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A binary mixed polymer brushes coating with adjusted hydrophobic property to control protein adsorption

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In this work, binary mixed polymer brushes based on poly[(2-methyl-2-oxazoline)-random-glycidyl methacrylate] (PMOXA-r-GMA) and poly (glycidyl methacrylate)-block-poly(N-isopropyl acrylamide)-block-poly(styrene) (PGMA-b-PNIPAM-b-PSt) were used as thermoresponsive coating to control the protein adsorption. Firstly, PMOXA-r-GMA was synthesized via cationic ring opening polymerization of 2-methyl-2-oxazoline followed by its copolymerization with glycidyl methacrylate (GMA); PGMA-b-PNIPAM-b-PSt was synthesized by reversible addition-fragmentation chain transfer polymerization of GMA, N-isopropyl acrylamide, and styrene sequentially. Then the mixed brushes were fabricated successfully by spin coating the mixture of PMOXA-r-GMA and PGMA-b-PNIPAM-b-PSt solutions onto silicon or glass substrates followed by annealing protocol using GMA segments as anchor. Afterward, the investigation for adjusting the hydrophobic property of mixed brushes was performed. The results showed that when the environmental temperature is lower than the lower critical solution temperature (LCST) of PNIPAM, the tethered PNIPAM blocks are swollen and could bring the hydrophobic PST block with optimum molar mass to the surface; when the environmental temperature is higher than the LCST of PNIPAM, the PNIPAM blocks are collapsed and could bring the hydrophobic block inside the hydrophilic PMOXA grafted polymer layer. Finally, the protein adsorption on mixed polymer brushes was investigated by fluorescein isothiocyanate-labelled proteins assay and ellipsometry. The results showed that under optimum molar mass of PST block, mixed brushes could adsorb proteins in large quantities below the LCST of PNIPAM, and display the resistance to protein adsorption above the LCST of PNIPAM.

Introduction

Controlling the hydrophobic interaction between surfaces and proteins in an aqueous environment is important for it could control the protein adsorption which is the first step of surface fouling.1–9 Stimuli-responsive surfaces are usually used to control the level of hydrophobic interactions between surfaces and proteins due to its switching of hydrophobic/hydrophilic properties upon environment stimulus.10–14 The responsive surfaces could be obtained by grafting one end of polymer chains to the surface forming polymer brushes. Among the polymer brushes, binary mixed polymer brushes composed of a hydrophilic part and an environmental responsive part have drawn more attention for its anti fouling ability could be adjusted by switching the surface property between hydrophilic and hydrophobic behavior through changing the environmental stimuli (temperature, pH, ionic strength (I), etc.).15–22 O. Hoy and coworkers have prepared the mixed polymer brushes made of a hydrophilic homopolymer, poly(ethylene oxide) (PEO, also known as poly (ethylene glycol) (PEG)), and an amphiphilic block copolymer, polystyrene-b-poly (acrylic acid) (PSt-b-PAA).23 In which, the PSt-b-PAA copolymer was grafted to the surface via the reactive PAA part, and the hydrophobic block, PST as a hydrophobic probe, is immiscible with PAA and PEG. They demonstrated that the hydrophobic property of created mixed brushes provided by PST probe can be adjusted via PST domains up or down with respect to the top surface through variation of the PAA swelling depending on external stimuli (pH, I, and the presence of Ca2+), and the actuation of the hydrophobic domains in the brushes can be used to tune bovine fibrinogen adsorption on and off.

Although PEO and its corresponding derivatives have been used widely as antifouling materials because of their great hydrophilic properties, PEO is known to undergo degradation especially in physiological environment.24 Recently, poly(2-methyl-2-oxazoline)(PMOXA), a peptidomimetic polymer, has attracted interest for its similar properties (e.g. hydrophilicity and biocompatibility) to PEG and less degradation than PEG.25–29 A. A. Cavallaro and coworkers have reported that the films developed via plasma deposition of 2-methyl-2-oxazoline (MOXA) could resist the adsorption of staphylococcus epidermidis and cell more than 90% regardless of type of substrate used.30 L. Bai and coworkers have developed poly(2-methyl-2-oxazoline-random-glycidyl methacrylate) (PMOXA-r-GMA) comb copolymers.31 The PMOXA based coating could then be prepared by a simple annealing
Scheme 1. Strategy of design for PMOXA-r-GMA and PGMA-b-PNIPAM-b-PSt mixed polymer brushes.

protocol of PMOXA-r-GMA on silicon/glass surfaces by using GMA segments as the anchor, because the epoxide groups of glycidyl methacrylate (GMA) segments can not only react with functional groups (i.e., Si-OH) on the surface of silicon/glass, but perform self-crosslinking each other as well.22-24 The prepared coatings possess strong stability and excellent bovine serum albumin (BSA) resistant ability as well. Stimuli-responsive polymer could undergo a rapid conformational change in response to an external stimulus (e.g., temperature, light, redox signal, magnetic field, pH, ionic strength, or a specific molecule), resulting in the switching of the surface functionality on/off.25-27 Among them, poly (N-isopropyl acrylamide) (PNIPAM), thermoresponsive polymer, has received extensive attention due to its lower critical solution temperature (LCST) of 32 °C close to body temperature. When the environmental temperature is lower than 32 °C, PNIPAM chains are swelling due to the strong hydrogen bond formed between the water molecules and the PNIPAM chains. When the environmental temperature is higher than 32 °C, the thermal dissociation of water molecules from the hydrated polymer chains can lead to the intrinsic affinity of PNIPAM chains, leading to the lessening of the hydrogen bond between water molecules and the PNIPAM chains; as result, PNIPAM chains shrink and display hydrophobic behavior.28-30

Inspired by the stability and low fouling ability of PMOXA based coatings and swelling/deswelling behaviors of PNIPAM chains with temperature, herein our purpose is to create a binary mixed polymer brushes, on which the adsorption of protein could be adjusted through the change of the hydrophobic/hydrophilic behavior of mixed polymer brushes with temperature in aqueous environment. Firstly, comb copolymer, PMOXA-r-GMA was synthesized via cationic ring opening polymerization (CROP) of MOXA followed by its random copolymerization with GMA according to our previous work;31 and poly(glycidyl methacrylate)-block-poly(N-isopropyl acrylamide)-block-polystyrene (PGMA-b-PNIPAM-b-PSt) was synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization of GMA, N-isopropyl acrylamide (NIPAM), and styrene (St) step by step. Then the mixed brushes based on PMOXA and PGMA-b-PNIPAM-b-PSt were fabricated by spin coating the mixture of PMOXA-r-GMA and PGMA-b-PNIPAM-b-PSt solutions onto silicon or glass substrates followed by annealing protocol. A strategy of design for mixed brushes is shown in Scheme 1. The hydrophilic character of the mixed brush is provided by highly hydrophilic PMOXA, which is end grafted to the surface by using GMA units existing in PMOXA-r-GMA as anchor. PGMA-b-PNIPAM-b-PSt is grafted to the surface by using PGMA block as anchor, where PGMA block tethered to PGMA anchor is a thermoresponsive polymer chain and serves as a macromolecular actuator to bring the highly hydrophobic PSt block to the surface upon the external temperature change. In PMOXA-r-GMA/PGMA-b-PNIPAM-b-PSt mixed brushes, the crosslinking of GMA segments next to the surface could strengthen the anchoring to the surface. In an environment where the tethered PNIPAM blocks are swollen (environmental temperature lower than the LCST of PNIPAM), the PSt hydrophobic domain exposed to the surface. Such a surface has strong interactions with hydrophobic entities. When PNIPAM block is not swollen (environmental temperature higher than the LCST of PNIPAM), the hydrophobic domain of the brush is hidden inside the hydrophilic PMOXA grafted polymer layer. Therefore, hydrophobic/hydrophilic behavior of mixed polymer brushes could be tuned by moving the hydrophobic domains up or down with respect to the top surface. Prepared PMOXA-r-GMA/PGMA-b-PNIPAM-b-PSt coatings were characterized by using X-ray photoelectron spectroscopy (XPS), variable angle spectroscopic ellipsometry (VASE), atomic force microscopy (AFM), and static water contact angle (CA) measurements in order to assess their composition, morphology, thickness, and hydrophilicity respectively. The fluorescein isothiocyanate-labeled protein assay and VASE were used for qualitative and quantitative evaluation of the adsorption of protein on mixed brush coatings respectively through varying the temperature.

Experimental

Materials

The water used in all experiments was deionized water. N, N-dimethylformamide (DMF, Sinopharm Chemical Reagents, Shanghai, China) was distilled under reduced pressure before use. 2, 2-Azobisis(2-methylpropionitrile) (AIBN) was recrystallized from methanol. Glycidyl methacrylate (GMA, 97%, Aladdin, Shanghai, China) and styrene (St, Sinopharm Chemical Reagents, Shanghai, China) were respectively passed through an activated basic alumina column to remove the inhibitor before use. N-isopropyl acrylamide (NIPAM, Aladdin, Shanghai, China) was recrystallized from hexane. S-1-dodecyl-S‘-α’-dimethyl-α’-acetetic acid triethiocarbonate (DDMAT) was synthesized according to the previously published procedure.42 Fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA), fibrinogen (FITC-Fib), and lysozyme (FITC-Lys) were prepared by mixing fluorescein isothiocyanate (FITC, Sigma-Aldrich, USA) with bovine serum albumin (BSA, pl ~ 4.8, Mw ~ 68 kDa, Sigma-Aldrich, USA), fibrinogen (Fib, pl ~ 5.8, Mw ~ 300 kDa, Sigma-Aldrich, USA) and lysozyme (Lys, pl ~ 10.8, Mw ~ 15 kDa, Sigma-Aldrich, USA) respectively at a molar ratio of 1 : 1 in Na2CO3-NaHCO3 buffer for 2 h, followed by thorough dialysis in pH 7.4 phosphate-buffered saline (PBS, 10 mM) for three days.31 Glass
wafers and microscope glass slides for FITC-immunoassay were bought from Shanghai Jinglun Industry Glass Co. (Shanghai, China). Silicon (111) wafers with a natural oxidized layer were received from Zhejiang Crystal Photoelectric Technology Co. (Zhejiang, China). Other reagents were all obtained from Sinopharm Chemical Reagents (Shanghai, China).

**Synthesis of polymers**

**Synthesis of PMOXA-r-GMA.** PMOXA-r-GMA was synthesized according to our previous work.\textsuperscript{31} By using the same procedure, methacrylate terminated poly (2-methyl-2-oxazoline) (PMOXA-MAA) was synthesized by CROP of MOXA followed by its end-capping with methacrylic acid (MAA), triethylamine (TEA). Then, the PMOXA-MAA underwent random copolymerization with GMA using AIBN as an initiator to get PMOXA-r-GMA copolymer. The detailed synthesis procedure is shown in ES\textsuperscript{†}. The feed molar ratio of PMOXA-MAA to GMA was maintained to 3:1. Relative number average molar mass (M\textsubscript{n}) of resultant PMOXA-MAA and molar ratio of PMOXA-MAA to GMA were calculated by \textsuperscript{1}H NMR. Synthesized copolymer PMOXA-r-GMA was named as PMG for convenience.

**Synthesis of PGMA-b-PNIPAM-b-PSt.** At first, a typical procedure of the synthesis of PGMA with a designed M\textsubscript{n} of 1000 g/mol controlled by feed ratio was as follows. GMA (0.500 mL, 3.70 mmol), DDMAT (0.2009 g, 0.500 mmol), AIBN (0.0410 g, 0.250 mmol), and DMF (4.0 mL) were introduced into a flame-dried Schlenk flask. The reaction mixture was then stirred at 60 °C under nitrogen for 12 h. After that, the mixture was precipitated in ice-cold ethyl ether, filtered, and dried in vacuum overnight. PGMA-b-PNIPAM was synthesized with a designed M\textsubscript{n} of PNIPAM block 8000 g/mol controlled by feed ratio, and a typical procedure was as follows. Produced PGMA (0.500 g, 0.500 mmol), NIPAM (4.000 g, 35.20 mmol), AIBN (0.0410 g, 0.250 mmol), and DMF (8.0 mL) were added into a flame-dried Schlenk flask. The reaction mixture was stirred at 60 °C under nitrogen for 12 h. Afterward, PGMA-b-PNIPAM was obtained by precipitating the mixture into ice-cold ethyl ether, then filtering, and drying in vacuum overnight. A series of PGMA-b-PNIPAM-b-PSt was synthesized with a varied M\textsubscript{n} of PSt block. A typical example of PGMA-b-PNIPAM-b-PSt with a designed M\textsubscript{n} of PSt block 1000 g/mol was as follows. PGMA-b-PNIPAM (4.50 g, 0.500 mmol), St (0.500 ml, 4.30 mmol), AIBN (0.0410 g, 0.250 mmol), and DMF (8.0 mL) were added into a flame-dried Schlenk flask. Then the reaction mixture was stirred at 60 °C under nitrogen for 12 h. After that the mixture was precipitated in ice-cold ethyl ether, filtered, and dried in vacuum overnight to get PGMA-b-PNIPAM-b-PSt. For convenience, PGMA-b-PNIPAM-b-PSt was named as PGN, triblock copolymers, PGMA-b-PNIPAM-b-PSt, according to the designed M\textsubscript{n} of PSt block with 1000, 6000, 8000 were denoted as PGN\textsubscript{6000}, PGN\textsubscript{8000}, and PGN\textsubscript{10000} separately.

**Surface modification**

Silicon/glass wafers were cut into 1 × 1 cm\textsuperscript{2} pieces and cleaned by sonication in ethanol (15 min), and then in deionized water (15 min). Subsequently, the wafers were cleaned in a piranha solution (7 : 3 v/v mixture of H\textsubscript{2}SO\textsubscript{4} (95–98%) and H\textsubscript{2}O\textsubscript{2} (30%)) for 60 min, rinsed extensively with water, ethanol, and then dried under nitrogen before use. Solutions of polymers with PGNs (PGNs\textsubscript{6000}, PGNs\textsubscript{8000}, and PGNs\textsubscript{10000})/PGM mass ratio of 10/0 (pure PGNs), 0/10 (pure PGM), 5/5, were prepared by mixing stock chloroform solutions of PGM and PGNS at 10 mg/mL. Spin coating of copolymer solutions (150 \(\mu\)L for 1 × 1 cm\textsuperscript{2}) was performed on all above substrates for 20 s at 2000 rpm under vacuum by an EZ24 SPIN COATER (LEBO SCIENCE, China). The coated substrates were subsequently annealed for 12 h at 110 °C. After annealing, substrates were allowed to cool to room temperature and rinsed with chloroform, alcohol, and deionized water to remove unattached polymer in succession and then dried with nitrogen. To compare with mixed brush PGNs/PGM coating, PGN/PGM brush coating were also prepared by using the same annealing protocol as above with PGN/PGM mass ratio of 10/0 (pure PGN), 5/5 (PGN/PGM), based on the stock chloroform solutions of PGN and PGM at 10 mg/mL.

**Characterizations**

**NMR spectroscopy.** The \textsuperscript{1}H NMR spectra of copolymers were recorded using a Bruker DMX-300 instrument at 300 MHz. The spectra were acquired at room temperature using a deuterated chloroform solvent and referenced to an internal tetramethylsilane (TMS) standard.

**X-ray photoelectron spectroscopy (XPS).** The XPS data were collected on a VG ESCALAB MK II X-ray Photoelectron Spectrometer (VG Scientific Instruments, England) with an Al (K\textsubscript{α}) X-ray source (1486.6 eV). All spectra were calibrated by setting the signal of the aliphatic C signal at 284.7 eV (rather than 285.0 eV), given the high proportion of aromatic carbon in the compounds.

**Ellipsometry.** The dry film thicknesses were measured by using a variable angle spectroscopic ellipsometer (M-2000, Woollam Co., Inc., Lincoln, NE) at room temperature. The measurements were performed in the spectral range of 370 – 1000 nm at two different angles of incidence (65° and 75°). The analysis software, CompleteEASE 4.81, was used to analyze all data. The reported thickness was the average of measurements made from at least three spots on the polymer-modified silicon wafer. To fit the ellipsometric data, the optical constants (refractive index, extinction coefficient) of Si (n = 3.865, k = 0.020) and SiO\textsubscript{2} (n = 1.465, k = 0) were used to determine the SiO\textsubscript{2} layer thickness of the freshly cleaned silicon surfaces. Each polymer layer was represented as a slab of uniform thickness having sharp interfaces and optical properties described by a Cauchy model (refractive index smoothly decaying with wavelength), assuming that the PMOXA-r-GMA and PMOXA-b-PNIPAM-b-PSt layers had refractive indices of 1.45 at 632 nm.\textsuperscript{45}

**Atomic force microscopy (AFM).** The surface topology and surface roughness values of the unmodified and polymer modified silicon surfaces were evaluated on a DI Multimode\textsuperscript{\textregistered} atomic force microscope (AFM) from Veeco Instruments (Mannheim, Germany). The microscope was operated in tapping mode using Si cantilevers with a resonance frequency of 273 kHz and a drive amplitude of 1.30 V at a scan rate of 0.3 Hz. The AFM images were analyzed and post-processed using the NanoScope software (Version 5.12) at room temperature.

**Water contact angle measurements (WCA).** The static water contact angles (WCA) of bare and modified silicon wafers were measured with a CA system (SL200KB, USA KINO Industry Co., Ltd, USA) at room temperature. The static contact angles were measured by 2 \(\mu\)L water droplets that were delivered to the surface...
using a microliter syringe, and the values were recorded after 1 s. Each sample was measured at three random locations on the modified silicon surface to check its uniformity. Data presented were then averaged by three independent measurements on different locations of samples, and the results were reported as mean ± standard deviation (SD).

**Switchable properties**

**Hydrophilicity.** To check the switchable hydrophilic nature of modified surfaces under different temperature, WCAs of bare and polymer modified silicon wafers were measured at the temperature of 0 °C or 38 °C individually. Firstly, the wafer was placed on a stage of certain temperature loaded with circulating water for 1 min (an additional thermostatic waterbath was used to control the temperature of circulating water, heating for 38 °C and ice water mixture for 0 °C); and then WCAs were measured using the same operation conditions as the part of WCA measurements.

**Hydrated thickness.** To evaluate thermoresponsive behavior of polymer modified silicon surfaces, thickness of coated substrates dealt with different temperature was measured. Firstly, polymer modified substrates were separately immersed in water of 0 °C or 38 °C for 2 h each and then freeze dried for 12 h. After that the thickness of each substrate was measured at three different spots on polymer modified wafer and the results were reported as mean ± SD. All the ellipsometric data were fitted using Cauchy layer model to get the thickness of the brushes as described in the part of Ellipsometry.

**FITC-protein adsorption assay.** FITC-BSA, -Fib, and -Lys were chosen as the model protein for the qualitative evaluation of protein adsorption on bare or modified glass wafer surfaces under different temperature. FITC-BSA was used as an example to describe this procedure. Firstly, uncoated or polymer coated glass wafers were separately immersed in FITC-BSA solution of PBS (pH 7.4, 10 mM; 1 mg mL⁻¹) of 0 °C and 38 °C and placed in the darkroom for 2 h. After that the respective wafers were washed with PBS and deionized water of 0 °C or 38 °C three times, respectively to remove unbound proteins, followed by drying under nitrogen. The process of the adsorption of FITC-Fib and -Lys on bare or modified glass wafer surfaces under 0 °C or 38 °C is the same as FITC-BSA. The fluorescence images of the FITC-BSA, -Fib, and -Lys adsorbed samples were examined using an optical microscope, Olympus BX81 (Olympus, Japan) equipped with a halogen lamp, filter U-MNG2 (λem = 470 – 490 nm, λs = 510 nm) and camera type DP72. The color intensity was measured using the image J software, and an average value was calculated. The relative adhesion amount of protein was determined qualitatively based on the color intensity of the fluorescence images.

**Quantitative analysis of protein adsorption.** In this study, the quantitative analysis of protein (BSA, Fib and Lys) adsorption on polymer brushes at 0 °C and 38 °C was performed by measuring ellipsometry thickness of bare and polymer modified silicon wafers before and after the protein (BSA, Fib and Lys) adsorption. The dry thicknesses of bare or modified silicon substrates were measured at room temperature firstly, then bare or polymer modified wafers were immersed in protein solution of PBS (pH 7.4, 10 mM; 1 mg mL⁻¹) of 0 °C for 2 h, washed with water of 0 °C for 3 times, dried with nitrogen and then the thickness after protein adsorption at 0 °C was measured at room temperature. The measurement process of ellipsometry thickness of bare and modified silicon wafers after protein adsorption at 38 °C was the same as following procedure mentioned above, only the temperature of protein solution and rinsing water was maintained 38 °C. The adsorbed mass (Δm, ng/cm²) of protein on the bare and polymer modified silicon wafers was calculated by the following equation

\[ Δm = \rho × Δh × S \]

where S is the cross-sectional area of the silicon wafers, \( ρ \) is the density of the proteins (the density of BSA, Fib, Lys are assumed to be 1 g/cm³), and Δh is the difference of thickness before and after the protein adsorption.

**Results and discussion**

**Preparation of polymer**

PMOXA-r-GMA (PGM) was synthesized via CROP of MOXA followed by its random copolymerization with GMA according to our previous work. The \( ^1H \) NMR result (Fig. S1 in ESI†) showed that Mn of PMOXA chain on PGM is about 4900 g/mol, and contour chain length of PMOXA side chains on PGM is about 20.1 nm (Table S1 in ESI†).

PGMA-b-PNIPAM-b-PSt with different design Mn of PST block (PGNS1k, PGNS6k and PGNS8k) was synthesized by RAFT polymerization of St with PGMA-b-PNIPAM-macro-RAFT agent (PGN). As shown in Fig. 1a, the resonance peaks a (δ ~ 2.6 ppm, 2.8 ppm) and b (δ ~ 3.5 ppm) were assigned to the protons of methylene and methine of the GMA epoxide group individually, while the peak c (δ ~ 3.8 ppm, 4.3 ppm) was assigned to the methylene connected to the epoxide groups. The signals of proton
from isopropyl group of NIPAM were detected at the resonance peaks of \( \delta \approx 4.0 \) ppm, and \( \delta \approx 1.2 \) ppm. These significant proton signals confirm the successful synthesis of PGN. The \( M_n \) of PGMA block in PGN is about 770 g/mol, determined by calculating relative peak areas corresponding the peak \( n \) from DDMAT (\( \delta \approx 0.8-0.9 \) ppm) and peak \( c \) (\( \delta \approx 3.8 \) ppm, 4.3 ppm); the \( M_n \) of PNIPAM block in PGN is about 7900 g/mol determined by calculating relative peak areas corresponding the peak \( n \) from DDMAT RAFT agent’s end \( CH_3 \) (\( \delta \approx 0.8-0.9 \) ppm) and peak \( i \) (\( \delta \approx 4.0 \) ppm). The theoretical chain length of PNIPAM block is 20.3 nm (Table S1 in ESI†). Fig. 1b shows the \( ^1H \) NMR of PGNS with varying \( M_n \) of PST block. In addition to all other corresponding peaks of PGN, a new significant peak was detected at 6.7 – 7.2 ppm, assigned to the phenyl group of styrene, confirming the successful synthesis of PGNS. The intensity of this peak (\( \delta \approx 6.7 – 7.2 \) ppm) increases by increasing designed \( M_n \) of PST block from 1k to 8k respectively. The \( M_n \) of PST block of PGNS\(_{1k}\), PGNS\(_{6k}\) and PGNS\(_{8k}\) are 1232, 5897, and 7925 g/mol respectively, determined by relative peak areas of DDMAT RAFT agent’s end \( CH_3 \) (\( \delta \approx 0.8-0.9 \) ppm) and phenyl group signal of styrene (\( \delta \approx 6.7 – 7.2 \) ppm), close to the designed \( M_n \) of PST block. The contour chain length of PGNS\(_{1k}\), PGNS\(_{6k}\) and PGNS\(_{8k}\) are 23.2 nm, 37.5 nm and 43.2 nm respectively. The detailed chain length calculation is shown in Table S1 of ESI†.

### Characterization of polymer modified surfaces

| Samples | Element mole percent (atom%) | \( f_{PGMS/PGM} \) | \( f_{PGNS/PGM} \) | Thickness (nm) | \( \Gamma \) (chain/nm\(^2\)) | Water contact angle (°) |
|---------|------------------------------|--------------------|-------------------|---------------|-----------------|------------------------|
| sample  | C1s  | N1s  | O1s  | Si2s | | | |
| bare    | 27.15 | 1.20 | 29.01 | 42.64 | | | 5.2±1.6 |
| PGM     | 40.46 | 5.18 | 25.32 | 29.04 | 2.0±0.1 | 0.24 | 9.4±1.8 |
| PGN     | 46.06 | 5.70 | 23.72 | 24.51 | 2.6±0.1 | 0.20 | 30.6±1.9 |
| PGNS\(_{1k}\) | 46.72 | 4.80 | 26.88 | 21.59 | 2.6±0.1 | 0.17 | 31.8±1.6 |
| PGNS\(_{6k}\) | 53.68 | 1.54 | 23.64 | 22.14 | 2.5±0.2 | 0.11 | 43.5±1.4 |
| PGNS\(_{8k}\) | 57.04 | 1.82 | 22.64 | 18.50 | 2.2±0.1 | 0.08 | 50.1±0.7 |
| PGN/PST (5/5) | 42.37 | 5.31 | 24.91 | 26.41 | 3.8/6.2 | 2.5±0.2 | 0.23(0.07/0.16) | 20.3±1.2 |
| PGNS\(_{1k}\)/PST (5/5) | 45.89 | 4.87 | 23.07 | 26.17 | 3.9/6.1 | 3.0±0.1 | 0.23(0.08/0.15) | 28.9±2.1 |
| PGNS\(_{6k}\)/PST (5/5) | 46.42 | 2.62 | 24.31 | 23.65 | 4.3/5.7 | 2.9±0.2 | 0.18(0.03/0.15) | 29.1±1.1 |
| PGNS\(_{8k}\)/PST (5/5) | 52.10 | 1.95 | 24.76 | 21.19 | 4.2/5.8 | 2.1±0.1 | 0.12(0.02/0.10) | 45.8±1.4 |

\( f_{PGMS/PGM} \) and \( f_{PGNS/PGM} \) are the mass ratio of PGNS (or PGN) to PGM in mixed polymer brushes calculated according to Eq. S4 and Eq. S5.

| Samples | Element mole percent (atom%) | \( f_{PGMS/PGM} \) | \( f_{PGNS/PGM} \) | Thickness (nm) | \( \Gamma \) (chain/nm\(^2\)) | Water contact angle (°) |
|---------|------------------------------|--------------------|-------------------|---------------|-----------------|------------------------|
| sample  | C1s  | N1s  | O1s  | Si2s | | | |
| bare    | 27.15 | 1.20 | 29.01 | 42.64 | | | 5.2±1.6 |
| PGM     | 40.46 | 5.18 | 25.32 | 29.04 | 2.0±0.1 | 0.24 | 9.4±1.8 |
| PGN     | 46.06 | 5.70 | 23.72 | 24.51 | 2.6±0.1 | 0.20 | 30.6±1.9 |
| PGNS\(_{1k}\) | 46.72 | 4.80 | 26.88 | 21.59 | 2.6±0.1 | 0.17 | 31.8±1.6 |
| PGNS\(_{6k}\) | 53.68 | 1.54 | 23.64 | 22.14 | 2.5±0.2 | 0.11 | 43.5±1.4 |
| PGNS\(_{8k}\) | 57.04 | 1.82 | 22.64 | 18.50 | 2.2±0.1 | 0.08 | 50.1±0.7 |
| PGN/PST (5/5) | 42.37 | 5.31 | 24.91 | 26.41 | 3.8/6.2 | 2.5±0.2 | 0.23(0.07/0.16) | 20.3±1.2 |
| PGNS\(_{1k}\)/PST (5/5) | 45.89 | 4.87 | 23.07 | 26.17 | 3.9/6.1 | 3.0±0.1 | 0.23(0.08/0.15) | 28.9±2.1 |
| PGNS\(_{6k}\)/PST (5/5) | 46.42 | 2.62 | 24.31 | 23.65 | 4.3/5.7 | 2.9±0.2 | 0.18(0.03/0.15) | 29.1±1.1 |
| PGNS\(_{8k}\)/PST (5/5) | 52.10 | 1.95 | 24.76 | 21.19 | 4.2/5.8 | 2.1±0.1 | 0.12(0.02/0.10) | 45.8±1.4 |

\( f_{PGMS/PGM} \) and \( f_{PGNS/PGM} \) are the mass ratio of PGNS (or PGN) to PGM in mixed polymer brushes calculated according to Eq. S4 and Eq. S5. The data obtained in dry film and expressed as mean ± SD (n = 3).
The XPS measurements were used to determine the surface composition of the polymer coatings formed on the silicon wafers. The XPS wide scan spectra and high-resolution C1s of bare, PGN, PGM, PGNS8k, PGNS1k, PGNS4k, PGN/PGM, PGNS8k/PGM, and PGNS4k/PGM modified silicon wafers are shown in Fig. S3 and S4 of ESI†, and the corresponding atomic percentage of elements on bare and modified substrates is listed in Table 1. Compare to the bare silicon surface, clear changes in carbon (C1s, 287 eV), nitrogen (N1s, 400.5 eV), oxygen (O1s, 533 eV), and silicon (154 eV for Si2s and 103 eV for Si2p) signals were observed in modified silicon surfaces. For instance, in PGM modified substrate, increment in carbon (C1s, 287 eV), nitrogen (N1s, 400.5 eV), and oxygen signals (O1s, 533 eV), with corresponding decrease in silicon (154 eV for Si2s and 103 eV for Si2p) signals confirm the successful immobilization of PGM on the substrate as reported previously.31

The intensities of the carbon (C1s, 287 eV) signal increase with increasing the molar mass of PST block from PGN to PGNS3k respectively. For the mixed polymer brushes of PGN/PGM, PGNS3k/PGM, PGNS5k/PGM, and PGNS8k/PGM (mass ratio of PGN (PGNS) and PGM was 5/5, the detail for determination of the ratio of PGNS and PGM was described in ESI†), the intensity of the carbon (C1s, 287 eV) and nitrogen (N1s, 400.5 eV) signal between the intensity of pure PGM and pure PGNS or corresponding PGNS modified silicon wafer, suggested the presence of both polymers on the silicon wafer. The area of O=C-N peak (288.6 eV) from amide bond of PNIPAM block or PMOXA part (Fig. S4 of ESI†) decreased with the increment of PST block from PGN/PGM to PGNS8k/PGM (Table S2 of ESI†), further indicated the presence of both PGM and PGN(PGNS) copolymers on PGN(PGNS)/PGM modified surface.

The relative mass fraction of PGN, PGM, and PGNS (fPGN, fPGM, fPGNS) in the mixed polymer brushes of PGN/PGM, PGNS3k/PGM, PGNS5k/PGM, and PGNS8k/PGM were calculated by using Eq. S5 and Eq. S6 of ESI† based on XPS results, and the results are represented in Table 1. Results demonstrated that the resultant mass fraction of PGN, PGM, and PGNS on the the mixed polymer brushes modified surfaces are close to feed mass ratio (5/5) (resultant mass fraction based on XPS results for other feed mass ratio of PGNS3k/PGM is described in Table S2), implying that the surface composition could be controlled by the concentration ratio of polymers in the mixed polymers solutions which were used to fabricate mixed polymer brushes coatings.

Furthermore, the dry thickness of polymer modified surfaces were determined at room temperature and results are given in Table 1. The results showed that dry thickness of all the polymer modified surfaces are in the range of 2 – 3 nm and it is higher than that of bare silicon wafer (~ 0 nm). The grafting density of polymers on these samples could be estimated by using Eq. S6–13 of ESI† based on ellipsometry thickness. As shown in Table 1, the grafting density of the PGM is 0.24 chain/nm². From PGN to PGNS8k the grafting density decreased from 0.20 chain/nm² to 0.08 chain/nm², suggesting that the polymer brushes with the larger PST block have the lowest graft density on the substrate. From PGN/PGM to PGNS8k/PGM the grafting density decrease from 0.23 chain/nm² to 0.12 chain/nm², the grafting density of PGM also decrease from 0.16 chain/nm² to 0.10 chain/nm² with increment of PST block’s molar mass, because of the steric hindrance of PST block.

**Fig. 2.** AFM images of bare, PGN, PGM, PGNS1k, PGNS4k and PGNS8k/PGM, PGNS5k/PGM, PGNS8k/PGM modified silicon wafer. The scan size was 2 × 2 μm², scale bar was 200 nm, and the vertical scale was 20 nm.
The WCA of bare and polymer modified silicon wafers were also measured at room temperature and results are depicted in Table 1. The value of WCA on bare silicon wafer is 52 ° representing its hydrophilic nature after dealing with piranha solution. The value of PGM modified silicon wafer is 9.4 °, confirming the hydrophilic properties of PMOXA chains as reported previously. While for PGN modified surfaces, WCA increased sharply to 30.6 °, because PNIPAM is more hydrophobic than PMOXA at room temperature. The WCAs of PGNS modified wafers are higher than PGN modified wafers, due to the presence of PSt block with hydrophobic nature, and the WCA increased from 31.8 °, 43.5 ° to 50.1 ° with increasing the molar mass of PSt block from PGNS1k to PGNS6k to PGNS8k respectively. For the modified silicon wafer with PGN/PGM brushes, WCA (20.3 °) was observed to be in between of pure PGM and pure PGN modified wafers. For modified silicon wafers with PGNS1k/PGM, PGNS6k/PGM, and PGNS8k/PGM, WCA values decreased comparing with their corresponding pure PGNS brushes, and WCA values increased from 28.9 °, 29.1 ° to 45.8 ° with increasing the molar mass of PSt block from PGNS1k, PGNS6k, to PGNS8k respectively. Taking into account of XPS, thickness, and WCA data, it is confirmed that silicon substrate could be modified successfully by PGM, PGN, PGNS and mixed polymer brushes of PGN/PGM, and PGNS/PGM with good surface coverage and controlled surface composition using annealing protocol.

To further examine the grafting of polymers on the silicon substrates, surface morphology was investigated by using AFM with tapping mode and results are illustrated in Fig. 2. The arithmetical mean deviation of the roughness profile (Ra) value increased from 0.294 nm of bare silicon to 5.05 nm of modified silicon wafers. The higher roughness values of the polymer modified surfaces comparing with bare substrate clearly indicated the successfully grafting of the polymers on the silicon surfaces. Compared with PGN (1.86 nm) or PGN/PGM (2.54 nm), the Ra of PGNS or PGNS/PGM increased with increasing the molar mass of PSt block, implying that hydrophobic segregated PSt domain would reach to the top exterior boundary of the brush with increasing the molar mass of PSt block. These results are consistent with the WCA data obtained at room temperature, in which WCA values increased with increasing the molar mass of PSt block from PGNS1k to PGNS8k in either PGN brush or PGNS/PGM mixed brushes coating.

**Switching of hydrophilic/hydrophobic properties**

The WCA measurement is the basic method to investigate the surface hydrophilicity that is commonly used to examine the antifouling properties of the material surfaces. Fig. 3a shows polymer modified silicon surfaces at 0 °C and 38 °C, respectively. WCA of PGM modified silicon surfaces exhibited negligible transition from 0 °C to 38 °C, anticipating the temperature independent nature of PMOXA. Also, very low WCA values (around 10 ° both at 0 °C and 38 °C) represent strong hydrophobic property of PMOXA. The WCA of PGN modified silicon surfaces was jumped from 20 ° to 40 ° by rising the temperature from 0 °C to 38 °C. The difference in WCA (∆WCA) between 0 °C and 38 °C represents thermostresponsive behavior of PNIPAM. Below the LCST of PNIPAM (0 °C), PNIPAM chains would swell and adsorb water, causing the lower WCA of PGN modified surface; while above the LCST of PNIPAM (38 °C), PNIPAM chains collapse, undergo water repellent properties, thus increment in the WCA was observed in PGN modified surface. As compared to PGN modified surface, increment in the WCA was observed for PGNS modified surface both at 0 °C and 38 °C, and the values of WCA increase with increasing the molar mass of PSt block from PGNS1k to PGNS8k. Moreover, ∆WCA from 0 °C to 38 °C is less than 5 °. These results indicated that the addition of PSt block in the PGNS modified surface could improve the hydrophobicity of the surface both at 0 °C and 38 °C. For the mixed polymer brushes of PGN/PGM, WCA varies from 16 ° to 22 ° by increasing the temperature from 0 °C and 38 °C, representing its thermostresponsive abilities but the extent of responsiveness is lower than pure PGN modified surface due to the presence of PMOXA side chains in PGM. As long as mixed polymer brushes of PGNS/PGM are concerned, very interesting behavior of WCA was observed. For instance, WCA of PGNS6k/PGM remains approximately the same both at 0 °C and 38 °C and its value is

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**Fig. 3.** WCA value (a) and hydrated thickness (b) at 0 °C and 38 °C for PGN, PGM, PGNS1k, PGNS6k, PGNS8k and PGN/PGM, PGNS1k/PGM, PGNS6k/PGM, PGNS8k/PGM modified silicon wafer. Data are expressed as mean ± SD (n = 3).

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respectively at 0 °C, while the values of hydrated thickness decrease almost the same as that of PGM brush. The hydrated thickness of polymer brushes of PGN/PGM, hydrated thickness increased as PNIPAM chains in PGNS at 38 °C respectively. For the mixed swelling of PNIPAM chains in PGNS at 0 °C and deswelling of PSt block was higher at 0 °C and lower at 38 °C. It might be due to swelling of PNIPAM chains in PGNS at 0 °C and deswelling of PNIPAM chains in PGNS at 38 °C respectively. For the mixed polymer brushes of PGN/PGM, hydrated thickness increased as compared to pure PGN brush both at 0 °C and 38 °C, and remained almost the same as that of PGM brush. The hydrated thickness of PGN/PGM coating remains constant both at 0 °C and 38 °C, and also the value of hydrated thickness was almost the same as that of PGN/PGM, illustrating little contribution of PSt block with low molar mass. For PGN/PGM, and PGN/PGM modified silicon wafers, hydrated thickness increases sharply to 15 nm and 16.5 nm respectively at 0 °C, while the values of hydrated thickness decrease at 38 °C both for PGN/PGM and PGN/PGM. Pure PGM brush modified silicon wafer remains hydrophilic irrespective to the change of temperature due to the presence of PMOXA chains in PGM. On the other hand, pure PGN brush shows swelling when T < LCST of PNIPAM and undergoes hydrophobic nature; by increasing the temperature (T > LCST), PNIPAM chains collapse and show strong hydrophobic behavior, representing the dominant role of PNIPAM in PGN. After being modified with PGN, the substrates displayed more hydrophobic comparing with pure PGN modified substrate, and this hydrophobic properties varied a little with the temperature and increased with increasing the molar mass of PSt block (Fig. 3a); meanwhile the increment in brush thickness with the molar mass of PSt block and the switchable behavior in thickness upon temperature change were observed (Fig. 3b). These results suggested that the hydrophobicity of PGN modified substrates was determined by PSt block, and the switchable behavior in thickness was determined by PNIPAM block. Although the contour chain length of PMOXA (20.1 nm) in PGM is similar to that of PNIPAM (20.3 nm) in PGN (Table S1 of ES1), the hydrated thickness of PNIPAM modified substrate is higher than that of PGN modified substrate at both 0 °C and 38 °C (Fig. 3b), and PGM modified substrate is more hydrophilic than PGN modified substrate at both 0 °C and 38 °C (Fig. 3a). Therefore, for PGN/PGM mixed polymer brushes coating, the hydrated thickness and hydrophilicity are determined by PGM part owing to its position in the mixed brush exterior, and kept almost the same at both 0 °C and 38 °C as shown in Scheme 2. For PGN/PGM mixed polymer brushes coating, though the total contour chain length of PNIPAM and PSt block in all PGNS is longer than that of PMOXA in PGM part (23.2 nm for PGN/PGM, 37.5 nm for PGN, 43.2 nm for PGM, Table S1 in ES1), the hydrophilicity/hydrophobicity of modified substrates are different. For PGN/PGM mixed brushes, the total contour chain length of PNIPAM and PSt block in PGN/PGM is a little longer than that of PMOXA in PGM, but the hydrated thickness of pure PGM is much higher than pure PGN at both 0 °C and 38 °C, leading to the dominant role of PGM in mixed brushes; hence the hydrated thickness and hydrophilicity are determined by PGM part, and kept almost the same at both 0 °C and 38 °C as shown in Scheme 2. For PGN/PGM mixed brushes, the total contour chain length of PNIPAM and PSt block in PGN/PGM increased greatly with increasing the molar mass of PSt block, meanwhile, the hydrated thickness of pure PGN/PGM is much higher than that of PGM at 0 °C, and lower than that of PGM at 38 °C (Fig. 3b), suggesting that PGN/PGM would dominate the modified substrate at 38 °C representing hydrophobicity of PSt block, and PGM would dominate the modified substrate at 38 °C representing hydrophilicity (Fig. 3a) as shown in Scheme 2. By further increases the molar mass of PSt in the mixed polymer brushes (PGN/PGM), the contour chain length of PSt block increased greatly, and the total contour chain length of PNIPAM and PSt block in PGN/PGM is much longer than that of PMOXA in PGM; moreover, both the hydrated thickness of pure PGN/PGM are higher than PGM at 0 °C and 38 °C (Fig. 3b) due to the high molar mass of PSt block, implying that PGNS would control the wettability of PGNS/PGM modified surface; consequently the PGNS/PGM modified substrate remains hydrophobic both at 0 °C and 38 °C as shown in Scheme 2. The polymer brush which had a
more higher hydrated thickness would occupy the top of the mixed brushes modified surface, therefore hydrophilic/hydrophobic property of the surface could be controlled by this exposed part. For PGNS/PGM mixed polymer brushes coating, the overall hydrophilic character of the mixed brush is provided by the highly hydrophilic PMOXA in the PGM. PNIPAM block is an inherently responsive polymer chain and serves as a macromolecular trigger to bring the PST hydrophobic part to the surface upon the change of external temperature above or below the LCST of PNIPAM. PST block is a hydrophobic probe. When PNIPAM is not swollen (e.g. at 38 °C higher than the LCST of PNIPAM), the hydrophobic probe with appropriate Mₙ of PST block (PGNSₙ) would be hidden inside the hydrophilic PGM layer; when PNIPAM blocks are more extended (e.g. at 0 °C lower than the LCST of PNIPAM), the hydrophobic probe of PST would be exposed to the surface. Consequently, with PGNSₙ/PGM mixed brushes, by tuning the environmental temperature from 38 °C to 0 °C, the surface behavior could be changed from hydrophilic to hydrophobic.

**Adsorption of protein**

The effect of switching between hydrophilic and hydrophobic properties of the developed system on its adsorption of protein was investigated. To qualitatively assess the protein adsorption, a fluorescent test was performed using FITC-BSA, FITC-Fib and FITC-Lys as the model proteins, respectively. Fig. 4 shows the surface images by fluorescence microscopy after the adsorption of proteins at 0 °C and 38 °C, respectively. The bar graphs are the relative quantitative analysis of the fluorescence intensities of proteins adsorbed on the modified surfaces.

![Fluorescence microscopy images and fluorescence intensity bar graph of the (a) FITC-BSA, (b) FITC-Fib and (c) FITC-Lys adsorption on PGM, PGN, PGN/PGM, PGNSₙ/PGM, PGNSₙ/PGM modified glass wafer at 0 °C and 38 °C. Scale bar is 50 μm. The fluorescence intensity of PGNSₙ at 38 °C sets as 100% for each protein. Data are expressed as mean ± SD (n = 3).](image-url)
corresponding fluorescence images in Fig. 4. Herein the green fluorescence illustrates the adsorption of protein. For PGM modified surfaces, no adsorption was detected for all three types of protein (BSA, Fib, and Lys) at either 0°C or 38°C, representing strong anti-fouling property of PMOXA. For PGN modified polymer brushes, strong adsorption of proteins with the fluorescence intensity of 40% for BSA, 52% for Fib, 50% for Lys was observed at 38°C, but by changing the temperature to 0°C, very little adsorbed protein was observed (fluorescence intensity was only 1% for BSA, 11% for Fib, 19% for Lys ) because of the protein-resistant ability of hydrated layer of swollen PNIPAM in PGN.\(^2\)\(^{50-52}\) After the block copolymerization of PGN with St (PGNS), the adsorption of three proteins on PGNS modified substrates was observed at 0°C, while by shifting the temperature to 38°C, protein adsorption amount was nearly as same as at 0°C, and the amounts of protein adsorbed on PGNS modified substrates increased with increasing the molar mass of PST both at 0°C and 38°C. In the mixed polymer brushes of PGN/PGM, no evident fluorescence was observed (fluorescence intensity was nearly 3% for BSA, 3% for Fib, 1% for Lys ) both at 0°C and at 38°C due to strong protein repellent property of exposed PGM. As long as mixed polymer brushes of PGNS/PGM were concerned, almost negligible amount of these three proteins adsorption was observed for PGNS\(_{8k}/\)PGM, both at 0°C and at 38°C. For PGNS\(_{6k}/\)PGM, strong adsorption of protein was observed at 0°C and its fluorescence intensity was 65% for BSA, 78% for Fib, 77% for Lys; while unconspicuous adsorption of protein took place by changing the temperature to 38°C (fluorescence intensity was 8% for BSA, 9% for Fib, 10% for Lys). These results further demonstrated that the exposed PST block with more hydrophobic at 0°C would result in strong protein adsorption and exposed PGM with more hydrophilic at 38°C would display strong protein repellent property on PGNS\(_{8k}/\)PGM mixed brushes as shown in Scheme 2. However, for PGNS\(_{6k}/\)PGM, where molar mass of PST block was maximum, the same intensive fluorescence on PGNS\(_{6k}/\)PGM was observed at either 0°C or 38°C for each protein (67% for BSA, 84% for Fib, 95% for Lys), due to the predominant role of exposed PST block irrespective temperature as shown in Scheme 2. In brief, the adsorption degree of proteins on PGNS/PGM mixed brushes, regardless of acidic or basic protein (BSA/Lys), large or small size protein (Fib/Lys) could be adjusted through the hydrophobic/hydrophilic interaction between protein and exposed part of mixed brushes via varing the temperature of environment.

The quantification of the adsorption of protein was performed by using VASE. To verify the reliability of this method, after measuring the dry thickness of polymer modified wafers, the same wafers were immersed in PBS solution (without protein) for 2 h at 0°C or 38°C, then its thickness was remeasured after being dried under nitrogen at 25°C. The results showed no considerable difference in thickness, suggesting the validity of this method. The detail of experiment and corresponding results were shown in ESI†. The ellipsometry thickness of polymer modified silicon wafers before and after BSA, Fib and Lys adsorption at 0°C and 38°C were shown in Fig. S8 of ESI†, and the amounts of protein adsorption calculated by Eq. 1 were shown in Fig. 5. As shown in Fig. 5, the amounts of protein adsorption on the PGM modified surfaces remain nearly same at either 0°C or 38°C, representing the strong protein repellent properties of PMOXA. The amounts of protein adsorbed on PGN at 0°C were lower than that adsorbed at 38°C, indicating that the the hydrophobic interaction between PGN and proteins could be adjusted through the thermoresponsive ability of PNIPAM. Similar to fluorescence microscopy results, the amounts of protein adsorbed on PGNS modified wafers increased with increasing the molar mass of PST block at either 0°C or 38°C. In the mixed polymer brushes of PGN/PGM, a little adsorption of protein (nearly 10 ng/cm\(^2\)) for BSA, 30 ng/cm\(^2\) for Fib, 10 ng/cm\(^2\) for Lys) was observed both at 0°C and 38°C. While in the mixed polymer brushes of PGNS\(_{6k}/\)PGM, little amount of adsorption (nearly 10 ng/cm\(^2\) for BSA, 60 ng/cm\(^2\) for Fib, 50 ng/cm\(^2\) for Lys) took place both at 0°C and at 38°C. However, by increasing the molar mass of PST in the mixed polymer brushes (PGNS\(_{8k}/\)PGM), a large amount of protein adsorption (207 ng/cm\(^2\)) for BSA, 700 ng/cm\(^2\) for Fib, 408 ng/cm\(^2\) for Lys) was observed at 0°C, but by changing the temperature to 38°C, the amounts of protein adsorbed on PGNS\(_{8k}/\)PGM decreased sharply (11 ng/cm\(^2\) for BSA, 83 ng/cm\(^2\) for Fib, 77 ng/cm\(^2\) for Lys), representing its strong temperature responsive antifouling properties. By further increase the molar mass of PST, considerable amounts of protein adsorbed on PGNS\(_{8k}/\)PGM were observed at either 0°C or 38°C, and the amounts of protein adsorbed on PGNS\(_{8k}/\)PGM are of the same order of magnitude at both 0°C and 38°C as shown in Fig. 5. These results were fairly consistent with the observations obtained from

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Fig. 5. The adsorption amount of (a) BSA, (b) Fib and (c) Lys on PGM, PGN, PGNS\(_{8k},\) PGNS\(_{6k},\) PGN/PGM, PGNS\(_{8k}/\)PGM, PGNS\(_{6k}/\)PGM, PGNS\(_{8k}/\)PGM modified silicon wafer at 0°C and 38°C, respectively, obtained by using VASE. Data are expressed as mean ± SD (n = 3).
fluorescence microscopy. At last, the PGNS$_{6k}$/PGM shows the most attracting thermally responsive property and excellent stability (Fig. S9, S10), having important implication in developing chemically modified surfaces.

Conclusions

In this study, a mixed polymer brushes coating with thermoresponsive ability was fabricated by spin coating the mixture of PMOXA-r-GMA and PGMA-b-PNIPAM-b-PSt solutions onto silicon or glass substrates followed by annealing protocol via GMA units as anchor part. Under the selected temperature (below or above the LCST of PNIPAM), the hydrophilic/hydrophobic behavior of mixed brushes in aqueous environment was mainly governed by the exterior part through varying the molar mass (i.e. chain length) of PSt block. When the hydrated thickness of PGNS$_{6k}$/PGM was below the LCST of PNIPAM, PNIPAM block was swelled. The hydrated thickness of PGMA-b-PNIPAM-b-PSt brush increased with increasing the molar mass of PSt. When the hydrated thickness of PGMA-b-PNIPAM-b-PSt with lower molar mass of PSt block (PGNS$_{6k}$) was lower than that of PMOXA-r-GMA, the surface showed hydrophilic behavior; when the hydrated thickness of PGMA-b-PNIPAM-b-PSt with higher molar mass of PSt block (PGNS$_{8k}$ and PGNS$_{12k}$) was higher than that of PMOXA-r-GMA, the surface showed hydrophobic behavior. When the environment temperature remained above the LCST of PNIPAM, PNIPAM block was collapsed. The surface only displayed the hydrophobic behavior when the hydrated thickness of PGMA-b-PNIPAM-b-PSt with maximum molar mass of PSt block (PGNS$_{18k}$). The reversible transition between hydrophilic and hydrophobic behavior was realized for PGNS$_{6k}$/PGM mixed brushes in an aqueous environment upon temperature change (lower or higher than LCST of PNIPAM) through the reversibly exposing hydrophobic fragment at its hydrophilic surface. Moreover, switching of the exterior part for PGNS$_{6k}$/PGM could be used to adjust the adsorption of proteins by tuning the environmental temperature (lower or higher than LCST of PNIPAM). This strategy of reversibly exposing fragment will lead to potential application in coating design.

Conflicts of interest

There are no conflicts of interest to declare.

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