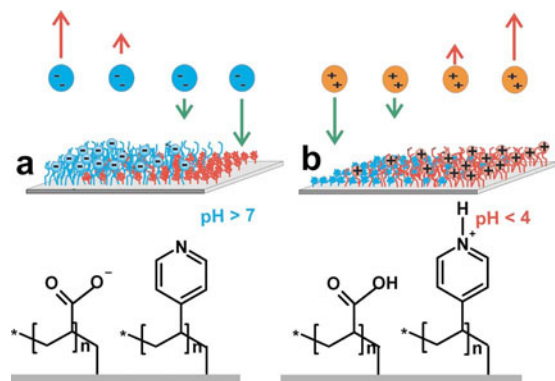


FIG. 1. (Color online) Scheme of interaction between proteins and PAA-mix-P2VP mixed-gradient brush.

On the other hand, PAA is dissociated at high pH and therefore charged negatively.³⁶ The surface is weakly charged at the moderate pH range of 5.0–8.0. The character of switching depends on the ratio between the polyelectrolytes.^{40,41} These effects are at the heart of inverse and reversible switching of the gradient of water contact angle on the surface of mixed brushes with a gradually changing ratio between the components of the brushes.⁴² Here, we report on the use of such mixed-gradient grafted polyelectrolyte brushes for stimuli-sensitive generation of surface gradients of proteins. The principle of this approach is illustrated in Fig. 1. Proteins are attracted to either uncharged or oppositely charged surfaces and are repelled from similarly charged surfaces (Fig. 1). Changes in pH result in reversible switching of both the gradient of the charge on the surface and the charge of the protein. This leads to a change of electrostatic interaction between the polymer surface and the protein.

II. EXPERIMENT

Carboxyl-terminated poly(*tert*-butyl acrylate) (PBA-COOH, number average molecular weight (M_n) = 42 000 g/mol, weight average molecular weight (M_w) = 47 000 g/mol) and carboxyl-terminated poly(2-vinylpyridine) (P2VP-COOH, M_n = 39 200 g/mol, M_w = 41 500 g/mol), synthesized by anionic polymerization, were purchased from Polymer Source, Inc. Polyglycidylmethacrylate (PGMA) of (M_n = 84 000 g/mol) was synthesized by free-radical polymerization of glycidyl methacrylate (Aldrich).⁴³ The polymerization was carried out in methyl-ethylketone (MEK, VWR) at 60 °C. AIBN (Aldrich) was used as the initiator. The obtained polymer was purified by multiple precipitations from MEK solution in diethyl ether.

Highly polished single-crystal silicon wafers of {100} orientation (Semiconductor Processing Co.) were used as a substrate. The wafers were first cleaned in an ultrasonic bath for 30 min, placed in hot piranha solution (3:1 concentrated sulfuric acid and 30% hydrogen peroxide—the mixture reacts violently with organic solvents, so handle with care) for 1 h, and then rinsed several times with MilliQ water.

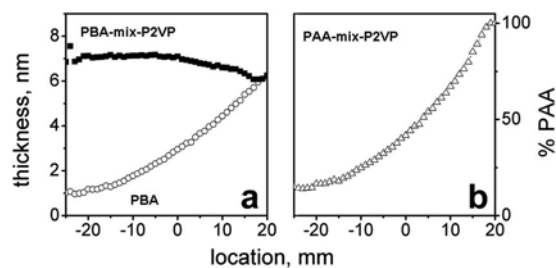


FIG. 2. (a) Thickness mapping for PBA (circles) the mixed PBA-mix-P2VP brush (squares) and (b) fraction of PAA in the PAA-mix-P2VP mixed-gradient polymer brush.

Gradient brushes consisting of two incompatible polymers PBA and P2VP were prepared and characterized via a two-step procedure as described elsewhere.^{37,44–46} A thin layer of PGMA (1.5 ± 0.1 nm) was deposited by spin coating from 0.01% solution in methylethylketone. Afterwards, the film of PBA-COOH was spin coated from 2% solution in toluene and annealed for 1 h on a specially designed stage with a one-dimensional temperature gradient so that the temperature of the stage changed gradually from 90 °C on the left-hand side of the stage to 130 °C on the right-hand side. The distance between these two edges was 50 mm. The temperature gradient was measured using thermocouples built into the stage. Upon heating, the esterification reaction results in the formation of a grafted PBA layer with a gradient of grafting density caused by a temperature dependence of the grafting kinetics. The ungrafted polymer was removed using Soxhlet extraction in toluene for 3 h. In the second step, a film (500 nm thick) of P2VP-COOH was spin coated on the top of the gradient PBA brush. The film was annealed at 150 °C for 8 h to graft P2VP-COOH. Afterward, the ungrafted polymer was removed by Soxhlet extraction in tetrahydrofuran (THF) for 4 h. We prepared the $X \times Y = 50 \times 20$ mm² sample of the gradient mixed brush (PBA-mix-P2VP) with the one-dimensional gradient of the brush composition (ratio PBA/P2VP) directed along the X axis. The PBA component of the mixed brush was then hydrolyzed upon treatment in benzene solution of *p*-toluene sulfonic acid monohydrate at 55 °C for 1 h.

The thickness of polymer layers was measured at $\lambda = 633$ nm with an angle of incidence of 70° with a SENTECH SE-402 microfocus ellipsometer (lateral resolution is defined by the beam spot of about 20 μ m) on each step of modification using a multilayer model described elsewhere.^{42,44,47} The grafted thickness of PBA gradually increased from about 1 to 7 nm from the left edge to right (Fig. 2). After grafting the second polymer, P2VP, the entire layer thickness is in the range of 6–7 nm.

Bovine serum albumin (BSA) was adsorbed from 0.25% solution in 0.01% phosphate buffered saline (PBS) buffer. The adsorption was performed for 5 h at pH ranging from 4 to 8.6. After adsorption experiments, samples were rinsed several times in water and dried by nitrogen flux. The thick-

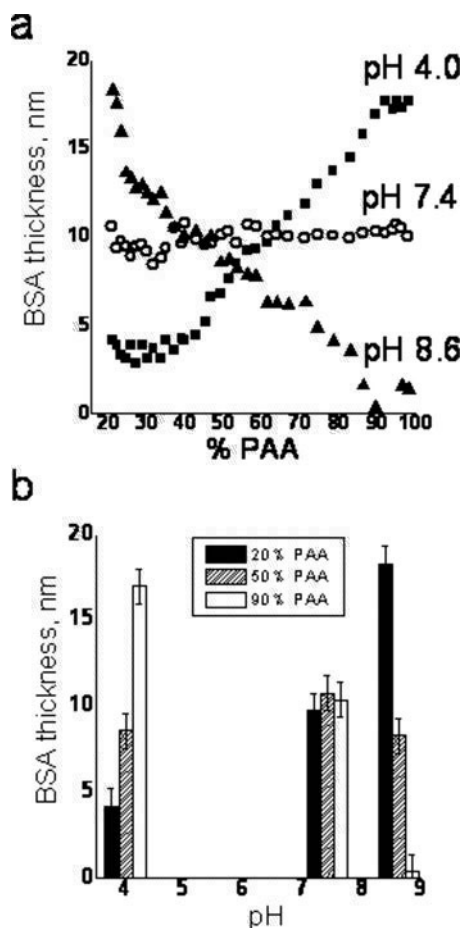


FIG. 3. (a) Adsorption of BSA on gradient mixed PAA-mix-P2VP polymer brush as a function of the brush composition at pH 4.0 (squares), pH 7.4 (circles), and pH 8.6 (triangles) and (b) as a function of pH of the surrounding environment at different brush compositions: 20% PAA (filled bars), 50% PAA (textured bars), and 90% PAA (open bars).

ness of adsorbed protein was evaluated as a difference between thicknesses obtained from ellipsometric mapping before and after adsorption.

III. RESULTS AND DISCUSSION

Synthesis of gradient PAA-mix-P2VP brush consists of several steps described in detail elsewhere.⁴² In the first step, a PBA layer with gradually changing thickness is prepared using annealing on the temperature-gradient stage (see Sec. II). The thickness of the gradient PBA brush synthesized in the first grafting step is presented as a function of the location on the sample (X coordinate) in Fig. 2(a) (cycles). The thickness of the layer gradually increases along the X axis from the left-hand side to the right-hand side, reflecting the change of grafting density of PBA. Afterwards, P2VP is grafted to the PBA-modified substrate. The entire thickness of the mixed brush (after the second polymer was grafted) is also presented in Fig. 2(a) (squares). The entire thickness of the mixed brush along the X axis has a virtually constant value of about 7 nm (dry film) corresponding to the grafting density of about 0.105 chains/nm². The distance between grafting sites of 3.8 nm is smaller than the gyration radius

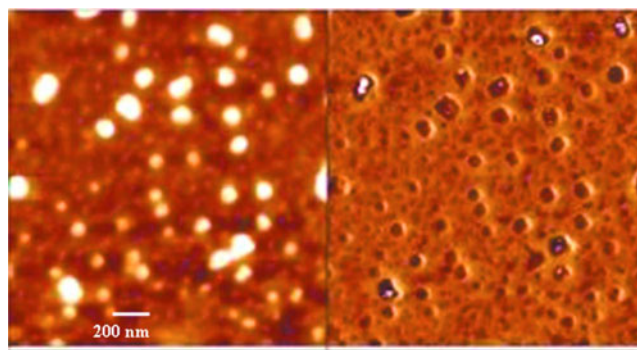


FIG. 4. (Color online) $1 \times 1 \mu\text{m}^2$ AFM images (left, topography; right, phase) of BSA, adsorbed on the 50:50 PAA-P2VP mixed brush at pH 4, root-mean-square roughness of 1.97 nm, and Z range of 60 nm.

(R_g) of PBA and P2VP polymer coils ($R_g \approx 5$ nm). Consequently, the polymer grafted film can be considered as a brushlike layer. Hydrolysis of the PBA component in the mixed brush yields PAA and, therefore, PAA-mix-P2VP brush. Ellipsometric data were used to calculate the composition of the PBA-mix-P2VP brush plotted in Fig. 2(b). The fraction of PAA, calculated as a ratio of thickness of PAA layer to entire brush thickness, gradually changes along the X axis of the PAA-mix-P2VP sample.

Experiments demonstrate that the amount of adsorbed (BSA) onto the gradient PAA-mix-P2VP brush changes with location on the brush sample and pH (Fig. 3). In the cases of asymmetrical brushes, adsorption of BSA is guided by the major component in the brush. For example, adsorption of the protein is weaker at high pH and stronger at low pH in locations with a predominating fraction of PAA [Fig. 3(a)]. The inverse behavior is observed in locations with a predominating fraction of P2VP. In this case, the protein adsorbs more strongly in locations with predominating P2VP [Fig. 3(a)]. In the case of nearly symmetric PAA/P2VP mixed polymer brush (ranging from 40% to 60% of PAA), adsorption is slightly reduced at low and high pH compared to pH=7.4. Thus, the adsorption of BSA increases with increasing fraction of PAA and P2VP at low and high pH, respectively [Fig. 3(b)].

A representative atomic-force microscopy (AFM) image of the BSA adsorbed layer at pH 4 is shown in Fig. 4. Details of the adsorbed layers' structure are beyond the focus of this article. This image is shown to prove a homogeneous distribution of structures (aggregates) formed by the adsorbed BSA on the brush surface within the scanned area (on this scale the BSA concentration gradient is very low).

In fact, adsorption of proteins on charged substrates is determined by the interplay of two major factors: electrostatic and nonelectrostatic factors. Electrostatic interactions cause adsorption of proteins on oppositely charged substrates, whereas the repulsion between similarly charged protein and substrate prevents the adsorption. However, in many cases, the amphiphilic character of protein molecules and

ion-exchange effects are driving forces of protein adsorption even on similarly charged surfaces (polyelectrolyte-mediated protein adsorption).⁴⁸

The values and signs of charges of protein and surface determine electrostatic interactions between them and depend on *pH*. P2VP and PAA are charged positively and negatively at low *pH* and high *pH*, respectively. BSA is a globular protein with hydrophobic core and hydrophilic shell composed of ionizable functional groups. BSA is charged positively and negatively below and above the isoelectric point (IEP) (IEP=4.9), respectively. Thus, at *pH*=4, PAA is only weakly negatively charged, whereas P2VP and BSA are positively charged. Coulomb repulsion between positively charged P2VP and BSA leads to reduced adsorption in P2VP-rich areas. Adsorption increases with increasing fraction of PAA due to a decrease in electrostatic repulsion between the surface and protein. A similar scenario can be assigned to high *pH* when PAA is negatively charged. In this case, electrostatic repulsion between negatively charged BSA and PAA reduces adsorption in PAA-rich areas. An adsorbed amount of protein increases with fraction of uncharged P2VP. Consequently, changes in *pH* affect the distribution of charges on the surface. This leads to the generation of the gradients of protein adsorption.

In neutral conditions (*pH* 7.4), P2VP and PAA form a polyelectrolyte complex, the surface is weakly charged, and the adsorption of protein is guided by nonelectrostatic interactions such as van der Waals and hydrophobic forces.⁴⁹ There are carboxyl, amine, and amide groups on the surface of BSA molecules. These groups are able to form donor-acceptor bonds with vinylpyridine ring or carboxylic groups of a grafted polymer layer. Therefore, BSA molecules interact with both P2VP and PAA and their complex. Most likely, this nonelectrostatic interaction dominates and is responsible for the weak effect of the mixed brush composition on BSA adsorption at *pH* 7.4. In all cases, the adsorbed protein forms a multilayered structure on the mixed brush surface. That can be caused by the interpenetration of polymer chains into a layer of adsorbed protein, formation of protein agglomerated, or secondary adsorption of protein onto a protein monolayer on the surface.^{50,51}

IV. CONCLUSIONS

We demonstrated that mixed polymer brushes with gradually changing composition can be successfully used to fabricate stimuli-sensitive surfaces when gradients of adsorbed proteins can be created, tuned, and inverted by external signals. We believe that the reported approach has important applications for fabricating protein surface gradients, combinatorial investigation of proteins and their interactions with polymers, as well as analytical applications in combinatorial tests of enzymatic activity or tests based on antibody-antigen recognition mechanisms. As compared to mixed SAMs the mixed brushes demonstrate a unique option to tune and switch protein adsorption gradients.

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