Electronic Supplementary Information

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

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Experimental

Materials

2-Methyl-2-oxazoline (MOXA, 99%, TCI, Tokyo, Japan) was dried by refluxing over CaH_2 and subsequently distilled prior to use. Glycidyl methacrylate (GMA, 97%, Aladdin, Shanghai, China) was passed through an activated basic alumina column to remove the inhibitor before use. Methyl tri-fluoromethanesulfonate (MeOTf, 98%, TCI, Tokyo, Japan) was distilled under reduced pressure and stored under nitrogen. Acetonitrile (ACN), methacrylic acid (MAA), triethylamine (TEA), isopropanol (IPA) and other reagents were obtained from Sinopharm Chemical Reagents (Shanghai, China). TEA was dried over potassium hydroxide and distilled before use. MAA was distilled under reduced pressure. 2,2'-Azobis(2-methylpropionitrile) (AIBN, Sinopharm Chemical Reagents, Shanghai, China) was recrystallized from methanol.

Synthesis of PMOXA-r-GMA

PMOXA-MAA. MOXA (5.03 g, 59.0 mmol), MeOTf (0.83 mg, 5.00 mmol), and 10 mL ACN were added into a dried glass tube at nitrogen atmosphere with a magnetic stir bar, then degassed by three freeze – pump – thaw cycles, then stirred at 80 °C in an oil bath for 22 h, the end-capping agents MAA (0.64 mL, 7.2 mmol) and NEt_3 (1.4 mL, 10 mmol) were added into the reaction tube, and then heated the solution at 70 °C in an oil bath for 36 h. The mixture was then cooled to room temperature, and the solvent was removed under reduced pressure. Afterward, the residue was dissolved in 20 mL of saturated $NaHCO_3$ aqueous solution and stirred for 30 min. Subsequently, the water in the mixture was extracted under reduced pressure, and 100 mL chloroform ($CHCl_3$) was added into the mixture, and the mixture solution was stirred overnight. Next, the mixture solution was dried over sodium sulfate overnight, filtered, and concentrated under reduced pressure. The concentrated solution was added dropwise into ice-cold diethylether to obtain the precipitate, and then the precipitate was dried in vacuo to get

the white solid macromonomer (PMOXA-MAA).

PMOXA-r-GMA. PMOXA-r-GMA with a 3/1 feed ratio of PMOXA-MAA and GMA was synthesized via free radical polymerization. As a typical example, PMOXA-MAA (1 g, 0.2 mmol), GMA (10 µL, 0.07 mmol), and AIBN (4.1 mg, 0.025 mmol) were mixed in 10 mL of isopropanol in a 20 mL dried glass tube equipped with a magnetic stir bar. The mixture was degassed via three freeze – pump – thaw cycles and placed in an oil bath at 70 °C for 24 h with vigorous stirring. The polymerization was quenched by cooling of the flask with cold water and exposure to air. Subsequently, the resulting viscous oil was diluted with 10 mL of chloroform, precipitated in cold diethyl ether, filtered off, and then dissolved in 10 mL of chloroform and precipitated again into diethyl ether. Next, the sample was dried in vacuum. Synthesized random copolymer PMOXA-r-GMA was named as PGM.

Characterization

^{1}H NMR of PGM. The ^{1}H NMR spectra of PMOXA-MAA and PMOXA-r-GMA are shown in Fig. S1. As shown in Fig. S1, the synthesized copolymer contains combined structural features of GMA and PMOXA supporting the controlled synthesis of the copolymer. The molecular mass of PMOXA-MAA determined from ^{1}H NMR was 4900 g/mol (degree of polymerization (DP) = 58). Molar ratio between PMOXA-MAA and GMA was 2.7:1, determined by calculating relative peak areas corresponding the PMOXA side chains ($\delta \sim 3.05$ ppm) and GMA groups ($\delta \sim$ 2.60 and 2.81 ppm).
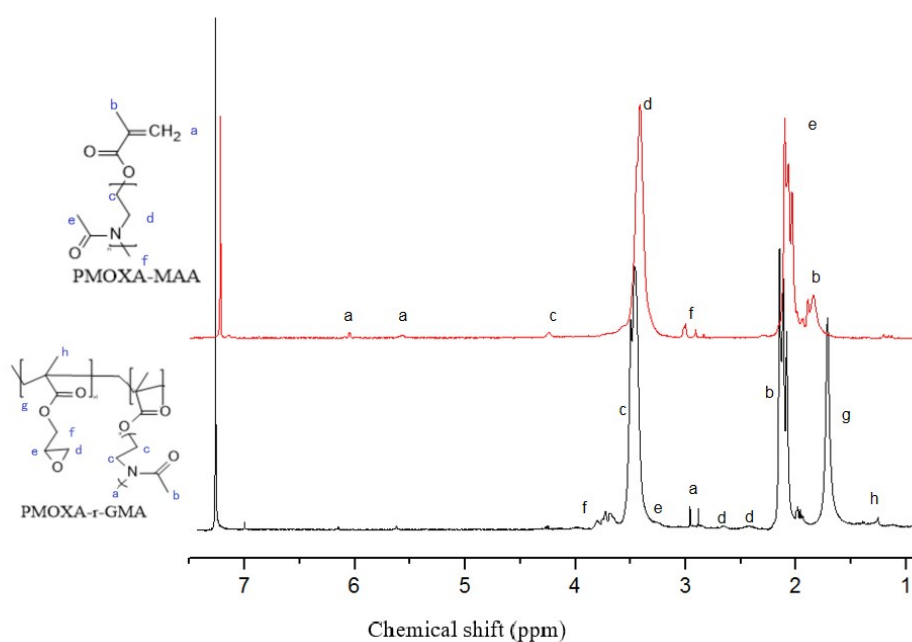

Fig. S1 ^{1}H NMR spectra of PMOXA-MAA and PMOXA-r-GMA.

Gel permeation chromatography (GPC) of PGN, PGNS$_{1k}$, PGNS$_{6k}$ and PGNS$_{8k}$. Number and weight average molar mass and molar mass distributions were determined by gel permeation chromatography (GPC) measurements on a Waters GPC system, which was equipped with a Waters 1515 HPLC solvent pump, a Waters 2414 refractive index detector, and four Waters styragel high resolution columns (HR4, HR2, HR1, HR0.5, effective molar mass range 5000 – 600000, 500 – 20000, 100 – 5000, and 0–1000, respectively). DMF was used as eluent at 35 °C, delivered at a flow rate of 1.0 mL min^{-1}. Polystyrene standards were used to generate the calibration curve. Molar mass distribution (PDI) of PGN, PGNS$_{1k}$, PGNS$_{6k}$ and PGNS$_{8k}$ determined by GPC are 1.10, 1.25, 1.39, 1.33,

respectively; the number-average molar mass of PGN, PGNS$_{1k}$, PGNS$_{6k}$ and PGNS$_{8k}$ determined by GPC are 10586 g/mol, 11957 g/mol, 14666 g/mol, 15831 g/mol, respectively.
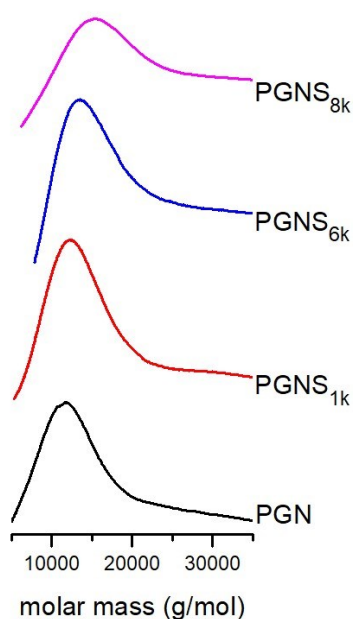

Fig. S2 GPC traces of PGN, PGN, PGNS$_{1k}$, PGNS$_{6k}$ and PGNS$_{8k}$.

In this work, approximately theoretical contour chain length for PMOXA side chains in PGM was about 20.1 nm calculated through Eq. S1.[1]

$$L = (L_{C-C} \times \sin\frac{\theta}{2} + 2 \times L_{C-N} \times \sin\frac{\theta}{2}) \times DP \qquad (\text{S1})$$

where L$_{C-C}$ and L$_{C-N}$ are the lengths of C-C (0.154 nm) and C-N (0.157 nm) bonds, respectively, θ is the corresponding bond angle of C-C-C (109.5) and C-N-C (107.28) in PMOXA side chains and DP is degree of polymerization of PMOXA measured by ^{1}H NMR.

Approximately theoretical contour chain length for PNIPAM block in PGNS was about 20.3 nm calculated through Eq. S2.[1]

$$L = 2(L_{C-C} \times \sin\frac{\theta}{2}) \times DP \qquad (\text{S2})$$

where L$_{C-C}$ is the length of C-C (0.154 nm) bond, θ is the corresponding bond angle of C-C-C (109.5) in PNIPAM block chains and DP (about 70) is degree of polymerization of PNIPAM block measured by ^{1}H NMR.

Approximately theoretical contour chain length for PSt block in PGNS (PGNS$_{1k}$, PGNS$_{6k}$, PGNS$_{8k}$) was about 2.9 nm, 17.2 nm, 22.9 nm calculated through Eq. S3 in which M$_n$ is 1232, 5897, 7925 g/mol and DP is 12, 59, 79 respectively.[1]

$$L = 2(L_{C-C} \times \sin\frac{\theta}{2}) \times DP \qquad (\text{S3})$$

where L$_{C-C}$ is the length of C-C (0.154 nm) bond, respectively, θ is the corresponding bond angle of C-C-C (109.5°) in PSt block chains and DP is degree of polymerization of PSt block measured by ^{1}H NMR. The contour chain length of polymers is shown in Table S1.

Table S1 Contour chain length of PGM, PGN, PGNS$_{1k}$, PGNS$_{6k}$, PGNS$_{8k}$.

	Contour chain length (nm)			
	PMOXA	PNIPAM	PSt	total
PGM	20.1 -		-	20.1
PGN	-	20.3	-	20.3
PGNS$_{1k}$	-	20.3	2.9	23.2
PGNS$_{6k}$	-	20.3	17.2	37.5
PGNS$_{8k}$	-	20.3	22.9	43.2

Differential scanning calorimetry (DSC). Differential scanning calorimetry measurements were carried out with a VP-DSC (Microcal, USA) from -50 to 100 °C (at a heating rate of $10\,°C \cdot min^{-1}$ for 3 times) at air atmosphere. The DSC data of third time was shown in Fig. S3. The glass transition temperature of PGN, PGNS$_{1k}$, PGNS$_{6k}$, PGNS$_{8k}$ are 69.2 °C, 73.6 °C, 78.0 °C, 78.1 °C.
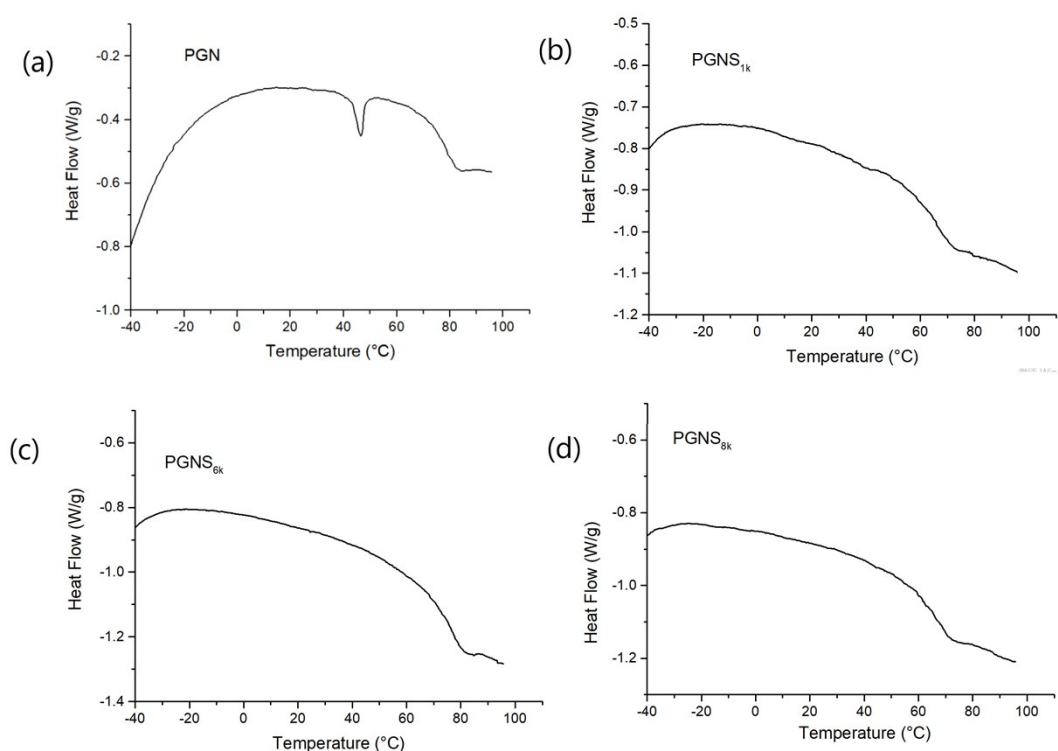
Fig. S3 DSC thermograms of (a) PGN, (b) PGNS$_{1k}$, (c) PGNS$_{6k}$, (d) PGNS$_{8k}$.

Surface characterization of the modified substrates. The X-ray photoelectron spectroscopy (XPS) spectra of wide scan for bare, PGN, PGM, PGNS$_{1k}$, PGNS$_{6k}$, PGNS$_{8k}$, PGN/PGM, PGNS$_{1k}$/PGM, PGNS$_{6k}$/PGM, and PGNS$_{8k}$/PGM modified silicon wafer are shown in Fig. S4. XPS spectra of the high-resolution C1s for PGM, PGN, PGNS$_{1k}$, PGNS$_{6k}$, PGNS$_{8k}$ and PGNS$_{1k}$/PGM, PGNS$_{6k}$/PGM, PGNS$_{8k}$/PGM modified silicon wafer are shown in Fig. S5. Peak area of O=C-N from for PGM, PGN, PGNS$_{1k}$, PGNS$_{6k}$, PGNS$_{8k}$ and PGNS$_{1k}$/PGM, PGNS$_{6k}$/PGM, PGNS$_{8k}$/PGM modified silicon wafer is shown in Table S2.
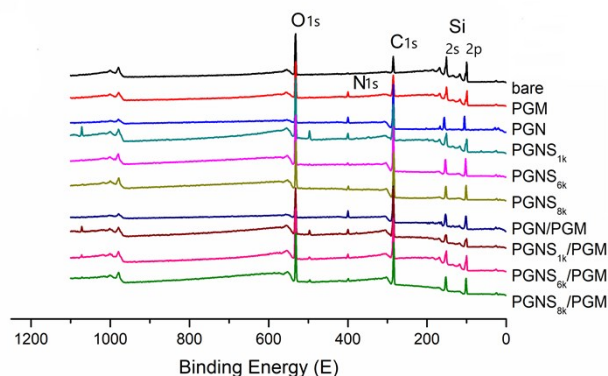
Fig. S4 XPS spectra of wide scan for bare, PGN, PGM, PGNS$_{1k}$, PGNS$_{6k}$, PGNS$_{8k}$ and PGNS$_{1k}$/PGM, PGNS$_{6k}$/PGM, PGNS$_{8k}$/PGM modified silicon wafer.
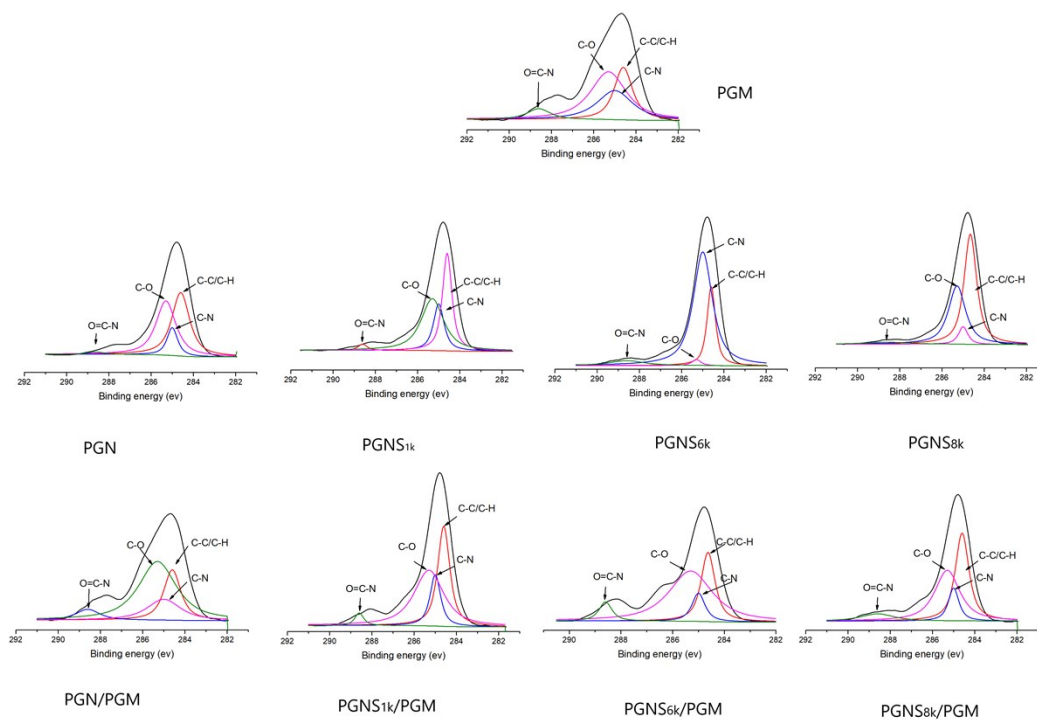

Fig. S5 XPS spectra of the high-resolution C1s for PGM, PGN, PGNS$_{1k}$, PGNS$_{6k}$, PGNS$_{8k}$ and PGNS$_{1k}$/PGM, PGNS$_{6k}$/PGM, PGNS$_{8k}$/PGM modified silicon wafer.

Table S2 Peak area of O=C-N for PGM, PGN, PGNS$_{1k}$, PGNS$_{6k}$, PGNS$_{8k}$ and PGNS$_{1k}$/PGM, PGNS$_{6k}$/PGM, PGNS$_{8k}$/PGM modified silicon wafer based on XPS.

	PGM	PGN	PGNS$_{1k}$	PGNS$_{6k}$	PGNS$_{8k}$	PGN/PGM	PGNS$_{1k}$/PGM	PGNS$_{6k}$/PGM	PGNS$_{8k}$/PGM
Peak area of O=C-N	16418.3	14980.03	12073.67	7502.12	6278.576	15500.83	14391.28	10098.57	8923.5

To search for appropriate composition ratio of PGNS and PGM on the mixed polymer brushes coating surfaces, a series of PGNS$_{6k}$/PGM mixed brushes coatings with different ratios of PGNS$_{6k}$ and PGM (0/10, 3/7, 5/5, 7/3, 10/0) were fabricated. Solutions of polymers with PGNS$_{6k}$/PGM mass ratio of 0/10, 3/7, 5/5, 7/3, 10/0, were

prepared by mixing stock chloroform solutions of PGM and PGNS$_{6k}$ at 10 mg/mL. Spin coating of mixed polymer solutions (150 μL for 1 × 1 cm^2) was performed on cleaned silicon or glass (immersed in piranha solution (7 : 3 v/v mixture of H$_2$SO$_4$ (95 − 98%) and H$_2$O$_2$ (30%) for 60 min, rinsed extensively with water, ethanol, and then dried under nitrogen) substrates for 20 s at 2000 rpm under vacuum by an EZ4 SPIN COATER (LEBO SCEINCE, 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.

Mass ratio of PGNS$_{6k}$/PGM based on XPS data are shown in Table S3. The surface composition of the binary mixed polymer brushes coatings was calculated from XPS C1s and N1s spectra. To check the mass fraction in PGNS/PGM, the peak area of carbon (C1s, 287 eV), nitrogen (N1s, 400.5 eV) was measured and compared to that present in pure PGM and PGNS modified brush, and the mass fraction was calculated by following equation

$$\frac{C_{binary}}{N_{binary}} = \frac{C_{PGM} \times f_{PGM} + C_{PGNS} \times f_{PGNS}}{N_{PGM} \times f_{PGM} + N_{PGNS} \times f_{PGNS}} \tag{S4}$$

$$f_{PGM} + f_{PGNS} = 1 \tag{S5}$$

where C_{binary} and N_{binary} is the peak area of the C1s and N1s components in the mixed brushes, C_{PGM} and C_{PGNS} are the C1s peak areas of pure PGM and PGNS components respectively, N_{PGM} and N_{PGNS} are the N1s peak areas of pure PGM and PGNS brushes respectively. While f_{PGNS}, f_{PGM} are mass fraction of PGNS, PGM in binary mixed polymer brushes respectively.

Table S3 Mass ratio of PGNS$_{6k}$/PGM brushes based on XPS

PGNS$_{6k}$/PGM[a]	Peak area of C1s	Peak area of N1s	f$_{PGNS6k}$/f$_{PGM}$[b]
0/10	190697.9	39248.72	-
3/7	168813.81	37955.18	1.9/8.1
5/5	175500.66	27341.87	4.3/5.7
7/3	181710.02	30351.23	6.6/3.4
10/0	140341.32	6749.11	-

[a] Feed mass ratio of PGNS$_{6k}$ to PGM in mixed polymer solutions
[b] Mass ratio of PGNS (or PGN) to PGM in mixed polymer brushes are calculated according to Eq. S4 and Eq. S5.

The grafting density of pure polymer brushes on the samples are calculated by the follow equation

$$\Gamma_{PGN} = \frac{\rho_{PGN} \times h_{PGN} \times N_A}{M_{n PGN}} \tag{S6}$$

$$\Gamma_{PGNS} = \frac{\rho_{PGNS} \times h_{PGNS} \times N_A}{M_{nPGNS}} \qquad\qquad (S7)$$

$$\Gamma_{PGM} = \frac{\rho_{PGM} \times h_{PGM} \times N_A}{M_{nPGM}} \qquad\qquad (S8)$$

where Γ_{PGN}, Γ_{PGNS}, Γ_{PGM} are PGN, PGNS, PGM polymer brushes' grafting density on the samples respectively, ρ_{PGN}, ρ_{PGNS}, ρ_{PGM} are density of PGN, PGNS, PGM polymer brushes (about 1.0 g/cm^3), h_{PGN} h_{PGNS}, h_{PGM} are the ellipsometric thickness of PGN, PGNS, PGM polymer brushes respectively, N_A is Avogadro constant, and M_{nPGN}, M_{nPGNS}, M_{nPGM} are the molecular mass of PGN, PGNS, PGM calculated according to results of ^{1}H NMR.

The grafting density of binary mixed polymer brushes on the samples are calculated by the follow equation

$$\rho_{binary} \times h_{binary} = \rho_{PGNS(PGN)} \times h'_{PGNS(PGN)} + \rho_{PGM} \times h'_{PGM} \qquad (S9)$$

$$\frac{f_{PGNS}}{f_{PGM}} = \frac{\rho_{PGNS(PGN)} \times h'_{PGNS(PGN)}}{\rho_{PGM} \times h'_{PGM}} \qquad\qquad (S10)$$

$$\Gamma'_{PGNS(PGN)} = \frac{\rho_{PGNS(PGN)} \times h'_{PGNS(PGN)} \times N_A}{M_{nPGNS}} \qquad (S11)$$

$$\Gamma'_{PGM} = \frac{\rho_{PGM} \times h'_{PGM} \times N_A}{M_{nPGM}} \qquad\qquad (S12)$$

$$\Gamma_{binary} = \Gamma'_{PGNS(PGN)} + \Gamma'_{PGM} \qquad\qquad (S13)$$

where Γ_{binary} is binary mixed brushes' grafting density, $\Gamma'_{PGNS(PGN)}$, Γ'_{PGM} are PGNS(PGN), PGM polymer brushes' grafting density in binary mixed brushes respectively, $\rho_{PGNS(PGN)}$, ρ_{PGM} are density of PGNS(PGN), PGM (about 1.0 g/cm^3), h'_{binary} is the ellipsometric thickness of binary mixed brushes, h'_{PGNS}, h'_{PGM} is assumed as thickness of PGNS(PGN), PGM in binary brushes, N_A is Avogadro constant, and M_{nPGNS}, M_{nPGM} are the molecular mass of PGNS(PGN), PGM calculated according to results of ^{1}H NMR. f_{PGNS}, f_{PGM} are gotten from Eq. S4 and Eq. S5.

Best ratio analysis. WCA, fluorescence microscopy image, and quantitative analysis of protein adsorption (described in corresponding part of main article) of PGNS$_{6k}$/PGM with different ratio were investigated, the related results are shown in Fig. S6. PGNS$_{6k}$/PGM with ratio of 5/5 showed the largest difference for WCA (Fig. S6a) and the amounts of protein adsorption (Fig. S6b and c) at 0 °C and 38 °C, implying that PGNS$_{6k}$/PGM mixed brushes with ratio of 5/5 possessed the best switchability towards the change of temperature.
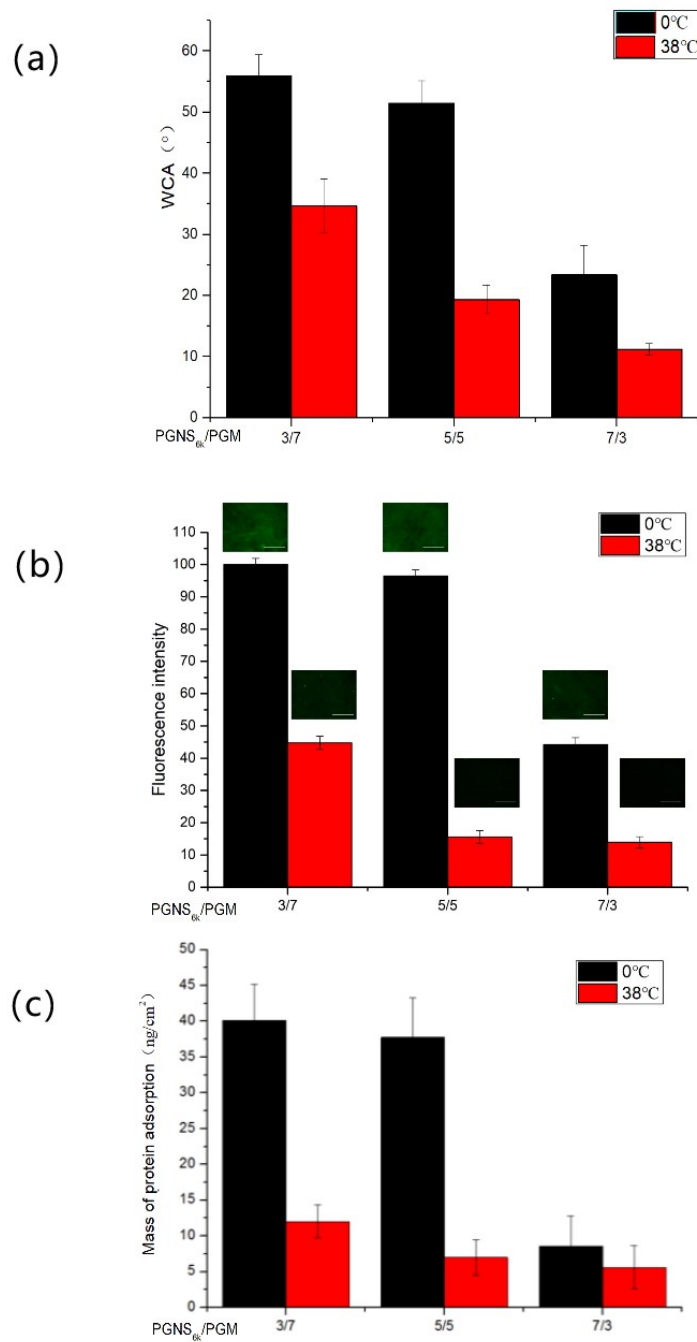
Fig. S6 WCA (a), fluorescence microscopy images and intensity of the FITC-BSA adsorption (b), mass of BSA adsorption (c) on PGNS$_{6k}$/PGM modified glass wafer with different ratio of 3/7, 5/5, 7/3 at 0 °C and 38 °C and scale bar in fluorescence microscopy images is 50 μm. Data are expressed as mean ± SD (n = 3).

S8

Adsorption of protein

To verify the reliability of VASE in the quantitative analysis for the protein adsorption, control analysis was measured as follow: First of all, the dry thicknesses of polymer coated glass wafers were measured by ellipsometry. And then the samples were separately immersed in PBS without protein (pH 7.4, 10 mM) of 0 °C or 38 °C and placed in the darkroom for 2 h. After that the wafers were washed with deionized water of 0 °C or 38 °C three times, followed by drying under nitrogen, then the thickness was measured at room temperature by ellipsometry. As shown in Fig. S7, the thickness of polymer modified wafers before and after solution treatment without protein was observed no difference. Fig. S8 shows the ellipsometry thickness of polymer modified silicon wafers before and after BSA, Fib and Lys adsorption at 0 °C and 38 °C.
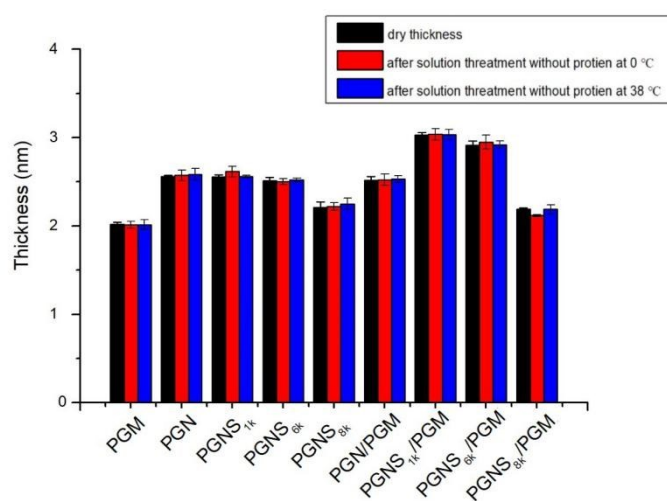

Fig. S7 Ellipsometry thickness of polymer modified silicon wafers before and after solution treatment without protein at 0 °C and 38 °C. Data are expressed as mean ± SD (n = 3).
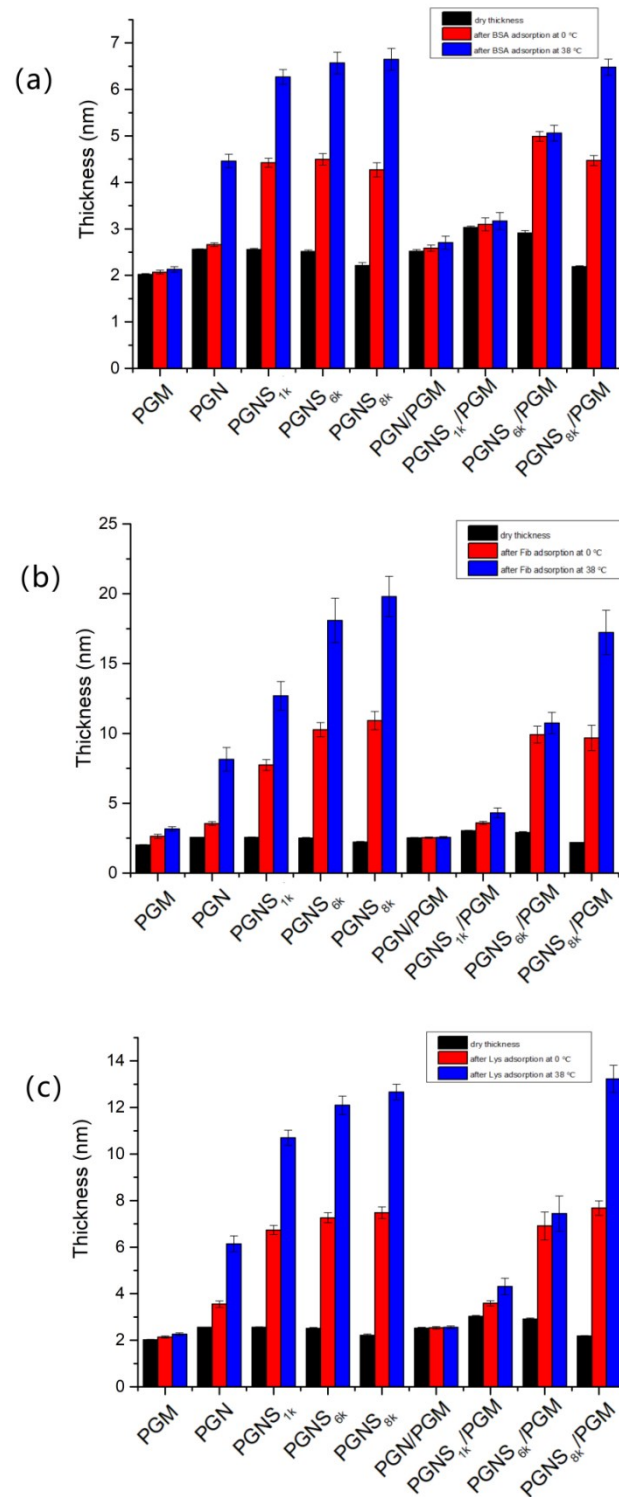
Fig. S8 Ellipsometry thickness of polymer modified silicon wafers before and after BSA(a), Fib(b) and Lys(c) adsorption at 0 °C and 38 °C. Data are expressed as mean ± SD (n = 3).

Coating stability test. $PGNS_{6k}/PGM$ coated glass wafers were used to investigate the coating stability. Firstly, $PGNS_{6k}/PGM$ coated glass wafers were soaked in PBS solution (pH = 7.4) at room temperature, and then the dry thickness of the coatings immersed for a certain time was measured by VASE; meanwhile, $PGNS_{6k}/PGM$ coated glass wafers after performing dry thickness measurements above were also immersed in BSA solution of PBS (pH 7.4, 10 mM; 1 mg mL^{-1}) of 0 °C or 38 °C for 2 h, washed with water of same temperature with BSA solution for 3 times, dried with nitrogen and the thickness after protein adsorption at 0 °C or 38 °C was measured at room temperature. All the ellipsometric data were fitted using Cauchy layer model to get the thickness of the brushes as described in the part of Ellipsometry in main text. The dry thickness of $PGNS_{6k}/PGM$ coating with immersion time are shown in Fig. S9; and protein adsorption amount (at 0 °C or 38 °C) on $PGNS_{6k}/PGM$ coated glass wafers with immersion time are shown in Fig. S10. The results indicated that $PGNS_{6k}/PGM$ coating is stable within 15 days.
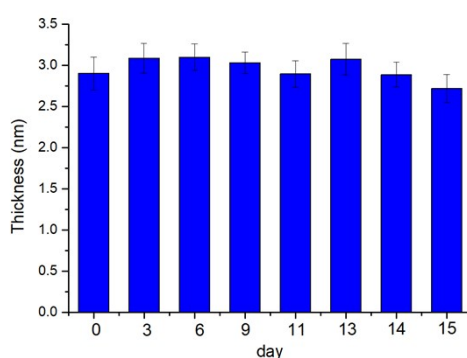

Fig. S9 Dry thickness of $PGNS_{6k}/PGM$ after immersing in PBS solution at room temperature for 15 days, obtained by using VASE. Data are expressed as mean ± SD (n =3).
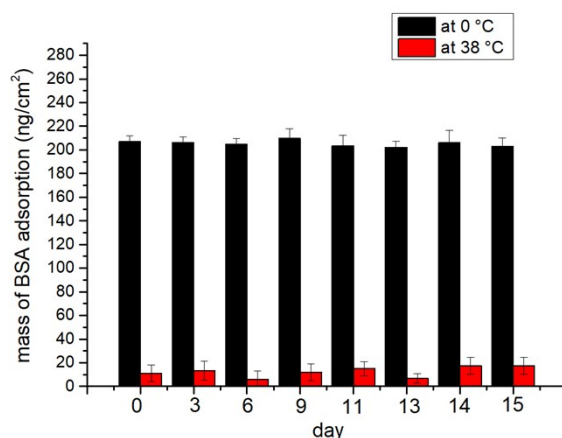

Fig. S10 The adsorption amount of BSA on $PGNS_{6k}/PGM$, $PGNS_{8k}/PGM$ modified silicon wafer after immersing in PBS solution at room temperature for 15 days at 0 °C and 38 °C, respectively, obtained by using VASE. Data are expressed as mean ± SD (n = 3).

References

[1] E.L. Adams, P.A. Kroon, G. Williamson, V.J. Morris, Carbohydr. Res. 2003, **338**, 771-780